

THE ACTIVITY PATTERN OF IDENTIFIED NEUROSECRETORY CELLS DURING FEEDING BEHAVIOUR IN THE LOCUST

JOACHIM SCHACHTNER AND PETER BRÄUNIG

*Institut für Zoologie, Technische Universität München, Lichtenbergstraße 4,
W-8046 Garching, Germany*

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Summary

In the locust, *Locusta migratoria*, the activity of identified efferent neurones of the suboesophageal ganglion was recorded extracellularly for long periods (hours or days). During recording, the animals were free to move in their enclosures. Activity of the cells appears to accompany feeding behaviour: they become active shortly before feeding starts and their activity persists during feeding. The cells have previously been shown to be serotonin-immunoreactive and to have a dense network of neurohaemal terminals on the sheath of the peripheral nerves supplying the mouthparts. The role of serotonin as a neuromediator involved in feeding activities is discussed for insects and other organisms.

Introduction

Although a wealth of information has accumulated during recent years on the modulation of behaviourally relevant neuronal circuits and/or effector systems by neurohormones such as biogenic amines and peptides, only a little is known about the integrative relationships between these circuits and the nerve cells producing and releasing these modulators or hormones. In most cases, the activity patterns of individual neurosecretory cells during defined behavioural sequences are not known. Insects provide excellent model systems which may be used to address these questions. First, there exists extensive knowledge about insect neuroethology. Second, the insect central nervous system (CNS) contains large, easily identifiable nerve cells, including neurosecretory cells that produce peptides or biogenic amines. Of the aminergic cells, the most intensively studied are those containing octopamine or serotonin (for a review, see Orchard, 1982; Evans, 1985; Nässel, 1987; Agricola *et al.* 1988).

Serotonin-like immunoreactivity can be detected in comparatively few nerve cells, which are distributed over the entire insect CNS. Most of these cells are interneurons, but a few immunoreactive efferent nerve cells have also been observed (Bishop and O'Shea, 1983; Tyrer *et al.* 1984; Nässel and Elekes, 1985; Flanagan, 1986; Hustert and Topel, 1986; Longley and Longley, 1986; Davis, 1987; Lange *et al.* 1988; Griss, 1989; Homberg and Hildebrand, 1989; Orchard *et al.* 1989; Van Haeften and Schooneveld,

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1992). In the locust CNS, the only efferent nerve cells with serotonin-like immunoreactivity are found in the suboesophageal ganglion (SOG). These are (1) neurones supplying the salivary glands, (2) the link nerve cells innervating mandibular muscles, and (3) the cells that establish a neurohaemal system called the satellite nervous system (SNS: Tyrer *et al.* 1984; Bräunig, 1987, 1988; Baines *et al.* 1990; Gifford *et al.* 1991). This latter group of six large, neurosecretory cells that contain serotonin-like immunoreactivity (three on each side of the SOG) establishes an extensive meshwork of terminal ramifications on the surfaces of all peripheral nerves of the SOG and brain that innervate the mouthparts. The function of the SNS is as yet unknown, but the morphology of a comparable system in cockroaches suggests that it participates in the control of feeding behaviour (Davis, 1987). This view is supported by observations that serotonin influences the activity of various peripheral target organs involved in feeding behaviour, including mandibular muscles, salivary glands, foregut and, possibly, sensory structures such as mechano- and chemoreceptors.

A critical test for this hypothesis is to find out whether the SNS neurones are active during feeding or during any other behavioural activities. Intracellular recordings from the SNS neurones during behaviour are not feasible, because it is impossible to expose the SOG without massive dissection of the head and mouthparts. For this reason, we further explored the anatomy of the SNS in the periphery in order to find means to record its activity selectively with extracellular methods in intact locusts. We describe a particular peripheral nerve in the head capsule, the labral median nerve (LMN), that permits such recordings. Using chronically implanted electrodes, we obtained the first long-term recordings (which lasted for hours to days) from insect neurosecretory cells. Our results show that the SNS is indeed activated during feeding, but not in any other observed behavioural context.

Materials and methods

Insects

For all experiments, males and females of *Locusta migratoria migratorioides* were used. The insects were maintained in a crowded laboratory culture under a constant light:dark cycle (12h:12h) and at constant temperature (28°C). They were fed on wheat seedlings.

Histology

Peripheral nerves were backfilled with 1–3% CoCl₂ for 18–48h at 6°C, the staining was developed in (NH₄)₂S, and the ganglia were fixed in alcoholic Bouin. After fixation, preparations were silver-intensified (Bacon and Altman, 1977).

Physiology and behaviour

For recording in dissected preparations, locust heads were mounted ventral side up and the SOG and the SNS were exposed as described by Bräunig (1987). The clypeus was also opened in order to record from both satellite-to-tritocerebral (ST) nerves and the LMN simultaneously using small metal hook or suction electrodes.

For implantation of electrodes, locusts were fixed in a metal block cooled to 4°C by Peltier elements. This immobilized the animals and prevented haemolymph pumping and clotting. A window was cut in the cuticle near the midline of the clypeus. Removal of a few airsacs and fatty tissue exposed the two labral anterior retractor muscles (M2; Snodgrass, 1928) and the labral median nerve between them (LMN, see Fig. 1 inset). For recording, the nerve was mounted on a small hook electrode formed from stainless-steel wire (diameter 30 µm) and isolated by a small piece of polyethylene tubing filled with Vaseline (method according to Wilkens and Wolfe, 1974, as modified by Hensler and Honegger, 1985). After implantation, the wound was closed with the dissected piece of cuticle and sealed with dental wax. Steel wires were used for myogram recordings. A myogram of the mandibular closer muscle (M9; Snodgrass, 1928) recorded simultaneously with LMN served as a monitor for feeding behaviour. This muscle was chosen because, of all the muscles involved in feeding, it is the most representative since mandibular closing movements appear to entrain the rhythms of all other mouthparts during feeding (Seath, 1977).

