

## Commentary

# The role of the arthropod stomatogastric nervous system in moulting behaviour and ecdysis

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### Summary

A possible role of the insect stomatogastric nervous system (STNS) in ecdysis was first implied in early studies reporting on internal air pressure build-up in the digestive tract and air swallowing during ecdysis. The frontal ganglion, a major component of the insect STNS, was suggested to play an important part in this behaviour. Recent neurophysiological studies have confirmed the critical role of the STNS in the successful completion of both larval and adult moults in insects. In aquatic arthropods, though much less studied, the STNS plays an equally important and probably very similar role in water swallowing. Water uptake is instrumental in splitting the crustacean cuticle and allowing successful ecdysis. Current data are presented in a comparative view that contributes to our understanding of the role of the STNS in arthropod behaviour. It also sheds light on the question of homology of the STNS among the different arthropod groups. New insights into the neurohormonal control of ecdysis, related to the STNS in both insects and crustaceans, are also presented and comparatively discussed.

Key words: crustacea, ecdysis, frontal ganglion, insect, neuromodulation, stomatogastric, ganglion.

### Introduction

The stomatogastric nervous system (STNS) of arthropods comprises the anterior portion of the nervous system, constituting a peripheral complex of ganglia and nerve fibres that innervate the visceral organs and are closely connected to the brain and endocrine system (Fig. 1).

Neuronal circuits within the STNS are capable of generating rhythmic motor patterns. These central pattern generator (CPG) networks in turn control the motion of the foregut and regulate food uptake and food transport. The generation and control of feeding-related behaviour is hence the primary physiological role of the STNS. In addition, however, the STNS of arthropods offers a leading system in the study of questions related to multi-functional neuronal circuits. The reason for this is first and foremost the need for the STNS to cope with various food types and food particle consistencies, and to generate different appropriate variations of the motor pattern. A second, and somewhat less acknowledged, reason is the important role that the arthropod STNS plays in another fundamental, yet very different, behavioural context beyond feeding – that of moulting and ecdysis.

In the current paper I provide a comparative view of the STNS of insects and crustaceans. I first deal with aspects of the anatomy and development of the system and refer to the question of homology of the STNS among the different arthropod groups. Next, I present a brief account of the much studied physiology of the STNS in crustaceans, followed by the more recent work in insect preparations. The latter has established the role of the insect frontal ganglion in moulting-related behaviour. Much less is known about the role of the gut and the STNS in the control of ecdysis in other arthropods. The next section, therefore, reviews what is known about ecdysis in decapod crustaceans. Finally, the STNS offers a leading model system in the study of neuromodulation and the effect of modulators on CPG circuits. Evidence for the role of ecdysis-related peptides in

the control of the STNS is presented, including a recently suggested new function of the crustacean cardioactive peptide (CCAP) in the early stages of moult in insects, and data on this and other peptidergic modulators in crustaceans are provided.

### The arthropod STNS: the question of homology

The STNS is of interest from an evolutionary point of view as it is regarded as a plesiomorphic character, being present in all higher invertebrates, starting with the annelids (e.g. Millott, 1943; Barna et al., 1997). Hence it offers a very suitable and attractive case for a comparative investigation of the nervous system.

Katz and Tazaki (Katz and Tazaki, 1992) referred to the question of homology as a first and important step in conducting a comparative study of the STNS (among crustaceans). They cited Roth's definition of structures in different species as being considered homologous if they had arisen from the same structure in a common ancestor (Roth, 1984). Another frequently used way by which to demonstrate homology is to show that the two structures in question have the same embryological origin (Thomas et al., 1984).

However, the need for 'the same embryological origin' (Thomas et al., 1984) is somewhat problematic. A growing number of studies have focused on the insect STNS as a model for nervous system development. Most research on insect stomatogastric system development has employed either the moth *Manduca sexta* (e.g. Copenhaver and Taghert, 1991) or the fly *Drosophila melanogaster* (e.g. Hartenstein et al., 1994; Forjanic et al., 1997). In his thorough review, Hartenstein (Hartenstein, 1997) reports that the insect STNS 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. Unfortunately, no such knowledge is available for the crustacean STNS.

