The Insect Frontal Ganglion and Stomatogastric Pattern Generator Networks

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Key Words

Central pattern generator · Feeding behavior · Frontal ganglion · Locust · *Manduca sexta* · Molting · Neuromodulation · Stomatogastric nervous system

Abstract

Insect neural networks have been widely and successfully employed as model systems in the study of the neural basis of behavior. The insect frontal ganglion is a principal part of the stomatogastric nervous system and is found in most insect orders. The frontal ganglion constitutes a major source of innervation to foregut muscles and plays a key role in the control of foregut movements. Following a brief description of the anatomy and development of the system in different insect groups, this review presents the current knowledge of the way neural networks in the insect frontal ganglion generate and control behavior. The frontal ganglion is instrumental in two distinct and fundamental insect behaviors: feeding and molting. Central pattern-generating circuit(s) within the

frontal ganglion generates foregut rhythmic motor patterns. The frontal ganglion networks can be modulated in-vitro by several neuromodulators to generate a variety of motor outputs. Chemical modulation as well as sensory input from the gut and input from other neural centers enable the frontal ganglion to induce foregut rhythmic patterns under different physiological conditions. Frontal ganglion neurons themselves are also an important source of neurosecretion. The neurosecretory material from the frontal ganglion can control and modulate motor patterns of muscles of the alimentary canal. The current and potential future importance of the insect stomatogastric nervous system and frontal ganglion in the study of the neural mechanisms of behavior are discussed.

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Introduction

The insect nervous system has been widely and successfully employed as a model system in the study of the neural basis of behavior [e.g. 1–4]. Hoyle [1] has noted the outstanding importance of the insect model system for a comparative neurophysiological approach to neural net-

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work analysis (as in this special issue of *Neurosignals*). The considerable potential of insect models arises, on the one hand, from the great accessibility and simplicity of the insect nervous and neuroendocrine systems. Both systems have been amply studied and are well characterized in insects [4–6]. On the other hand, insects are capable of demonstrating complex and very carefully controlled behavioral processes that are relatively easily studied in the laboratory. The many similarities and common principles shared by the nervous systems of simple and higher organisms are by now a well-known and widely accepted fact [e.g. 7, 8].

A second key aspect of research on insect neural networks is that insights into the basis of insect behavior have important practical consequences. The arsenal of safe and cost-effective insecticides is shrinking for various reasons (e.g. insecticide resistance and unacceptable side effects). New strategies for insect pest management, more effective and above all ecologically safe ones, pose an ongoing challenge. Detailed knowledge of the neural and neuroendocrine mechanisms underlying insect behaviors can serve the urgent need to define alternative targets.

The insect stomatogastric nervous system (also referred to as the enteric or stomodeal nervous system in early work) is present, in some form or another, in all known insect species [9, 10]. It serves to innervate the anterior parts of the insect digestive tract [11–21]. Ample work and very rich literature has been dedicated to this system in Crustacea ([22], see also the article by Hooper, pp. 50–69, in the present issue). Mainly due to its small cell number and (relatively) simple behavioral output, the stomatogastric nervous system in lobsters and crabs has served for several decades as a leading model in the study of the neural control of rhythmic behavior. Our knowledge of the equivalent insect system (the question of homology is an important one but beyond the scope of the current work) is lagging far behind. Furthermore, as the stomatogastric nervous system is present in all higher invertebrates, starting from annelids [23–26], it offers a very suitable and attractive case for evolutionary or comparative investigations of the insect nervous system.

This review summarizes the present knowledge on the insect stomatogastric nervous system and, specifically, one of its principle components, the frontal ganglion (FG). Following a short description of the anatomy of the system in different insect groups, I briefly present aspects of the development of the insect stomatogastric nervous system. Both issues have been covered previously in detailed reviews (see below). The current paper focuses on the neural basis for the control of two fundamental behav-

iors in an insect's life: feeding and molting. As will be described, the stomatogastric nervous system, and specifically the FG, plays a critical role in both behaviors. The current state of our knowledge of the neural networks that generate foregut rhythmic movements will be presented. Finally, the importance of neuromodulation and neurosecretion in the insect stomatogastric nervous system will be discussed.

The Insect Stomatogastric Nervous System: Gross Anatomy and Development

The anatomy of the stomatogastric nervous system in insects reflects its function and thus varies mainly according to the mode of feeding of the species. As would be expected, the system is generally reduced in liquid-feeding insects, where foregut movement is mainly myogenic [27, 28] but is more evident in insects feeding on solid foodstuffs, where movements of the foregut are more complex, i.e. both myogenic and neurogenic [29–32]. In the case of holometabolous insects, where the mode of feeding is closely tied to the insect's developmental stadium, we should also expect some changes in the stomatogastric nervous system along with the insect's ontogeny. According to Snodgrass [33], 'it is impossible to give a general description applicable to all its (the stomatogastric nervous system) numerous variations in different insects'.

