Typical ventilatory pattern of the intact locust is produced by the isolated CNS

H.P. Bustami *, R. Hustert

Institut für Zoologie und Anthropologie der Universität Göttingen, Berliner Str. 28, 37073 Göttingen, Germany

Received 18 January 2000; received in revised form 27 January 2000; accepted 27 January 2000

Abstract

Ventilatory rhythms of locusts are generated in the central nervous system (CNS). The primary oscillator or central pattern generator (CPG) is located in the metathoracic ganglion. We studied the different patterns of ventilation by recording long-term efferent discharges from the isolated metathoracic ganglion.

Two different basic patterns occur: continuous ventilation and discontinuous ventilation. These patterns can be found in the isolated nerve cord as well as in intact animals. In intact animals sensory feedback usually elicits high frequency continuous ventilation as is the case in most physiological experiments. Many studies of ventilation-associated interneurones were performed under what we call stressed conditions i.e. with strong sensory feedback. Under these conditions many interneurones may be recruited which probably do not belong to the basic CPG. In isolated nerve cords of locusts we recognised the two basic types of ventilation. This provides an experimental approach to the origin of rhythmogenesis in ventilation. We can now examine single interneurones under less stressed or even discontinuous ventilatory conditions in the isolated CNS.

We suggest the dominance of intrinsic rhythmogenesis of ventilation in the metathoracic ganglion of locusts. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Ventilation; Locusts; CPG; Isolated nerve cord

1. Introduction

Ventilation in insects is one of the basic behaviours of rhythmical functions of the body providing a sufficient supply of the tissues with oxygen. Depending on the insect body size ventilatory mechanisms range from passive gas exchange by diffusion to active convection in the tracheal system, mainly mediated by co-ordinated motor activity (Chapman, 1998). This motor activity depends on patterned neural control, which in locusts is generated by a central pattern generator (CPG) that is supposed to be located in the metathoracic ganglion (review: Burrows, 1996). The neural patterns from this ventilatory CPG exhibit a wide range of bursting frequencies. They depend mainly on behavioural or metabolic conditions influencing the animal. A stressed locust will show strong and high frequency abdominal ventilation movements. By contrast a locust at rest shows ventilation patterns of slow and discontinuous abdominal pumping movements. Between these extremes transitional patterns exist. They originate from the underlying CPG. It is a network — of partly known interneurones (Ramirez and Pearson, 1989) — that provides the rhythms to the motor neurones which supply the ventilatory muscles.

Backed by the basic rhythmicity of this CPG, metabolic, hormonal, sensory, neural and behavioural influences can modify the rhythmic output and many of these aspects have been studied.

In locusts, Ramirez and Pearson (1989) analysed pattern generation in rapid ventilation studying CPG-related interneurones with rhythm resetting properties (i.e. they depolarised in phase with extracellular ventilatory activity and current injection led to a reset of the rhythm). These interneurones are located in the metathoracic ganglion.

Relations of physical activity to ventilation (oxygen...
consumption of the dragonfly (Harrison and Lighton, 1998), and for running in different cockroach species (Herreid and Full, 1984).

The influence of hypoxia and hypercapnia on ventilatory movements has been studied in the aquatic insect Corydalus cornutus (Kinnamon et al., 1984). The relation of internal physiological parameters as pH, pCO₂ and tracheal gas levels has been studied in relation to the ventilation rate in the grasshopper Melanoplus differentialis and the locust Schistocerca americana (Krolikowski and Harrison, 1996). It could be shown that pCO₂ influences ventilation rates in locusts. Temperature changes cause transitions from continuous to discontinuous CO₂ release in honeybees (Lighton and Lovegrove, 1990). The relationship of respiratory patterns to water loss in Drosophila melanogaster have been characterised (Williams et al., 1997; Williams and Bradley, 1998), including genetic differences in ventilation patterns developed by populations of Drosophila bred under varying humidity conditions.