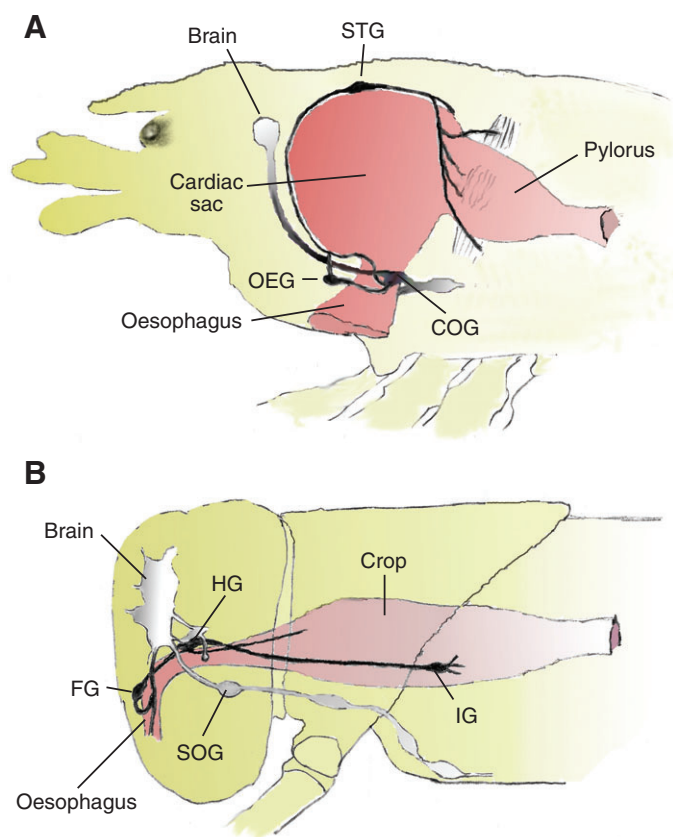


Fig. 1. The arthropod stomatogastric nervous system. The stomatogastric ganglia and associated nerves are shown (black) in lobster (A) and locust (B), including their nerve connections to the rest of the nervous system (shaded grey) and to the targets they innervate (schematic representation only). COG, commissural ganglion; FG, frontal ganglion; HG, hypocerebral ganglion; IG, ingluvial ganglion; OEG, oesophageal ganglion; SOG, suboesophageal ganglion; STG, stomatogastric ganglion.

The question of homology between the insect and the crustacean STNS is better answered by addressing the phylogenetic relationship between the two groups. An autonomous nervous system consisting of a pharyngeal part and an intestinal part is present in most flatworms. However, it should first be established that this is indeed the 'same structure in a common ancestor' as appears in Roth (Roth, 1984). Insects and crustaceans are sister groups whose lineages diverged over 500 million years ago. Previous reports have been uncertain regarding whether these evolved from a common arthropod ancestor or, alternatively, whether they evolved independently from annelid worms (e.g. Osorio and Bacon, 1994). In contrast, a crustacean-insect close affinity was postulated based on the synthesis of several well-sampled molecular loci, and supported by a comprehensive data set of morphological, developmental, ultrastructural and gene order characters (e.g. Strausfeld, 1998; Giribet et al., 2001; Strausfeld et al., 2006). In accordance with these studies, a more recent analysis by Andrew and Strausfeld (Andrew and Strausfeld, 2008) strongly supports a monophyletic clade that includes hexapods (including insects) and crustaceans (based on neuromorphological characters in a Bayesian framework).

The STNS of different arthropods may thus be in correspondence or even similar in position, structure and, above all, function. The following sections deal mainly with the latter aspect, introducing

evidence for similarities in the physiological function of the STNS. The focus here is on the crustacean stomatogastric ganglion (STG) and the insect frontal ganglion (FG), as these are the principal components of the system in the two groups (Fig. 1), both of which have attracted much research.