For long-term recording, operated insects were kept in a wire mesh box (10cm×10cm×20 cm). To simulate normal environmental conditions, this box was placed inside a larger one containing other gregarious locusts. Both boxes were kept in an insulated chamber at constant temperature (28°C) with a light:dark cycle of 12h:12 h. Chronic recording started the day after operation because we observed that most locusts had recovered from surgery after this period. Animals which had not resumed feeding during this period usually did not feed again and died on the second or third day.

Five experiments with chronically implanted hook electrodes were successful. For each experiment, 8–12h of recording were made per day. Data were stored on an FM tape recorder (Racal Store 7 DS) for later processing with a computer-based analysing system (CED interface, Spike 2 software). From each experiment, at least five feeding periods were chosen and analyzed in detail.

Results

Anatomy

To make chronic recordings of SNS nervous activity during behaviour we had to find a peripheral nerve with the following properties. (1) It had to run close to the cuticle so that it could easily be exposed by minimal dissection. (2) It always had to receive innervation from the SNS. According to previous investigations, the branching pattern of the system in the periphery is rather variable from insect to insect (Bräunig, 1987) and not all nerves reliably receive SNS innervation on their most distal ramifications. (3) It preferably had to contain the axons of only a few other neurones with their activity being clearly distinguishable from that of the SNS collaterals.

One particular peripheral nerve in the head, the labral median nerve (LMN, Fig. 1) was found to be particularly well suited for *in vivo* recording because it fulfils all three criteria: it runs just beneath the clypeal cuticle, it receives SNS innervation from both sides of the suboesophageal ganglion *via* the ST branches of the SNS, and it contains only two other

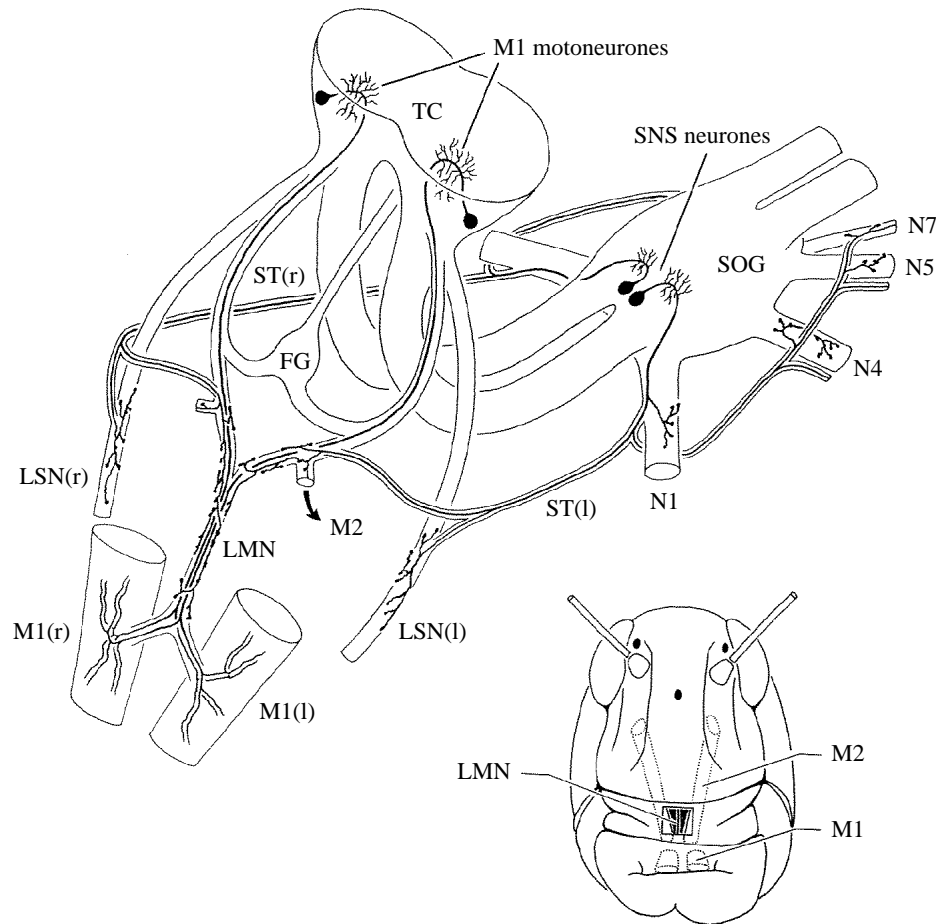


Fig. 1. Relationship between the labral median nerve and the satellite nervous system. The labral median nerve (LMN) contains two axons from motoneurons located in the tritocerebrum (TC) that innervate both left and right M1 muscles [M1(l), M1(r)]. In addition, it receives innervation on both sides by the satellite nervous system *via* the two ST nerves [ST(l), ST(r); ST, satellite-to-tritocerebral nerves]. Note that in this schematic representation only one of the three SNS neurones is shown on each side of the suboesophageal ganglion (SOG), that the diameter of the satellite nerves is greatly exaggerated, and that distances are greatly foreshortened. The inset shows a frontal view of a locust head to indicate the position of the labral muscles M1 and M2 and the LMN. FG, frontal ganglion; LSN(l), LSN(r), left and right labral sensory nerves; N1, N4, N5, N7, peripheral nerves of the SOG (nerves 2, 3, 6 and 8 are not shown).

axons (Fig. 1). These two axons belong to two motoneurons each of which innervates the labral compressor muscles of both sides [M1, Snodgrass, 1928; innervation of both ipsilateral and contralateral muscles appears to be a property common to most tritocerebral labral motoneurons (P. Bräunig, unpublished results)]. All of the six backfills of the LMN showed the two M1 motoneurons, one in each tritocerebral lobe of

Table 1. Summary of the chronic experiments

Experiment	Age (days)	Sex	t (days)	t_{rec} (h)	Feeding bouts	Spike classes
1	7	F	2	15	9	2
2	14	F	2	13	20	3
3	40	M	1	11	12	1
4	14	F	3	28	14	3
5	28	F	1	11	17	4

t , duration of the stable LMN record; t_{rec} , duration of tape-recorded activity; feeding bouts, total number of feeding bouts from each experiment recorded on tape.