Detailed morphological studies of the stomatogastric nervous system of specific insect groups were first provided by Orlov [11, 34] for the larvae of the beetle *Oryctes*. An anatomical description was also given by Willey [12] for *Periplaneta americana* and other Blattaria. Dando et al. [16] first described the system in *Schistocerca gregaria*, and Kirby et al. [20, 21] in *Acheta domesticus*. Investigations of the stomatogastric nervous system of lepidopterous insects (*Manduca sexta*) were provided by Borg et al. [35] and Bell et al. [36]. These and others were reviewed in detail by Penzlin [10] and Chapman [37]. More recently, the stomatogastric nervous system in some Dipteran species [38–40] and in *Apis mellifera* [41] was also described.

In all the studied insects the stomatogastric nervous system consists of a series of small ganglia that are closely associated with the brain, the corpora cardiaca and corpora allata and the anterior portion of the gut (fig. 1). The FG is a principal component of the stomatogastric system in most insect orders [10, 37], accordingly, this ganglion has attracted much research. The best detailed account of

Fig. 1. Sectional diagram of an Orthopteran insect head, showing the position of the cephalic stomatogastric ganglia, the frontal and hypocerebral ganglia (black) relative to the major neural and endocrine centers. Modified from Ayali et al. [45].

the cellular structure of the insect FG was given for *P. americana* [42–44] and *Acheta* [20, 21].

Two insect preparations which, in recent years, have been at the focus of neurophysiological studies of the stomatogastric nervous system will be discussed in greater detail below. Ample work on the different Orthopteran species has set the ground for recent investigations of the FG of the desert locust *S. gregaria* [45–47], and recent studies on the tobacco hornworm *M. sexta* [48, 49] are based on previous work on lepidopterous insects.

In locusts, the FG lies in the forehead, on the dorsal side of the pharynx, in front of the brain. It is connected to the tritocerebrum of the brain by the paired frontal connectives (fig. 2a). Posteriorly, a recurrent nerve passes from the FG 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 (APN), median (MPN) and posterior pharyngeal nerves (PPN) – branch onto the dilator muscles of the gut in a rostrum to caudal order, making the FG the major source of foregut muscles innervation (fig. 2a). The median 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 FG is encased by a neural

lamella. It is characterized by a central neuropil surrounded dorsally and laterally by a single or double layer of neurons [see figure 1 in 45]. The cell bodies are 25–50 μm in diameter. Their number is estimated to be about 100, depending on the exact methods used [18, 19].

The FG in both larval and adult *Manduca* is connected to the tritocerebrum by the paired frontal connectives (fig. 2b). Two additional nerves exit the *Manduca* ganglion; a single recurrent nerve that runs posteriorly from the FG to innervate the muscles of the pharynx and esophagus, and an anteriorly directed frontal nerve which innervates the buccal musculature. Unlike the locust, the *Manduca* stomatogastric nervous system does not feature a hypocerebral ganglion after the embryonic stage [48]. During adult development there is much rearrangement of foregut musculature accompanying the formation of the cibarium. In adult moths, the FG innervates the muscles of the cibarial pump. The frontal nerve innervates most of the pump dilators and the recurrent nerve innervates the pump compressors (fig. 2b); [49]. In *Manduca* fifth-instar larvae and adults, the FG is 160 μm in diameter and contains about 35 neurons, arranged in a single layer. The neurons range in diameter from about 20 to 45 μm , and a number of them have been identified in both larvae and adults [48–50].

A growing number of studies present the insect stomatogastric nervous system as a model for nervous system development. Most research on insect stomatogastric system development employed either *M. sexta* [51–55], or *Drosophila melanogaster* [56–58]. Ganfornina et al. [59] focused on the embryonic development of the locust stomatogastric nervous system. Using a number of specific molecular markers, these authors studied morphogenesis and some aspects of neuronal differentiation in the locust system. In his thorough review, Hartenstein [60] reports that the insect stomatogastric nervous system is derived from a small neuroectodermal placode located in the foregut. All cells within this placode give rise to neural cells whose precursors migrate and finally re-aggregate to form the different stomatogastric system ganglia.

Physiological Role of the Insect Stomatogastric Nervous System and Frontal Ganglion

In his review article, dedicated primarily to the structure of the stomatogastric nervous system, Penzlin [10] writes: ‘Much more experimental work is necessary to gain a better consolidated insight into the control function of the stomatogastric nervous system’. More than a de-

Fig. 2. A schematic drawing of the FG of the locust (**a**) and *Manduca* (**b**). In each panel, the upper half shows the ganglion nerves and the lower half the muscles they innervate. Caudal and rostral sides are also indicated.

cade later, in *The Neurobiology of an Insect Brain*, Burrows [2] made the following comment: ‘... virtually nothing is known of their (the stomatogastric or stomodeal ganglia) physiological actions, in stark contrast to their counterparts in crustacea.’