## The physiological role of the STNS in the control of feeding-related behaviour

Many decades of research have provided very detailed knowledge of the structure and function of the neural networks in the crustacean STNS (Fig. 1A) (Harris-Warrick et al., 1992) (reviewed by Selverston, 2008). Rather than beginning with an analysis of the behaviour of the foregut and then elucidating the neural substrates underlying it, the early work on this preparation began with analysis of the motor patterns generated *in vitro*. The *in vitro* crustacean STNS preparation very quickly became a leading model for the way in which rhythmic motor patterns are generated by well-defined neural networks. The study of this system as an archetype of pattern-generating circuits somewhat overshadowed its behavioural role in the intact animal. It still remains to be revealed fully the extent to which the complex repertoire of motor patterns displayed *in vitro* corresponds to a similarly complex behaviour in the intact animal (Turrigiano and Heinzel, 1992).

The crustacean foregut is composed of four compartments – the oesophagus, the cardiac sac, the gastric mill and the pylorus. The neural networks controlling the muscles of the different compartments are distributed among the ganglia of the STNS (Fig. 1A). The motor neurons innervating the muscles of the gastric mill and pylorus are found within the STG, the major component of the system. The gastric mill muscles rhythmically open and close a set of three teeth in a behaviour that resembles chewing. Two modes of activity can be observed in live animals *via* videoendoscopy: either simultaneous closing of the teeth, or sequential movements of the lateral and medial teeth to produce a cut and grind motion (Heinzel, 1988). These movements can be shown to be correlated with bursts of spikes in the appropriate motor neurons when recorded from isolated preparations. The pyloric network controls the dilator and constrictor muscles of the pylorus, producing a wave of peristaltic contractions from front to back that move the food particles toward the gut.

In principle, most pattern variations recorded from the isolated crustacean STNS preparation can be referred to as modes of 'fictive feeding'. This term, although regularly used for mollusc preparations and others, is not commonly attributed to the crustacean STNS. The reason is, again, the fact that the preparation was initially studied in great detail *in vitro* before observations began on the behavioural patterns of the intact animal. One example, directly relating STNS motor patterns to feeding, is the report on the effect of food intake on the rhythmic masticatory movements of the lobster gastric mill (Clemens et al., 1998). Another example of a motor pattern with clear behavioural attributes is that of a unique motor pattern generated jointly by neurons of different networks, which was described as swallowing-like behaviour (Meyrand et al., 1991).

Most early studies on the function of the insect STNS examined the effects of ablating the FG (Fig.1B) on the subsequent behaviour and development of the insect (Penzlin, 1985) [see also Ayali (Ayali, 2004) and references therein]. Overall, from these many studies, one can deduce that the insect FG is instrumental in passing food through the foregut and in crop emptying. More recent studies have provided insights into the neurophysiology of the FG.

Two types of rhythmic foregut movement and, accordingly, two FG feeding-related rhythmic motor patterns were described in fifth instar larvae of *M. sexta* (Miles and Booker, 1994). The first constitutes posteriorly directed waves of foregut peristalsis, which are generated by phase shifts between rhythmic bursts of activity in the anterior and posterior constrictor muscles. The second type of pattern, correlated with the accumulation of food within the crop, is characterized by the synchronous constriction of muscles along the entire oesophageal region.

In the desert locust a rhythmic motor pattern consistent with a rostrum-to-caudal peristalsis wave in the foregut muscles 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 ceases as soon as the locust gut is full (Zilberstein and Ayali, 2002). Between meals, the frontal ganglion pattern is often totally inhibited.

Unlike the case of the larval moth, and more similar to the locust, the adult *Manduca* cibarial pump motor programme is only displayed during feeding. 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 (Miles and Booker, 1998).