Age is given as days past the final moult.

F, female; M, male.

the brain (Fig. 1). No other neurones were labelled either in the brain or in the frontal ganglion. In spite of their small axon diameter and the long distance from the filling site and the SOG (distances are greatly foreshortened in the schematic drawing of Fig. 1), quite surprisingly in one of the six preparations two SNS neurones were faintly labelled in the SOG.

Physiology

In principle, the LMN could receive innervation from all six SNS neurones, but, according to the inherent variability of their peripheral branching pattern (Bräunig, 1987, 1988, unpublished observations), this is not necessarily the case. This may explain why we observed only 1–4 SNS units in our recordings (Table 1), but this discrepancy in numbers may also be caused by technical problems. The first possible problem is damage to axons during electrode implantation. Second, because of the small diameter of SNS collaterals, spikes from individual SNS cells might not be recognizable above the noise level. Third, spikes deriving from different cells may have a similar shape and amplitude, and may accordingly be regarded as members of the same spike class (this might explain why in Fig. 5B unit 2 appears to be twice as active as unit 1). All three factors would reduce the number of distinguishable spike classes.

Recordings from head preparations

The morphological features described above are reflected in recordings obtained from preparations of the locust head in which both ST nerves and the LMN were recorded simultaneously (Fig. 2A). Such recordings show two distinct classes of spikes. The smaller spikes in the LMN recording correspond to units in either the left or right ST nerve. They therefore represent the SNS activity arriving in the LMN with a fixed latency following the ST spikes. The two large spikes belong to the two tritocerebral motoneurones. This is shown by experiments in which the LMN and the M1 muscles were recorded simultaneously (Fig. 2B). Spikes of the two motoneurones frequently have almost identical shapes and amplitudes (e.g. both motoneurones are active in Figs 2, 3).

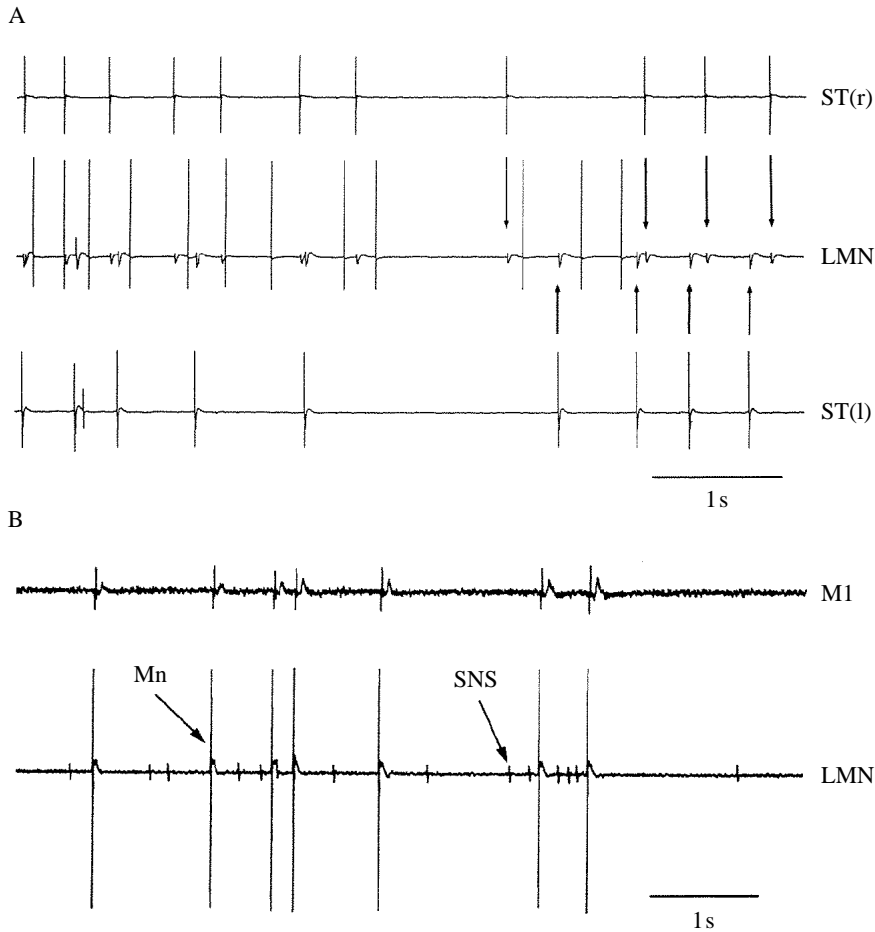


Fig. 2. (A) Simultaneous recordings from LMN and both left and right ST nerves (nerves containing only fibres of the SNS cells, see Fig. 1). Note the correlation between SNS spikes in the ST records and the small-amplitude spikes in the LMN record (arrows). (B) Simultaneous recordings from LMN and M1. Note the correlation between the high-amplitude M1 motoneurone spikes (Mn) in the LMN record and M1 activity. The small-amplitude spikes belong to the SNS.