In spite of the above statements, the function of the insect stomatogastric nervous system has been the focus of many studies for more than 100 years [61, 62]. Most of these studies, however, examined the effects of ablating

the FG on the subsequent behavior and development of the insect [10, and references therein].

Roussel [63] reported a role for the FG in the control of cardiac rhythm in *Locusta migratoria*. These findings were repeated in the sweet potato hornworm, *Agrius convolvuli*, where the FG was also found to control heartbeat [64, 65]. This control is exerted via a pair of anterior cardiac nerves that branch off the FG visceral nerve to innervate the dorsal vessel.

Most previous results have indicated that the FG is instrumental in the processes of growth, water balance and molting [e.g. 66–80]. These findings have been partially supported by recent neurophysiological work which has concentrated on the role of the FG in the control of two fundamental behaviors in the life of insects, feeding and molting [45–50].

The Role of the Frontal Ganglion in the Control of Feeding-Related Behavior

Frontal ganglionectomy caused a decrease in feeding activity and food intake in *S. gregaria* [30, 67, 69], *L. migratoria* [72], *Gryllus bimaculatus* [68] and *P. americana* [73, 81, 82]. Food was reported to accumulate in the foregut, and fecal output was markedly reduced [30, and references therein]. Similar results were reported in Lepidoptera, including adult *Heliothis zea* [78] and *M. sexta* larvae [79]. Overall, from these multiple studies, one can deduce that the insect FG is instrumental in passing food through the foregut and in crop emptying.

The larval *Manduca* gut is constantly active. The FG neurons were found to innervate all the larval foregut muscles and the ganglion was reported to be both necessary and sufficient for producing the motor patterns of the foregut [48]. Two types of rhythmic foregut movements and, accordingly, two FG rhythmic motor patterns were described in fifth-instar larvae. The first are posteriorly directed waves of foregut peristalsis, which are generated by phase shifts between rhythmic bursts of activity in anterior and posterior constrictor muscles (fig. 3a). The second type of pattern is characterized by synchronous constriction of muscles along the entire esophageal region. The latter motor pattern was reported to be correlated with accumulation of food within the crop [48]. Presumably it serves to pack the food particles and prevent food from reverting from the crop. In both the described rhythmic patterns, buccal constrictor activity preceded esophageal constrictors.

In contrast to the larvae of most Lepidoptera, locusts are generalist feeders, consuming a wide variety of foods of different composition and form. This wide range, together with the more complex morphological structure of their foregut (in comparison to the *Manduca* larvae), has led to more complex foregut peristaltic behavior in locusts. Rhythmic activity is not always demonstrated by the locust foregut muscles [46]. Most interestingly, as is the case in the *Manduca* larvae, the intact locust shows two types of FG motor patterns [46]. However, in the locust only one of these patterns could be related to feeding behavior: a rhythmic 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. 3b). This ‘food passage’ behavior can be recorded from the FG nerves in association with the beginning of a feeding bout. 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 [46]. Between meals, the FG pattern is often totally inhibited; 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 both between the FG and the ongoing ventilation motor pattern of the locust [46]. It thus appears that gut movements may also participate in ventilation, probably as a means to help with hemolymph circulation.

Unlike the case of the larval moth, and more similar to the locust, the adult *Manduca* cibarial pump motor program is only displayed during feeding [49]. Unless the moth is feeding, the muscles of the cibarial pump are silent. The FG activity pattern that generates rhythmic pumping movements in the moth’s cibarium is initiated by chemical stimulus to the proboscis of the moth.

Much work is still needed in order to elucidate the role of the FG neural networks in insect feeding-related behavior. As is apparent from the rather limited neurophysiological data available, new insights could be gained by comparing the stomatogastric nervous system in the locust and moth preparations and in the different developmental stages of the holometabola.

The Role of the Frontal Ganglion in the Control of Molt-Related Behavior

As already mentioned, in addition to feeding, the foregut and insect stomatogastric nervous system play a critical role in at least one other aspect of insect life: the molt. A molting insect displays a stereotypical set of behaviors that culminate in the shedding of the old cuticle at ecdysis. Ecdysial behavior has been extensively characterized in crickets [83, 84], locusts [85–87] and moths [88–91]. In all these insects, as the molt approaches, the insect ceases all feeding-related activity and searches for a suitable site for ecdysis. For example, both *S. gregaria* and *Manduca* larvae stop feeding and become quiescent approximately 24–36 h before ecdysis [50, 87, 92]. This is part of a preparatory or pre-ecdysis phase, which includes motor patterns that are aimed at loosening and eventually splitting the old cuticle. Next, the insect extracts itself from its old cuticle, followed by an expansion period, during which the new cuticle is stretched and shaped and the wings are expanded and folded.