#### The role of the insect FG in moulting-related behaviour

Arthropod growth occurs through multiple stages. At the end of each stage the animal must moult in order to produce a new exoskeleton (cuticle), and then shed the remains of the exoskeleton from the previous stage by means of a behaviour called ecdysis. A successful moult is critical to the animal's survival. A moulting insect (Fig. 2) displays a stereotypical set of very carefully controlled and timed behavioural patterns. Ecdysial behaviour can be divided into distinct phases, which have been extensively characterized in various insects [crickets (Carlson, 1977); locusts (Hughes, 1980); moths (Reynolds, 1980); and flies (Clark et al., 2004)]. In all these insects, as the moult approaches, the insect ceases all feeding-related activity. 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. This is followed by an expansion period, during which the new cuticle is stretched and shaped and the wings are expanded and folded.

Hence, there are two stages during ecdysis in which the insect needs to exert pressure on the body wall (Reynolds, 1980). The first is during rupture of the old cuticle; and the second is when expanding the new cuticle after emergence (Fig. 2D). A principal mechanism for achieving this is that of internal air pressure build-up in the digestive tract *via* air swallowing (Fig. 2A,C). Frontal ganglionectomy was reported to result in either failure by the insect to escape the old cuticle or failure to successfully expand body appendages, and specifically wings (e.g. Bell, 1983; Miles and Booker, 1994; Ayali, 2004). The dynamics of the air-swallowing motor programme can be monitored by recording from foregut dilator muscles or from motor neurons in the FG (Hughes, 1980; Elliot, 1980; Zilberstein and Ayali, 2002). The results of such recordings support the exclusive control of the FG over air-swallowing behaviour (see also Carlson and O'Gara, 1983; Bell, 1983; Miles and Booker, 1994; Miles and Booker, 1998) (reviewed by Ayali, 2004).

Another critical moult-related function of the FG was reported in *M. sexta*, for which the gut was suggested to be involved in removal of moulting fluids from the space between the old and new

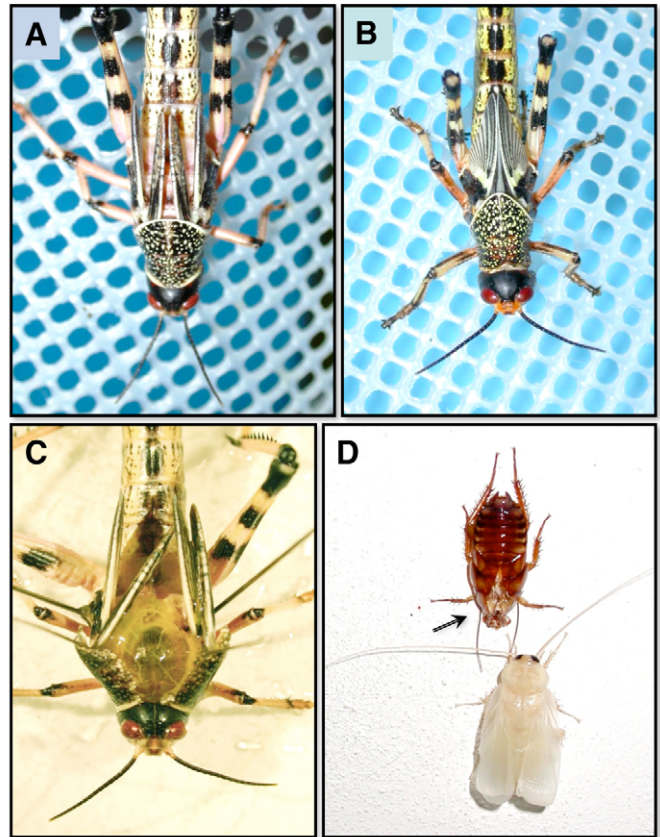


Fig. 2. (A) A fifth instar larvae desert locust, *Schistocerca gregaria*, at pre-ecdysis hanging upside down from a perch after air swallowing. Notice the swelling of the body and the parted wing buds (compare with the mid-instar locust shown in B). The head is strongly tilted as a result of the inflated foregut and crop. (C) A locust at the stage shown in A was surgically opened mid-dorsally to expose the fully inflated foregut and balloon-like crop, normally used to exert pressure and split open the old cuticle at ecdysis. (D) A newly-ecdysed adult cockroach, *Periplaneta americana*, (bottom) is at the final stages of expansion of the new cuticle, extending the antenna, legs and wings. The exuvium (old cuticle, top) is suspended in the ecdysis posture, showing the pronotal split of the old cuticle (arrow). All photographs by A.A.