Chronic recording

When recording from the LMN using electrodes implanted in intact locusts, the same two classes of spike amplitude (large motor spikes and small SNS spikes) can be recognized (Fig. 3). However, artefacts are caused by movements of the insect and, more frequently, by activity of muscles located close to the recording electrodes. Both types of artefacts are often similar in amplitude to the small SNS spikes, i.e. just above the noise level. For this reason, it is not possible to recognize the relevant signals simply by using a window discriminator circuit. For the same reason, recognizing SNS spikes by means of

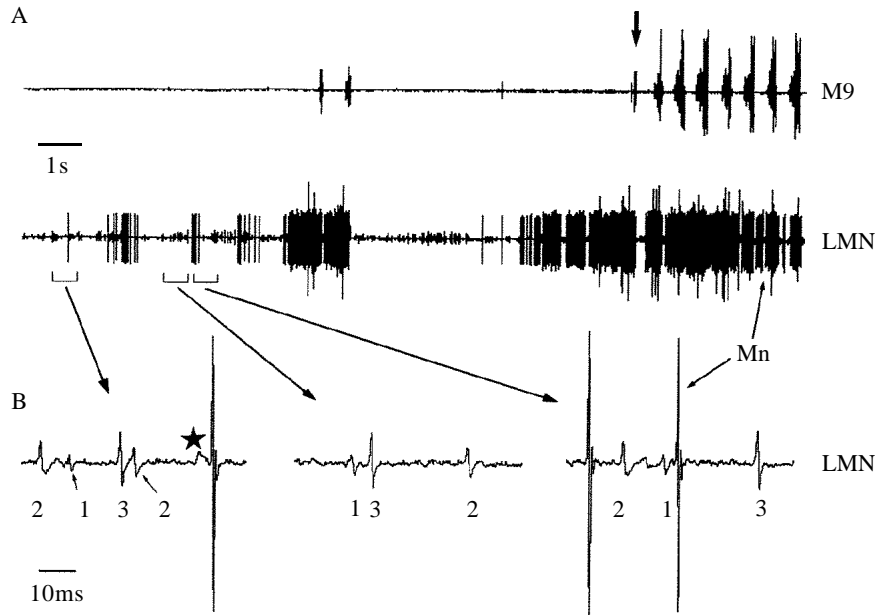


Fig. 3. (A) Simultaneous recording of M9 and LMN activity before and at the beginning of a feeding bout. The large spikes in the LMN trace belong to the M1 motoneurons (Mn), whereas the small events represent either SNS potentials (compare with Fig. 2) or some non-specific signals (not distinguishable at this time resolution). The arrow in the M9 trace marks the start of feeding. (B) Three extracts (note arrows) from the LMN trace on a faster time base. In this record, three different small SNS spike classes were present (numbered 1–3). The star marks an artefact (see text).

spike-form detection software was also unreliable because even slight baseline deflections disturbed the template-matching process.

These problems, however, could be overcome because SNS activity always started before feeding activity (see below), when most head muscles appear to be inactive. In this pre-feeding period (PFP in Table 2), SNS spikes could be more easily detected and assigned to classes on the basis of spike amplitude and shape (Fig. 3). Spikes classified in this fashion remained stable and recognizable for the entire recording period (see Table 1). The occurrence of particular spike-forms during behaviour was analyzed by visually inspecting the digitized spike trains on the computer screen. Only by using this time-consuming method were we confident of successfully capturing almost all relevant signals. This method was successful except for short periods when activity in M1 motoneurons was high (e.g. Fig. 3). At such times, the small-amplitude SNS spikes were obscured by the large-amplitude motor spikes. Because the M1 muscles are active both before and during feeding, our measurements of overall SNS activities are probably slight underestimates.

As an additional control experiment, we attempted to make chronic recordings simultaneously from LMN and from the nerve supplying the salivary glands. The latter nerve is known to contain the axons of only two large efferent cells (Altman and Kien,

Table 2. *Characteristic data for the five feeding bouts (A–E) analysed from each of the five chronic experiments*

Experiment	A		B		C		D		E	
	M9 (s)	PFP (s)	M9 (s)	PFP (s)	M9 (s)	PFP (s)	M9 (s)	PFP (s)	M9 (s)	PFP (s)
1	786.23	14.42	667.13	25.86	276.55	10.96	265.60	25.61	98.35	23.80
2	126.40	12.10	52.01	29.86	183.65	26.69	157.54	24.21	107.90	21.91
3	76.48	13.05	79.82	25.00	115.42	7.35	200.7	11.91	188.1	6.12
4	740.54	15.40	129.54	1.84	178.60	24.69	162.00	29.80	88.52	17.98
5	116.24	29.98	164.80	18.63	70.70	21.46	138.56	17.47	236.70	3.95

M9, duration of feeding bout; PFP, time difference between the onset of SNS activity and the beginning of M9 activity.

Note the variable length of M9 and PFP among the different feeding bouts even of the same animal.

1979) but is also known frequently to receive innervation from the SNS (Bräunig, 1987, unpublished observations). In the one locust successfully tested to date, the small units recorded from LMN were matched by units that occurred in the recording of the salivary gland nerve with a constant temporal relationship. Also, these units common to both nerves, together with all the other small-amplitude units recorded from LMN, showed the typical pattern of SNS activity (see, for example, Fig. 5). This provides additional evidence that the small units recorded from LMN indeed represent SNS spikes.