Fig. 3. FG feeding- and molting-related motor patterns in *Manduca* (**a**) and the locust *S. gregaria* (**b**). **a** The motor pattern recorded from the adult moth cibarial and our swallowing pump compressor (large units) and dilator muscles during feeding. Data are courtesy of CI Miles. **b** Simultaneous extracellular recordings of locust FG frontal connective (FC) and median pharyngeal nerves (MPN) during feeding and air swallowing behavior (lower trace). The boxed areas are shown in faster sweep speed on the right.

Hence, there are two stages during ecdysis in which the insect needs to exert pressure on the body wall [88]. The first is during rupture of the old cuticle, the second when expanding the new cuticle and wings after emergence. The principal mechanism for doing this is by filling the gut with air. De Bellesme [93] was the first to show that the pronounced enlargement of freshly emerged dragonflies

was accomplished by internal air pressure built up in the digestive tract [18]. Since then, air swallowing during ecdysis has been reported in a number of different insects [49, 76, 85, 88, 94]. The FG was reported to be important for this behavior in several species [14, 15, 49, 50, 66, 71, 75–77].

Hughes [74, 75] reported that the success of the imaginal ecdysis of the desert locust depends on inflation of the gut with air. The dynamics of the air-swallowing motor program during the imaginal ecdysis was monitored by electromyogram (EMG) recordings made from foregut dilator (extrinsic) muscles [74, 75]. Elliot [95] accompanied the EMG recordings during the course of the locust molt by intracellular recordings from a small number of motor neurons in the FG, supporting the fact that the ganglion is indeed the source of the molt-related foregut motor pattern [95]. Frontal ganglionectomy abolished air swallowing immediately [74]. In the cricket, Carlson and O’Gara [76] have also described the FG’s exclusive control over air-swallowing motor patterns and its importance for a successful molt. Ayali et al. (unpublished results) have recently confirmed these findings by testing the effects of ablating the ganglion of fifth-instar larval locusts, 48 h before the imaginal molt, on the probability of successful ecdysis. One hundred percent 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.

Zilberstein and Ayali [46] report a strong interaction between the locust FG and ventilation pattern generator circuits during ecdysis. Throughout the molt process, the FG and ventilatory patterns are totally synchronized, except for the very short period when air-swallowing behavior is activated. During air swallowing, a different pattern emerges that resembles the feeding-related pattern in many aspects (fig. 3b). This uncoupling of the ventilation and FG rhythms could be mimicked by experimental manipulation [46].

Air swallowing was also reported in Lepidoptera. Bell [77] suggested that the FG also plays a role in *M. sexta* eclosion, i.e. ecdysis to the adult stage. The FG was involved in swallowing air at the time of eclosion; frontal ganglionectomy abolished air swallowing immediately, leading to defects in eclosion and in expansion of the wings.

Recent work on *M. sexta* has revealed that the FG plays a critical role in the successful completion of both larval [50] and adult molts [49]. At both stages, the FG controls a foregut motor pattern that is used to remove molting fluids from the space between the old and new cuticle prior to ecdysis. Cornell and Pan [96] were the first to suggest that the gut played a role in the removal of molting fluids. At adult ecdysis, or eclosion, removing the FG resulted in difficulty or failure to shed the old cuticle [49]. The FG is activated about 6 h before the adult moth emerges from the pupal case. The crop initially fills with

molting fluid, then air. After eclosion, as the moth hangs in a position to expand its wings, the FG is again activated, producing a distinct air-swallowing motor pattern that lasts about 90 s (fig. 3a). During this period, the wings visibly expand. Miles and Booker [49] report that the few ganglionectomized individuals that successfully emerged from the pupal cuticle were unable to expand their wings. The motor pattern recorded from the FG at the time of eclosion is similar to that displayed during feeding (fig. 3) [49].

It is interesting to note that in aquatic arthropods (Crustaceans) the stomatogastric nervous system probably plays an equally important role in swallowing fluids in order to split the old cuticle. This has as yet not been investigated, though it was implied in many reports [e.g. 97–100]

Stomatogastric Neural Circuits

Electrophysiological studies of the stomatogastric nervous system of insects have been very rare. Möhl [32] was the first to conduct investigations of neural activity in the insect system. His work on *A. domesticus* was followed by Hertel [101], who studied the stomatogastric nervous system of *P. americana*. The latter described spontaneous nervous activity in the caudal parts of the system, most of which could be traced to the FG [101, 102]. As was also later confirmed by Pandey and Habibulla [103], spontaneous neuronal activity could be recorded from an isolated FG in vitro. Hertel and Penzlin [104] demonstrated spontaneous rhythmic burst activity in the stomatogastric nervous system of *P. americana* and *Blaberus craniifer*. Again, these authors suggested that the rhythm is generated in the FG and from there disseminates throughout the stomatogastric system. However, no physiological function could be assigned to this activity [10].