cuticle prior to ecdysis (Cornell and Pan, 1983). This precedes the critical phase of air swallowing. Hence, the crop initially fills with moulting fluid then air (Miles and Booker, 1998; Bestman and Booker, 2003). Fluid swallowing of a different nature may also be assumed to take part in the moulting of some aquatic insects (in this case substituting for air-swallowing behaviour).

#### Ecdysis in decapod crustaceans: the role of the gut

In her detailed account of the moulting cycle of the spiny lobster, Travis (Travis, 1954) reports that, 'The stomach becomes turgid and greatly swollen during this period and in *Panulirus* swells to the extent that it occupies over half of the cephalothorax.' Travis goes on to suggest that, 'This would cause pressure to be exerted on the surrounding regions and is, in all probability, the actual cause of swelling and lateral expansion of the thorax.' This description is very similar to what can be seen and has been described in insects.

As in insects, the ecdysis behaviour of decapod crustaceans too can be largely divided into two major phases: a preparatory phase, and a rapid active phase, during which the animal withdraws from the exuvium – the old cuticle (Passano, 1960; Phlippen et al., 2000)



(Fig. 3). Again, as in insects, all feeding-related behaviour is terminated toward ecdysis, when the animal retreats to a safe place and ceases nearly all movement. This was reported, for example, in several lobster and crab species (Skinner and Hill, 1987; Kuramoto, 1993). Prawns were reported to stop feeding for 3–4 days pre-moult (Petriella, 1986). Body swelling through water uptake appears to be a key step in all accounts of crustacean moult, beginning about one h before ecdysis and completed by 2 h after it. In lobsters, the animals increase their body weight by approximately 10% and this increase is entirely accounted for by the uptake of water (Travis, 1954). The increased hydrostatic pressure is essential for loosening and lifting the carapace before ecdysis can occur (Fig. 3A). Based on the fact that water uptake continues through the final stages of ecdysis, it might also be important (again as in insects) for expanding the new cuticle and appendages (Fig. 3D).

Similar to the case of insect air swallowing, it can be suggested with reasonable confidence (though it has never been directly tested) that successful crustacean ecdysis is dependent on water swallowing (Drach, 1939; Passono, 1960). As mentioned, it is largely accepted that most water uptake occurs through the lining of the digestive tract (Mykles, 1980), i.e. it is the result of water swallowing. However, there is very little reference in the literature to the specific motor patterns associated with water-swallowing behaviour. Kuramoto reported that although feeding stops at least 1 day before lobster ecdysis, chronically implanted electrodes nonetheless recorded a slow stomach rhythmic pattern until 1–2 h before ecdysis (Kuramoto, 1993). This was followed by periods of distinctive stomach electrical activity characterized by short trains of bursts. The change in pattern paralleled a steep change in heartbeat, and preceded the ecdysis-related patterns recorded from skeletal muscles. This temporal organization may reflect the precise

timing of water swallowing, followed by an intense water uptake that results in the need for increased heart activity, which in turn is followed by the onset of ecdysis itself. One cannot avoid speculating that this temporal organization is similar in its general features and in the relative timing of events to insect air swallowing (Hughes, 1980; Zilberstein and Ayali, 2002; Ayali, 2004) (with the exception of the dramatic increase in haemolymph volume that is absent in insects).