SNS activity during feeding behaviour

The activity of the mandibular closer muscle (M9) and the SNS during a typical feeding bout is shown in Figs 3 and 4. During feeding, M9 is rhythmically active with about 1–2 bursts of motoneurone spikes per second (Seath, 1977; Fig. 3A). The onset of this bursting activity is always sudden, whereas towards the end of a feeding bout the frequency of M9 bursts slows and eventually changes to arrhythmic widely separated short bursts of activity (Fig. 4). These latter events occur when the locust has actually stopped feeding, so that it is difficult to define the end of feeding clearly on the basis of M9 activity in myograms. We therefore arbitrarily defined the end of a feeding bout to be when the interburst interval between M9 bursts had changed to twice the length observed during feeding.

Analysing all 25 feeding bouts reveals that the activity of the SNS clearly correlates with feeding behaviour and shows a characteristic pattern. The SNS activity in most cases starts about 10–30s before, and persists during, feeding (Figs 3–7; Table 2). Only in a few cases is the time interval between the onset of SNS activity and the onset of feeding shorter (Table 2). The length of this pre-feeding activity period (PFP) is correlated neither with the length of the following feeding bout nor with the interval between successive feeding bouts. During the first 40–60s of feeding, the firing rate of the satellite cells is about twice that in the remaining feeding bout. This behaviour was observed for all feeding bouts irrespective of their considerable variation in length (bouts varied from approximately 1 to 15min). Several seconds before the feeding activity ends, the activity

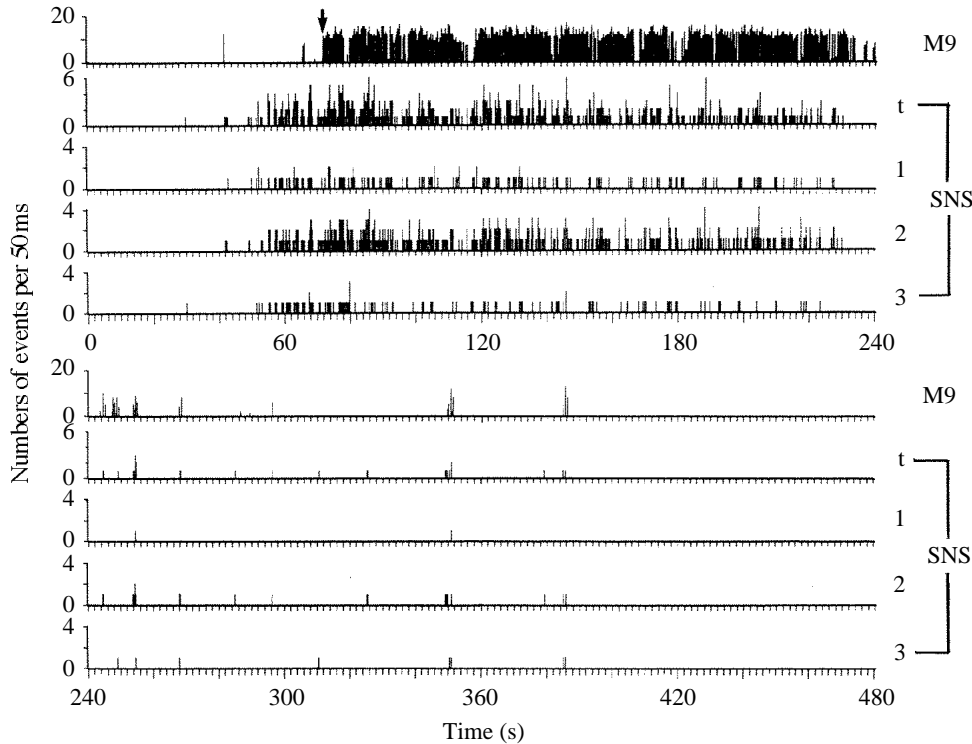


Fig. 4. Time histogram of M9 and SNS activity of one feeding bout (the one shown in Fig. 3). The first SNS trace (t) shows the activity of all SNS spike classes detected, whereas the following three traces show the activity of the individual spike classes (spikes 1–3). Note that maximum SNS activity occurs near the onset of feeding (arrow).

of the SNS stops. Short bursts of SNS activity may occur up to a minute before and after feeding (Figs 4, 6). However, apart from these pre- and post-feeding bursts, and the activity during feeding, no SNS activity was detected in any of the experiments.

These general features of SNS activity during feeding are summarized in Fig. 5A. To compare different feeding bouts, we chose five 30s intervals to describe the time-dependent occurrence of the SNS spikes. Plotting the activity of the three distinguishable SNS units of the feeding bout shown in Fig. 3 in this fashion results in the histogram of Fig. 5B. Analysis of other feeding bouts from the same experiment shows a similar activity pattern: although the total number of spikes within each spike class in the respective intervals varies between different feeding bouts, changes of activity between intervals show the same trend (Fig. 6).

In principle, the same features can be recognized when comparing SNS activity patterns from different locusts (Fig. 7). In Fig. 7A, the activities of all SNS units from experiment 4 (Fig. 6) are combined and displayed. Fig. 7B–E shows comparable plots for four other locusts (five feeding bouts each). Although overall SNS activities vary considerably among individuals (note the different ordinate scales), there is a clear