More recent reports for both *M. sexta* [48] and *S. gregaria* [45] have confirmed the early work and established the presence of a central pattern-generating circuit in the insect FG. The larval *Manduca* ganglion was spontaneously active and produced a bursting firing pattern in the total absence of descending or sensory inputs [48] (fig. 4a). Interestingly, the pattern demonstrated by an in vitro fully isolated preparation resembled that recorded prior to its isolation (characterized by phase shifts between rhythmic bursts recorded from the different efferent nerves, or a fully synchronized bursting pattern (Miles, pers. commun.).

A completely isolated in vitro locust FG also generated a robust and consistent spontaneous rhythmic motor pattern that could last for many hours (fig. 4b) [45]. The in

Fig. 4. a Simultaneous recordings from the frontal nerve (FN), recurrent nerve (RN), and frontal connective (FC) in a fully isolated FG dissected from a larvae *M. sexta*. **b** Simultaneous recordings from the frontal connective (FC), median and posterior pharyngeal nerves (MPN and PPN, respectively) in a fully isolated locust FG in vitro. The panels on the right show 1 burst of activity played at a higher sweep speed to reveal phase relations between different members of the FG central pattern generator.

in vitro pattern was found to be independent of the donor locust's physiological or developmental stage. It was characterized by multi-unit bursts of action potentials that could be recorded from the various FG motor nerves. Ayali et al. [45] have defined the locust FG 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. Interestingly, in another closely related Orthopteran, the cricket *Teleogryllus oceanicus*, an isolated FG in vitro

was reported to generate rhythmic activity only for 1 h [76]. Furthermore, in vitro rhythmic activity was exhibited only if the ganglion was dissected out of a molting insect and was characterized by a normal air-swallowing frequency [76].

The locust FG contains around 100 neurons [105], in between the numbers reported for the ganglion of other Orthoptera, such as the cockroach (circa 80 neurons [106]) and the cricket (150 neurons [21]). Are all these neurons members of the pattern-generating network(s)? In most of the central pattern-generating systems investigated, the pattern-generating circuit consists of interneurons, though in some preparations the motor neurons

themselves participate in generating the rhythm (e.g. the stomatogastric nervous system of Crustacea [107, and references therein]). Kirby et al. [21] suggest that no more than 25% of the neurons in the FG of *A. domesticus* are interneurons. Aubele and Klemm [19] described 19 neurons located in the locust FG that send their axons to innervate foregut muscles via the frontal connectives and their branches. Similar numbers of small-size neurons (motor neurons) were also reported by Elliott [95]. The rather limited number of rhythmic units included in FG nerve recordings [45], and some preliminary intracellular survey of the ganglion neurons in which the majority of cells proved to be either silent or tonically active (Ayali, unpublished results), are both consistent with the idea that only a relatively small number of the ganglion neurons take part in the FG rhythmic motor pattern. Hertel and Penzlin [104] suggested that the FG spontaneous rhythmic activity in *P. americana* is generated within the ganglion's neuropil in a pair of neurons whose cell bodies are located outside the ganglion, in the protocerebrum. These neurons send their axons to the FG via the nervous connectives.

Our knowledge of the neuronal characteristics or the cellular properties of members of the insect FG neural circuit is limited. In *Manduca*, only a few of the 35 FG neurons have been explored, using intracellular pipette recordings [48–50]. A number of motor neurons have been identified by correlating their spiking activity with specific muscle excitatory junction potentials. Nothing, however, is known on the synaptic connections between the network members and on their role in generating the rhythmic pattern. The locust FG has proved to be a challenging system for intracellular analysis [45, 95]. However, the limited intracellular recordings made from locust ganglion neurons demonstrate the presence of some properties which are considered to be important for central pattern generation, such as bursting, plateau potentials and post-inhibitory rebound. Recently, Shefi et al. [108] developed a culture preparation of dissociated locust FG neurons. Intracellular recordings from unidentified isolated FG neurons in culture just as they started to regenerate their neuronal processes, also revealed some of the neurons' endogenous properties, such as spontaneous firing and post-inhibitory rebound (Ayali, unpublished results). Extracellular recordings from two-dimensional networks in cultures of FG neurons revealed rhythmic bursting (Ayali et al., unpublished results).

Some information on the synaptic properties of the FG central pattern generator networks can be gained from sporadic evidence regarding effects of cholinergic agonists

and antagonists on the insect FG's electrical activity ([104, 109, 110]; Ayali and Dekel, unpublished results). Acetylcholine is an important transmitter in insects. It has also been reported to be a neurotransmitter in the stomatogastric nervous system of the earthworm [25] as well as in Crustaceans [111–113]. Acetylcholine and nicotine showed dose-dependent stimulation of the FG rhythm in *P. americana* [104]. Hertel et al. [110] reported on dose-dependent effects of different organophosphorous insecticides on spontaneous burst frequency of isolated ganglia. Increasing concentrations resulted in shortening of the inter-burst intervals, up to total loss of rhythmicity. Similar results were obtained in *S. gregaria* by bath application of the acetylcholinesterase inhibitor paraoxon to a rhythmically active, isolated FG in vitro (fig. 5; Ayali and Dekel, unpublished results). Penzlin [10] reviewed evidence of high acetylcholinesterase activity in the neuropil of the FG in *P. americana*. However, the cockroach stomatogastric nervous system ganglion cells could not be histochemically stained.