The work of Clemens and colleagues is the only report to date of changes in motor network expression, specifically STNS networks, related to moulting behaviour in a decapod crustacean (Clemens et al., 1999). Studying the rhythmic motor output of freely behaving lobsters, their report is somewhat similar to that of Kuramoto (Kuramoto, 1993). The pyloric moult-related motor pattern, as can be monitored by electromyogram recording of different pyloric muscles, is characterized by a significant elongation of cycle period and burst duration, starting about 24 h before ecdysis. This becomes even more pronounced as the time of ecdysis approaches, changing into a less organized or 'erratic' pattern once ecdysis is initiated. The periods of short trains of stomach burst activity reported by Kuramoto (Kuramoto, 1993) to appear 1–2 h before ecdysis, and attributed to water swallowing, are not mentioned in the report by Clemens and colleagues (Clemens et al., 1999), though there may be some indication of their presence in the increased variability in pyloric periods and the changes in inhibitory relationship between pyloric neurons reported to take place just prior to ecdysis. Clemens and colleagues (Clemens et al., 1999) tend to relate the moult-specific modifications in the pyloric pattern to a substantial reduction of oxygen in the blood. As will be discussed at length in the next section, there are, however, other sources of modulation to be considered.

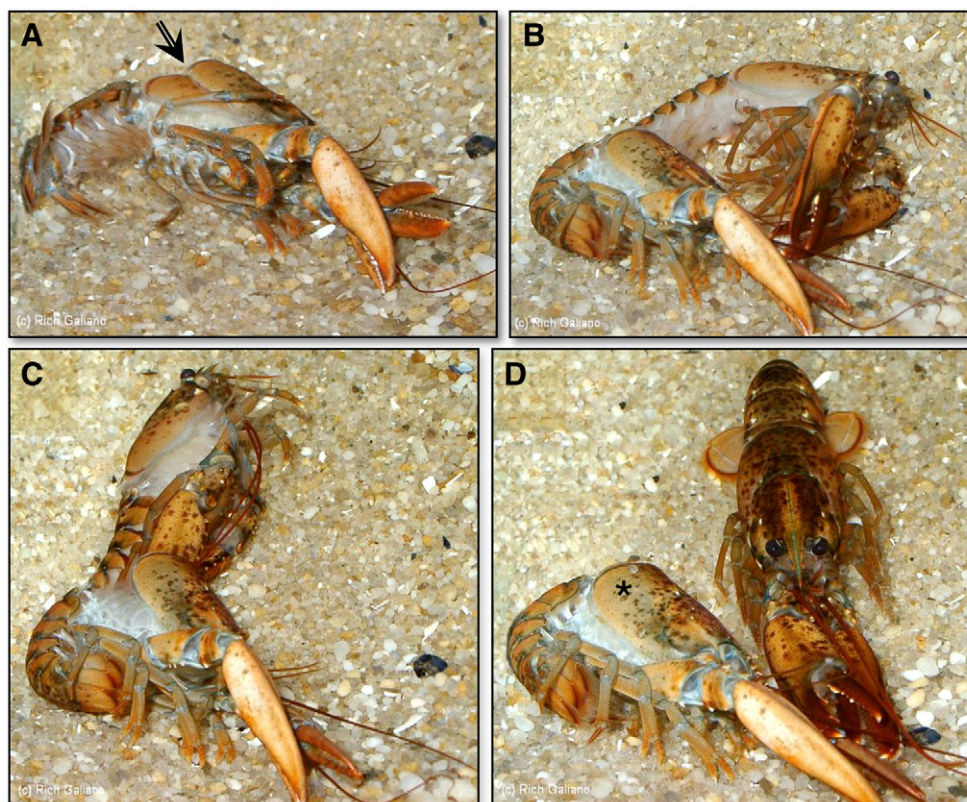


Fig. 3. Ecdysis of the American lobster, *Homarus americanus*. The swelling of the water-inflated stomach and the lifting of the carapace (A, arrow) cause the animal to roll onto its side. As in insects, throughout the process (B,C), and up to the time of complete withdrawal (D), the animal retains an elevated internal pressure (as a result of water uptake), which is used for expansion of the new cuticle (D, asterisk marks the old exuvium). Images are courtesy of Rich Galiano.