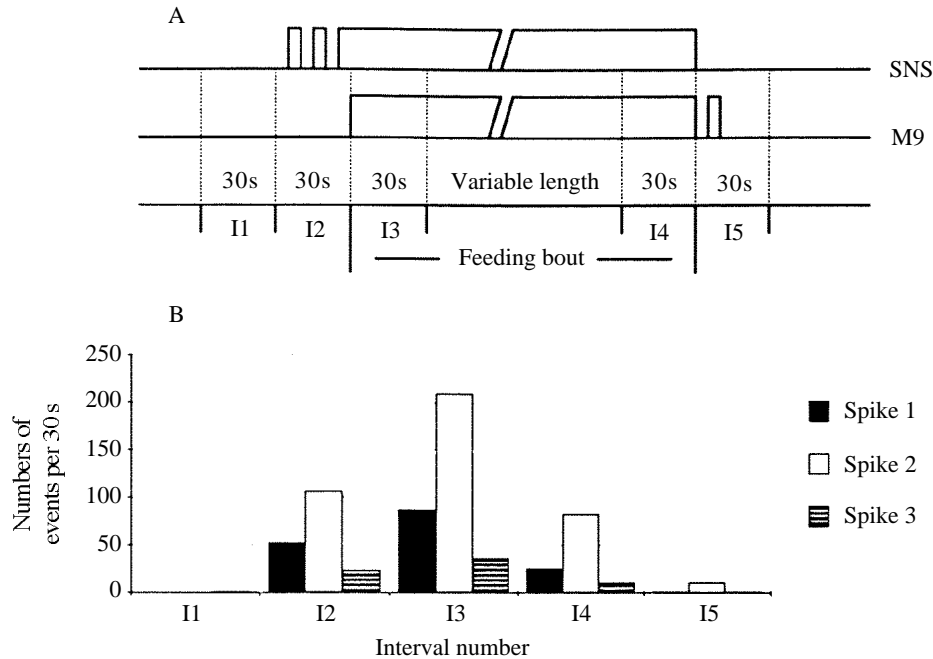


Fig. 5. (A) Summary diagram for the activity of the SNS during a feeding bout (M9). Given the general pattern of the SNS, we chose five intervals (I1–I5, each 30s long) to describe the time-dependent occurrence of the SNS spikes during feeding behaviour. The middle section is of variable length: the feeding bout can vary in duration from approximately 1 to 15min. (B) The activities of the spike classes (spikes 1–3) during the feeding bout shown in Fig. 4 plotted within these intervals.

tendency for maximum activity to occur around the onset of feeding (intervals 2 and 3). There is also a clear trend for SNS activity to decline towards the end of the feeding bouts (compare intervals 3 and 4). Finally, little or no activity is observed long before (interval 1) or after feeding (interval 5).

Discussion

The great majority of previous studies of insect neurosecretory cells investigated the large groups of cells located in the pars intercerebralis and the pars lateralis of the brain that supply the retrocerebral glandular complex. In principle, neurones located in the ganglia of the ventral nerve cord are much easier to study at the single-cell level, because they exist as single cells or in small groups and are thus much easier to identify. In spite of this, only a few studies have examined the functions of such cells (e.g. Adams and O'Shea, 1981; O'Shea and Adams, 1981; Taghert and Truman, 1982; Thompson and Bacon, 1991). However, none of these studies addresses the question of how neurosecretory cells are activated in different behavioural contexts.

We have chosen the SNS of the locust as a model system to investigate this question,