Thus, although ample evidence suggests an important role for acetylcholine in the insect FG, as yet the data do not distinguish between cholinergic synaptic transmission and cholinergic modulation.

Neurosecretion and Neuromodulation in the Stomatogastric Nervous System

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 is a fundamental question in neurobiology. Substantial progress has been made by studying rhythmic behaviors and the central pattern generator circuits that generate them [114, 115]. It is now clear that the nervous system can alter the properties of central pattern generators, via both descending as well as sensory inputs, to elicit many different motor patterns [e.g. 22, 116–121]. Thus the motor patterns of the insect foregut are expected to be multiple and complex, and the FG central pattern generator controlling these motor patterns is expected to generate various motor outputs, depending on the animal's physiological and behavioral state.

Neuromodulation of the Frontal Ganglion Central Pattern Generator

As already described, the insect stomatogastric nervous system plays a major role in two behavioral contexts: feeding and molting. Both behaviors (especially the latter)

Fig. 5. Simultaneous recordings from the median and posterior pharyngeal nerves (MPN and PPN, respectively) in a fully isolated locust FG in vitro. Data show the FG rhythm in control conditions, 5 and 10 min after bath application of 1mM of paraoxon (PO). The organophosphorous compound PO inhibits the enzyme acetylcholinesterase irreversibly.

are made up of a complex set of motor patterns that need to be carefully coordinated and controlled.

Volumetric feedback from the gut has been suggested in several preparations and undoubtedly takes part in the control of the stomatogastric nervous system and feeding-related motor output. Sensory information mediated via stretch receptors from the gut wall has been shown to be instrumental in the control of feeding in the fly [122–125]. Volumetric feedback from the crop and hindgut was

reported to interact in the regulation of meal size in crickets [126] and locusts [127]. Clarke and Langley [13] reported that in *L. migratoria*, the FG forms a link in the passage of nervous impulses originating from the stretch receptors of the pharynx and passing via the posterior pharyngeal nerve, FG and frontal connectives to the brain. Consistent with these early reports [see also 20], Zilberstein and Ayali [46] found that the amount of food present in the locust gut modulates the frequency of the

FG rhythm. The FG and foregut feeding-related rhythmic patterns were totally inhibited when the entire gut seemed replete with food [46]. In *M. sexta* the pattern of foregut activity has also been shown to vary with the amount of food present in the foregut and crop [48].

The ascending signals of gut sense organs (e.g. stretch receptors) could either produce inhibition directly or generate central inhibition [79]. Miles and Booker [48] showed that isolating the FG of *Manduca* larvae from the brain results in alteration of the FG rhythmic pattern. As reported for other insect central pattern generators [128], inputs from the brain were shown to have an inhibitory effect on the locust ganglion in an in vitro FG-brain preparation. The locust FG rhythmic pattern emerged only after severing the frontal connectives coming from the brain [46]. Other neuronal inputs to the FG have also been suggested. As already mentioned, Zilberstein and Ayali [47] reported on interactions between the FG and the thoracic ventilation central pattern generator, which are mediated via the frontal connectives. Backfilling the frontal connectives resulted in staining several neurons in the subesophageal ganglion and in thoracic ganglia all the way to the metathoracic ganglion, in which a single neural cell body was stained (Zilberstein and Ayali, unpublished finding). The metathoracic ganglion is where the locust ventilation central pattern generator resides [129].

Feeding-related motor patterns in the stomatogastric nervous system could also be controlled by humoral factors or regulatory neurohormones released into the circulation. These can be similar or different to those acting in classic synaptic transmission in the above-mentioned neuronal pathways. Release of humoral factors that play a role in cessation of locust feeding and involvement of chemoreceptors of the foregut was already suggested by Bernays and Chapman [130]. Ayali et al. [45] reported that application of hemolymph collected from locusts with a very full gut and crop to an isolated FG in vitro inhibits an ongoing rhythm.

Chemical modulation of FG motor patterns may also be working via localized release of neuromodulatory substances into the ganglion neuropil. In their electron microscopic and immunohistochemical study of the FG of *P. americana*, Ude et al. [131] reported a high content of neurosecretory material limited to the FG neuropil. These authors suggested that the autonomous control of the FG over vegetative function is influenced (i.e. modulated) by extrinsic neurosecretory cells establishing contact with intrinsic neurons within the ganglion. In *A. domesticus*, Kirby et al. [21] described neurons with cell bodies in the brain's median neurosecretory area that send their axons

to the FG, and suggested that these neurons are the source of the neurosecretory material described by many authors in the FG [see references in 21].