### Ecdysis-related peptides: neuromodulation of the arthropod STNS during the moult

Almost five decades ago Passano offered a description of a close correlation and even similarity between the endocrine control of the moult cycle in insects and crustaceans, suggesting a general theory of arthropod moult control (Passano, 1960). This issue was revisited by Chang who presented the generally same conclusion, though placing somewhat more emphasis on the known differences between the two groups (Chang, 1993). In the years between these two studies, our understanding of the subject in insects has undergone a major revolution, due to the identification of the insect eclosion hormone (EH) (Truman and Riddiford, 1970). As we now know, this was the first in a series of semi-revolutions that have taken place in the field, mostly in the last decade (Zitnan et al., 1996; Zitnan et al., 2007; Truman, 2005). As I shall review in brief below, our understanding of the peptidergic control of ecdysis in crustaceans is nevertheless still awaiting a major breakthrough.

According to the current view, successful performance of the insect ecdysis sequence and its distinct motor patterns requires the expression of specific genes and the release of multiple regulatory molecules (for a review, see Zitnan et al., 2007). Ecdysis-related gene expression is controlled by ecdysteroids from the prothoracic glands and gonads, while the activation and execution of each behaviour is under the control of peptide hormones produced by Inka cells and neuropeptides within the central nervous system (CNS). The subsequent humoral signalling between Inka cells and the CNS results in the central release of multiple neuropeptides and activation of neuronal circuits for pre-ecdysis and ecdysis (reviewed by Ewer and Reynolds, 2002; Truman, 2005).

The ecdysis sequence is activated by the initial release of pre-ecdysis-triggering (PETH) and ecdysis-triggering (ETH) hormones from Inka cells. The initiation of pre-ecdysis behaviour and the transition to ecdysis are regulated by stimulatory and inhibitory factors released within the CNS. These include EH, which is released both centrally into the brain and CNS and into the circulation, and establishes the temporal organization of ecdysis behaviour and the progression from one phase to the next. Thus, in order to activate each subunit of the moult set of programmed behaviours, specific neural elements, including the STNS networks, are targeted at precise time points.

Carlson and O'Gara (Carlson and O'Gara, 1983) reported that the cricket FG could generate spontaneous activity *in vitro* only if isolated from the near-moult, ecdysing or expanding insect. This state of 'activation' washed 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 moult: haemolymph collected from moulting larvae and applied to a larval FG–foregut preparation altered the ongoing feeding motor pattern to resemble that observed in moulting larvae. A rhythmic motor pattern that resembles air swallowing could be generated in isolated heads of animals 24–30 h prior to eclosion by application of EH (Miles and Booker, 1998). Interestingly, in the locust, haemolymph collected from non-feeding pre-moult larvae inhibited FG rhythmic activity (Ayali et al., 2002), and Zilbertstein and colleagues reported that EH transiently inhibited the FG rhythmic pattern *in vitro* (Zilbertstein et al., 2006). These different and somewhat contradictory observations can be settled by considering the different and complex effects of the various insect ecdysis-related peptides, and suggesting the crucial role of exact timing of the experimental manipulations.

According to the current scheme, crustacean cardioactive peptide (CCAP) has a key role in maintaining the ecdysis motor programme in insects (Ewer and Reynolds, 2002; Truman, 2005; Zitnan and Adams, 2005). However, findings in flies and moths regarding the regulative role of CCAP are not fully consistent and call for further investigation (Zitnan et al., 2007). Bestman and Booker observed that CCAP generated increased motility and in many cases peristalsis in *Manduca* during the larval–larval moults (Bestman and Booker, 2006). They suggest that the role of this modulation is to control the timing of moulting fluid ingestion. In locusts, Zilbertstein and colleagues 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* (Zilbertstein et al., 2006). The modulatory effects of CCAP on the FG motor circuits were dependent on the behavioural state and physiological context; and the action of CCAP was reported to depend on pre-exposure to ETH, which is thought to be released before CCAP *in vivo*. A picture similar to that seen in Fig. 2C was obtained by applying CCAP directly onto the FG and inducing intense air swallowing. These results imply a novel role for CCAP in generating air-swallowing behaviour during the early stages of ecdysis (Zilbertstein et al., 2006).