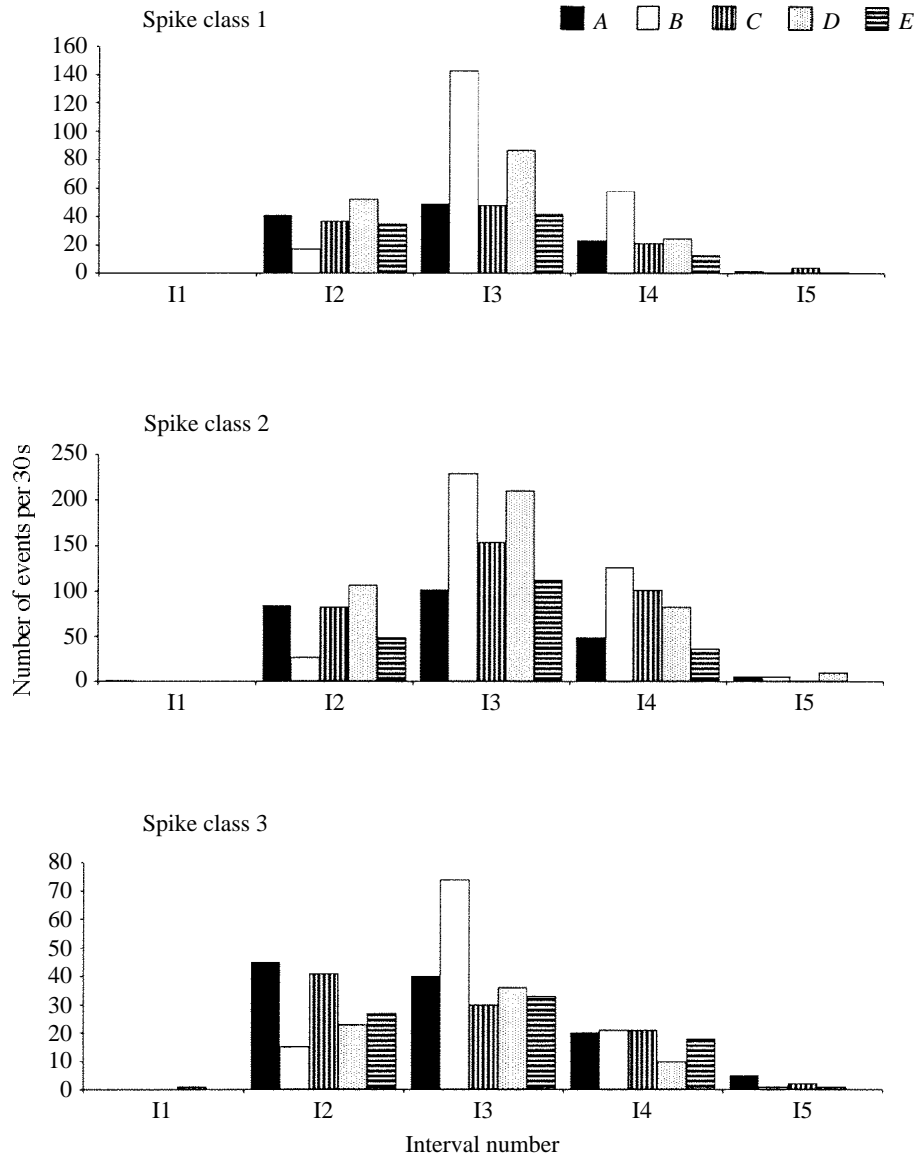


Fig. 6. Comparison of the activity pattern of the three spike classes 1–3 shown in Fig. 4 during five different feeding bouts (A–E) of this animal. Note the different activity profiles of units 1–3 during different feeding bouts (intervals I1–I5 as defined in Fig. 5A).

because its unique morphological properties allow the activity of a well-defined group of six neurosecretory cells of the ventral nerve cord to be recorded for long periods. Our investigation also contributes to the analysis of the neural circuits underlying feeding behaviour which, at least in insects, are only poorly understood (Blaney and Simmonds, 1987).

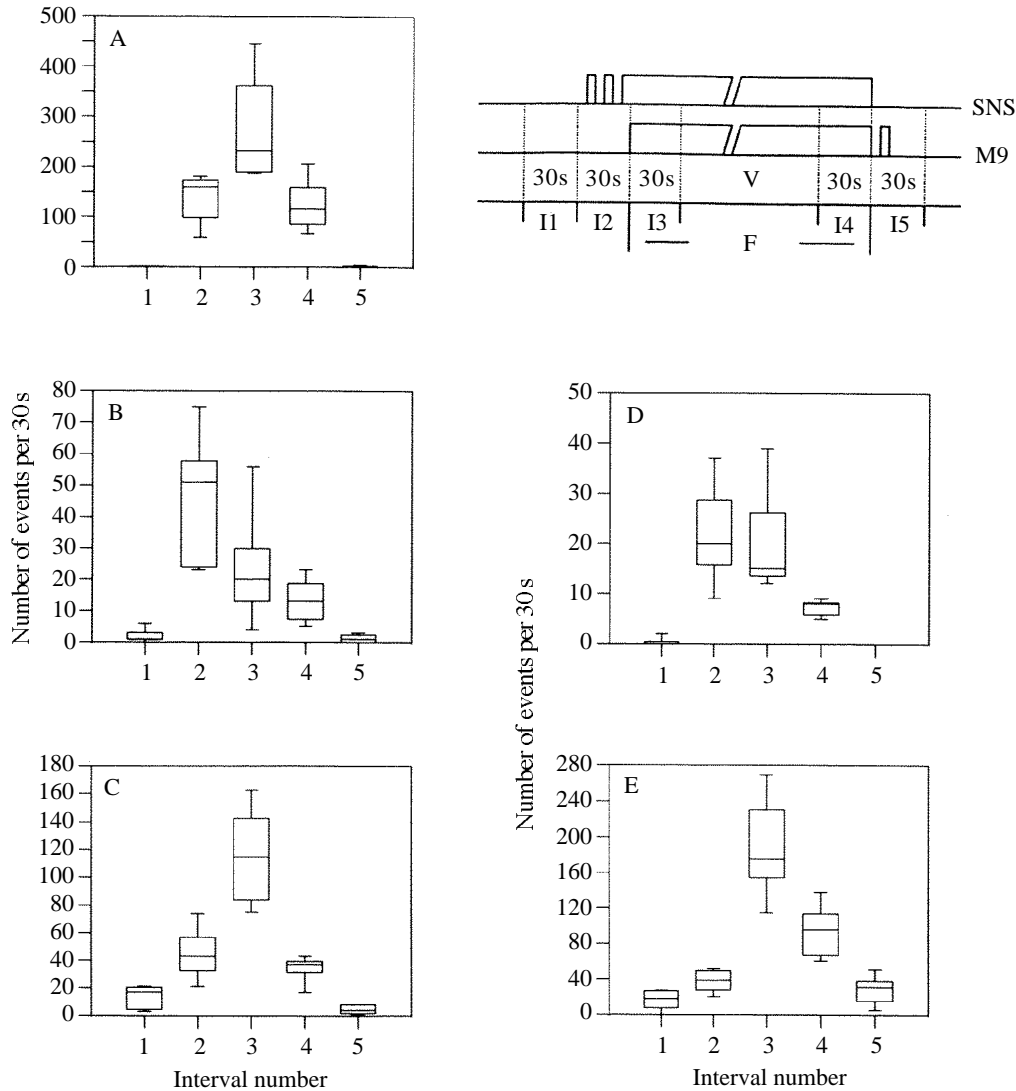


Fig. 7. (A) Summary of the results shown in Fig. 6 in the form of a box-and-whisker-plot (the box shows the data of the second and third quartiles of the ordered series of data, the whiskers extend between the minimum and maximum values of this series, and the horizontal bar represents its median). For each interval I1–I5 (see Fig. 5), the activity of all SNS spike classes from all five feeding bouts is summed. (B–C) Plots for the other four experiments (experiments 1, 2, 3 and 5 in Table 1). The inset repeats the definition of intervals I1–I5.