Two candidates for feeding-related FG neuromodulation will be discussed below: first, peptides of the allatostatin peptidergic family, and second, the biogenic monoamine octopamine.

Allatostatins are a large group of neuropeptides identified and localized by immunocytochemical means in many different insect species [132–137]. Besides their original physiological role as inhibitors of juvenile hormone production, members of this rich family of peptides have been reported to demonstrate inhibition of myotropic activity [138, and references therein]. Maestro et al. [134] have observed allatostatin-immunoreactive neurons in the tritocerebrum of *Blattella germanica*, with axons projecting and branching into the FG. This is of great interest in the light of recent data by Zilberstein and Ayali [47], who reported modulatory effects of allatostatin on the rhythmic output of an isolated locust FG in vitro. Furthermore, as described in the next section, FG cells that contain allatostatin immunoreactivity have been reported in various insects. The question whether the FG network is auto-modulated by neurosecreting neurons from within the ganglion is very intriguing.

Octopamine is an important modulator of neural function and behavior in insects. Octopamine modulation of insect rhythmic behavior has been repeatedly described [e.g. 139, 140, 141]. In relation to feeding behavior, in a recent study Miles and Booker [142] suggested that the dramatic decline in foregut activity demonstrated by *M. sexta* larvae parasitized by the braconid wasp *Cotesia congregata* was due to a sharp increase in hemolymph octopamine. Octopamine may also be a modulator of feeding and gut motor patterns in normal healthy larval moths.

In accordance with the above report, application of octopamine to an isolated locust FG in vitro disrupted all rhythmic activity [47]. Braunig [143] reported on subesophageal, octopaminergic dorsal unpaired median neurons which project into the FG. The ganglion is reached via the frontal connectives. Additional axon collaterals project into the numerous side branches of the nervous corporis cardiaci III, which innervate the hypocerebral ganglion and several pharyngeal dilator muscles. Thus one can speculate that octopamine, which is often correlated with arousal state in insects [144–146] and plays an important role in locust flight [145, 147, 148], will work to inhibit feeding-related behavior during demanding and stressful physiological states.

It is interesting to note that a role in the regulation of the stomatogastric nervous system was suggested for another biogenic amine, histamine. Horner et al. [149] reported that the histamine-immunoreactive terminals in the neuropil of the FG of *G. bimaculatus* have a neurosecretory appearance. This observation was also confirmed in the cockroach *Leucophaea maderae* [150].

The same mechanisms that act to shape feeding-related motor patterns in the insect stomatogastric nervous system also apply to the molt. Hughes [75] suggested a role for volumetric feedback in regulating the air-swallowing motor program during ecdysis. This was confirmed by Zilberstein and Ayali [46], who punctured the fully inflated gut of an adult locust when it was expanding its wings just as it fully emerged. A characteristic FG air swallowing rhythm was instantaneously initiated to compensate for loss of air pressure in the gut.

A number of peptide hormones are currently believed to be involved in the control of insect ecdysis behavior [151]. Carlson and O'Gara [76] reported that the cricket FG could generate spontaneous activity in vitro only if isolated from near-molt, ecdysing, or expanding insects. This state of 'activation' wore out within 1 h, during which the ganglion generated an air-swallowing pattern. In *Manduca* there is also evidence for foregut and FG modulation during the molt: hemolymph collected from molting larvae and applied to a larval FG-foregut preparation altered the ongoing feeding motor pattern to resemble that observed in molting larvae. A rhythmic motor pattern that resembles air swallowing could be generated in isolated heads of animals 24–30 h from eclosion by application of eclosion hormone (EH) [49]. Interestingly, in the locust, hemolymph collected from non-feeding pre-molt larvae inhibited FG rhythmic activity [45], and Zilberstein and Ayali [47] have reported that eclosion hormone transiently inhibited the FG rhythmic pattern in vitro. These different and somewhat contradictory observations can be explained by considering the different and complex effects of the various insect ecdysis-related peptides [151–154], and by suggesting that exact timing is crucial for the experimental manipulations. Zitnan and Adams [155] suggest that the initiation of pre-ecdysis behavior and the transition to ecdysis are regulated by stimulatory and inhibitory factors released within the central nervous system (CNS) after the initial actions of pre-ecdysis-triggering and ecdysis-triggering hormones. These factors, including EH, which is released both centrally into the brain and CNS and into the circulation, set the temporal organization of ecdysis behavior and the progression from one phase to the next. Thus, to activate each

subunit of the molt set of programmed behaviors, specific neural elements, including the FG neural network(s), are targeted at precise time points.

According to the current scheme, crustacean cardioactive peptide (CCAP) plays a role in maintaining the ecdysis motor program in insects [151]. Bestman and Booker (pers. commun.) have observed that CCAP generated increased motility and, in many cases, peristalsis in the molting larvae foregut. In locusts, Zilberstein and Ayali [47] demonstrated a modulatory effect of CCAP on the rhythmic pattern of the FG: a dose-dependent excitatory effect was obtained by application of CCAP to an isolated locust ganglion in vitro. In accordance with these reports, Dirksen and Homberg [156] observed CCAP immunoreactivity in locust brain neurons that gave rise to extensive arborization within the FG neuropil as well as in a FG neuron.