Similar to the traditional scheme mentioned above for insects, Phlippen and colleagues reported a dramatic release of CCAP into the haemolymph of crayfish and crabs during late stages of ecdysis (Phlippen et al., 2000). These authors suggested that CCAP is responsible for many of the changes in behaviour seen during ecdysis. CCAP is accumulated in the pericardial organs (crab) or the ventral nerve cord (crayfish) in very large amounts in late pre-moult stages. It is possible that it is stored in order to meet the high demand for release of the neurohormone during ecdysis (Phlippen et al., 2000). Although there is no direct reference to a possible role for CCAP in water-swallowing and water-uptake behaviour, CCAP is known to have direct myotropic actions on several visceral muscle systems in crustacea (Dirksen, 1994; Dirksen, 1998). Moreover, CCAP is a potent modulator of the pyloric rhythm of the STG (Weimann et al., 1997). It was reported to activate the pyloric rhythm of the crab, *Cancer borealis*, in most silent preparations, and to alter the phase relationships of pyloric motor neuron firing in all preparations. It was suggested that enhanced motor neuron firing and increased gain in neuromuscular junctions are likely to operate coordinately in response to hormonally released CCAP. Hence, as in the case of insects, it is possible that CCAP plays an additional, early, role in generating water-swallowing behaviour during the early stages of ecdysis. This hypothesis, however, requires experimental verification.

An additional neuroendocrine factor with a potential role in crustacean ecdysis is the hyperglycaemic hormone (CHH). Chung and colleagues (Chung et al., 1999) reported that ecdysis in the crab, *Carcinus meanas*, is associated with a precisely timed surge in circulating CHH, released from endocrine cells in the gut. These cells become strongly immunoreactive during pre-moult, and the CHH surge is a result of the complete discharge of cellular peptide content. It was suggested that the CHH surge is related to the dramatic water uptake. Indeed, when crabs were injected with physiologically relevant quantities of CHH, a precocious initiation of significant water uptake was observed, which resulted in accelerated ecdysis. Though the precise mechanism of CHH actions is not known, Chung and colleagues (Chung et al., 1999) reported that the increased water absorption was the result of a dramatic increase in water swallowing.



### Summary: open questions and future challenges in the study of the arthropod STNS

Our current state of knowledge of the arthropod STNS, and particularly that of insects and crustaceans, is in many ways complementary: what we are lacking in network characterization and neuron identification in insects has been achieved in great detail regarding the cellular properties, synaptic connections, neuromodulation, molecular characterization to the level of ion channels, and even gene expression in crustaceans. The insects, on the other hand, offer the behavioural context: very well studied and described behavioural patterns in different intact animals. Studies of neuronal and network patterns in the intact animal are very few in crustaceans, and our understanding of the functional and behavioural significance of much of the wealth of information acquired in the *in vitro* preparations is still very limited.

Hence the challenges before us are very clear: we need to advance in characterizing the stomatogastric neural networks in insects at all levels. This will open up new avenues in the currently limited comparative investigation of the STNS among different insect groups. It may also provide new insights into the important question of homology of the STNS among the different arthropod groups. Progress in understanding STNS neuronal mechanisms will also offer new strategies for the manipulation of behavioural patterns, moulting and ecdysis behaviour in particular.

Guided by the behaviourally relevant information obtained from insects, we now need to extend investigations of the intact animal among different crustaceans. This will add new perspectives to our in-depth *in vitro* studies. It may also open up new vistas on already much studied issues, such as neuromodulation, multi-functionality and network re-organization, which are critical for our deeper understanding of the role of the STNS in moulting and ecdysis-related behaviour.

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