SNS activity during behaviour

According to our results, the SNS is only active during feeding behaviour. We therefore provide positive evidence for the hypothesis put forward by Davis (1987). He speculated that the homologous system of serotonergic neurones in the cockroach

Periplaneta americana might be active during feeding. Our results also fit in with observations that, in *Rhodnius prolixus*, haemolymph serotonin levels rise in association with feeding behaviour (Lange *et al.* 1989). However, for this blood-feeding insect, apart from neurones of the SOG (Flanagan, 1986; Lange *et al.* 1988), other possible sources of haemolymph serotonin have been described (Orchard *et al.* 1989).

SNS activity was not observed at any time except during feeding behaviour. One has to bear in mind, however, that the experimental environment used here prevented all reproductive behaviour (e.g. copulation, oviposition). During ordinary locomotion, the SNS was certainly not active. It has been claimed, however, that sense organs involved in flight are modulated by serotonin and that haemolymph serotonin levels rise during flight (Koller *et al.* 1990). Apart from neurones in the stomatogastric nervous system (Klemm *et al.* 1986; Konings *et al.* 1988), only the SOG in locusts contains efferent serotonergic cells (see Introduction), so we tested our insects in a tethered flight situation. Under these conditions, the SNS was always inactive, so it is unlikely to be a source of haemolymph serotonin during flight.

Serotonin, a feeding modulator in insects

The physiological results of the present study strongly suggest a role for serotonin in feeding behaviour in the locust as well as in other insects which have similar serotonergic systems (see Introduction). Several lines of evidence suggest that serotonin acts as a modulating substance in feeding behaviour. The morphological features of the system and previous reports concerning its potential targets support this view.

The meshwork of SNS terminal ramifications is only found on the surfaces of the peripheral nerves that innervate the mouthparts. As already discussed by Davis (1987), this rather unusual topology of the system provides a means of loading numerous local haemolymph lacunae close to potential target organs. This means of distribution may be required by the short half-life of the mediator. Available data on the stability of serotonin after release into the haemolymph show that it is degraded rapidly (Lange *et al.* 1989; Sloley and Downer, 1990), so if it were to be released into the circulatory system far away from the target structures it would probably never reach them in an active form. The special morphology of the SNS could, in principle, ensure that serotonin reaches the appropriate mouthpart structures without flooding the entire haemolymph volume with this neuromediator.

Potential targets for modulation might be the muscular and sensory systems involved in the orchestration of feeding. For instance, it has been shown that serotonin modulates the activity of the locust mandibular closer muscle by increasing its contraction amplitude as well as its rates of contraction and relaxation (Baines *et al.* 1990). In crickets, this muscle has been shown to possess an adenylate-cyclase-linked 5-HT₂-like receptor (Baines and Downer, 1991).

Biogenic amines modulate the activity of mechanoreceptive sensory cells in several invertebrate species (Pasztor and MacMillan, 1990; Ramirez and Orchard, 1990; Gascoigne and McVean, 1991). Only indirect evidence exists for the modulation of chemoreceptive sensilla (for a review, see Blaney *et al.* 1986). It is of special interest, however, that serotonin may influence insect chemoreceptive epithelia (Küppers and

Thurm, 1975). In locusts, feeding leads to a decrease in contact chemoreceptor sensitivity (Bernays and Chapman, 1972; Bernays *et al.* 1972). These authors suggested that an unknown humoral factor released from the storage lobes of the corpora cardiaca caused this change. Because the SNS was unknown at that time, it might be worthwhile to investigate the role of serotonin released from this neurohaemal system in this context. It is interesting to note that, in the locust, other efferents involved in feeding are also serotonergic. These are the SOG neurones that supply the salivary glands (Altman and Kien, 1979; House and Ginsborg, 1985; Gifford *et al.* 1991) and efferents located in the frontal ganglion that innervate the gut (Klemm *et al.* 1986). Serotonin is an important neurotransmitter in both organs (Huddart, 1985; Baines and Tyrer, 1989).

Serotonin, a feeding modulator in other phyla

A role of serotonin in feeding behaviour has also been shown in groups other than arthropods. In the nematode *Caenorhabditis elegans*, injection of serotonin is able to elicit feeding behaviour (Horvitz *et al.* 1982). It has been suggested that serotonin is responsible for the orchestration of feeding behaviour in leeches (Lent and Dickinson, 1984). Large serotonergic efferents, the Retzius cells, elicit pharyngeal pumping, biting, mucus secretion and softening of body wall muscles, thereby increasing the ability of the body wall to distend during ingestion of a large blood meal. The Retzius cells, together with another pair of serotonergic efferents in the SOG of the leech, probably cause secretion of saliva.

Serotonergic cells are also involved in controlling feeding behaviour in molluscs (for a review, see Walker, 1986). The source of serotonin is a pair of giant cells, the metacerebral cells, which have been found in most mollusc species. Serotonin released from these cells acts centrally on follower motoneurons (most of which innervate feeding muscles) as well as on the peripheral buccal musculature. In *Aplysia californica*, chronic extracellular recordings show that these cells are only active during feeding and are quiescent during interfeeding periods (Kupfermann and Weiss, 1982; for further references, see Rosen *et al.* 1989; Kemenes *et al.* 1990).

In vertebrates, it is assumed that serotonin plays a more modulatory role in feeding. Serotonin seems to be involved at every stage of the feeding pattern, i.e. in the ingestion process itself, in post-ingestive events and in post-absorptive operations (for a review, see Blundell, 1992). In vertebrates, serotonin may act *via* different pathways, as indicated by the distribution of serotonergic neurones and by its different physiological actions (for a review, see Anwyl, 1990). A very interesting observation is that release of hypothalamic serotonin may occur prior to and during feeding. This means that serotonergic neurones have to be active before and during feeding activity (Schwartz *et al.* 1990). This parallels the results of the present study, as well as those for other invertebrate species.

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