The Insect Stomatogastric Nervous System and Frontal Ganglion as a Source of Neurosecretion

In contrast to the rather limited studies on other aspects of insect stomatogastric nervous system physiology, very rich literature is available on neuropeptides and other neurosecretory substances in cells of the stomatogastric system. Nevertheless, early reports regarding the presence of neurosecretory cells in the FG were somewhat controversial [10, and references therein].

Peptide-producing neurosecretory cells have been described in the FG of a number of lepidopterous insect species. Bounhiol et al. [157] described two such cells in *Bombyx mori*. This was supported by similar findings in *Manduca* [35, 36] and *Diatraea grandiosella* [158]. No specific role was suggested for these cells. Different degrees of activity in diapausing and non-diapausing pupae indicate a close relationship to developmental processes. Recently Duve et al. [135] reported on two pairs of large FG cells in *Helicoverpa armigera* that demonstrate allatostatin immunoreactivity, with one of the pairs showing colocalization with allatotropin [see also 137]. The axons of all four cells project to the brain via the frontal connectives and to the foregut via the recurrent nerve [136]. It is thus suggested that peptidergic neurons in the FG play a major role in regulating foregut motility ([135], see also previous section).

Penzlin [10] gave a thorough report of evidence for the presence of unidentified neurosecretory cells in the stomatogastric nervous system of different Orthoptera (mainly *Periplaneta* and locusts). A considerable amount of data regarding neurosecretory (mostly peptidergic) cells in the FG has accumulated since that review article was

published. Cell bodies that show immunoreactivity to a myotropic neuropeptide, Lom-AG myotropin I were detected in the FG of *L. migratoria* [159]. Locustatachykinin I (another myotropic neuropeptide) immunoreactivity was also found in cells of the FG of the locust [160, 161]. Myoshi and Endo [162] investigated the FMRFamide-containing peptidergic neurons in the stomatogastric nervous system of the American cockroach by immunoelectron microscopy. Immunoreactive cell bodies were located in both the ingluvial and frontal ganglia.

Luffy and Dorn [163] reported the presence of seven pairs of serotonergic perikarya in the FG of *Carausius morosus*. These authors also reported a strong excitatory effect of 5-HT on the isolated midgut. This was also observed in *Teleogryllus* by Cooper and He [164]. In another cricket, *G. bimaculatus*, serotonin and other major biogenic amines (octopamine, dopamine) were reported to be synthesized and metabolized in the FG [165], where the amount of serotonin was found to be highest.

Thus insect FG neurons are an important source of neurosecretion. The neurosecretory material from the FG can modulate muscles of the alimentary canal, thus providing an additional route for the stomatogastric nervous system control of gut motor patterns in feeding- and molting-related behavior.

Finally, there is even evidence for the presence of insulin in the FG of *M. sexta* [166], though no specific role for this has yet been suggested.

Concluding Remarks

Studies of identified neurons in insects have provided unsurpassed data on neural mechanisms of behavior [4]. In order for the insect FG and stomatogastric preparation to reach its full potential, much more work is needed in identifying and characterizing members of the FG central pattern generator in *Manduca* and the locust (for which much information is already available), as well as in other insect preparations.

A comparative approach in future research is important in order to elucidate the physiological significance of the FG and stomatogastric nervous system in insect behavior. More specifically, comparative studies across different insect species as well as different developmental stages are needed. The FG of Lepidoptera consists of circa 35 neurons, while that of the Orthopteran insects features three times this number or even more. Comparing the neuronal composition of the FG (and other stomatogastric ganglia) between these groups will shed light on the

role of the different components in generating and controlling behavior. Comparing the stomatogastric nervous system of the larval and adult stages in holometabolous insects is also an important question with implications for developmental neuroscience. Since the same set of neurons drive both the larval foregut and the adult cibarial pump, as neurons are neither added nor removed from the FG during adult development [49], it will be interesting to determine how the functions of the larval neurons change during adult development.

Finally, the question of motor program selection or the choice of motor pattern is fundamental in the study of the nervous system [167]. Hence, much of the appeal of the insect stomatogastric nervous system as a future neurobiological model system arises from its important dual role in feeding- and molting-related behavior. The specific motor output of defined neural circuits within the system must be generated at the appropriate time, in full coordination with other complex behavioral patterns. Hence, neuromodulation and the interaction between neural centers are fundamental concepts in understanding this system. Following the investigations described herein, comparing the feeding- and molting-related FG motor patterns, the way they are generated within the stomatogastric nervous system and the neural and chemical factors that shape and control them, is the mission we are currently engaged in.

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