One typical ventilatory pattern of resting or quiescent insects has been termed discontinuous ventilation (DV) which often can be found in insects as, for example, in desert ants (Lighton, 1990; Lighton and Berrigan, 1994), grasshoppers (Harrison et al., 1995), honeybees (Lighton and Lovegrove, 1990) and other insect species (Kestler, 1984). The endogenous activity of the CPG undisturbed by afferent feedback has been never been studied in greater depth (but see: Case, 1961; Komatsu, 1982). In most of the previous studies on the ventilatory network the distortion of the abdomen caused by dissection has provoked unnatural afferent feedback to the basic rhythm generator neurones, mainly resulting in stressed ventilation. Therefore we have tried a new approach along with the “traditional” method of searching interneurones of the CPG intracellularly: ventilation patterns from an isolated nerve cord might be comparable to patterns found in intact resting animals. In that case the motor output from an isolated CNS should nearly represent the pure endogenous activity of the ventilatory CPG. Long-term experiments with the isolated locust CNS are able to characterise the wide range of “unstressed motor patterns”, which were also shown for intact animals in earlier studies (Hustert, 1975). Thus a major goal of our recent examinations was to establish a basic preparation for further experiments on the intracellular level of the network (CPG) that produces endogenous ventilatory patterns, to describe those patterns and to compare them with the results of earlier studies that have been done on ventilation in locusts and in various species of the hexapods.

2. Material and methods

2.1. Animals

Three species of locusts, Schistocerca gregaria, Locusta migratoria and Taeniopoda eques were used in this study. They are all inhabitants of dry or desert areas. Taeniopoda eques was used for better comparison with respiration studies on this grasshopper (Harrison et al., 1995). Locusta migratoria and Schistocerca gregaria were bred in our colony (gregarious form) in cages with a food supply of wheat. Taeniopoda eques were reared similarly from egg pods kindly provided by Dr. Jon Harrison, Arizona State University.

2.2. Recording efferent discharges of ventilatory nerves in an isolated nerve cord

2.2.1. Preparation

Before isolating the nerve cord the animals were narcotised by cooling them down to 0°C. The head was cut off as were the rear segments of the abdomen. The guts were removed and the dorsal part of the animal was cut away with lateral incisions. The ventral part containing the nerve cord was fixed with insect needles in a small petri dish on Sylgard (Dow Corning) and flooded with saline. Then the nerve cord was isolated by detaching the peripheral nerves from the prothoracic to behind the first unfused abdominal ganglion (i.e. actually the fourth abdominal ganglion since the first three ones are fused with the metathoracic ganglion). This part of the central nervous system (CNS) was transferred to another small petri dish and superfused in oxygenated locust saline (according to Clements and May (1974), pH 6.8, with sucrose). Ventilatory activity from isolated nerve cords was monitored at the relevant efferent nerve stumps. This persisted reliably for at least 3 h, extending often to 4 or 5 h. A tracheal supply was maintained in some experiments (Fig. 4) with the two major ventral tracheal trunks remaining intact at the isolated ganglion and the more distant cut ends of which were positioned to open into the atmosphere above the saline’s surface (Fig. 1).

2.2.2. Recording efferent activity on tape

Rhythmic activity was recorded with suction electrodes from the stumps of the ventral or the median nerves of the third abdominal ganglion which is fused with the metathoracic ganglion. The body of the suction electrode consisted of a glass tube tapering at both ends, over which a piece of narrow plastic tubing could slide to produce an airtight seal. The longer piece of tubing was connected to a syringe that could produce suction pressure. A small piece of tubing fitted over the other end, where also a silver lead made contact between the inner saline and a coaxial cable that lead to the preamplifier. The tubing was drawn out and
cut to a diameter that was suitable for sucking in nerves with a tight fit in the tip. Bursting of ventilatory motoneurons was recorded from the proximal stumps of ventral abdominal nerves with mainly expiratory activity and of the median nerves with mainly antagonistic inspiratory activity. During in situ preparations the abdominal pumping movements correspond to the observed output, i.e. ventilatory bursts.

External conditions during the recordings (temperature, saline, pH, oxygen supply) were kept stable in a narrow range. Temperature was measured regularly with a thermometer. The initial level and concentration of the saline was kept stable by replacing carefully evaporated H₂O with distilled water when working without bath perfusion. We used a buffered saline (Clements and May, 1974) with the pH set at 6.8. By superfusing the nerve cord with oxygenated saline the accumulation e.g. of CO₂ could be excluded (in the experiments without maintained tracheal supply) and the supply of oxygen was kept stable. Saline levels of less than 1 mm above the ganglion further insured sufficient oxygen supply and CO₂ release.

For comparison, efferent patterns were recorded in some experiments (not illustrated here) under varied conditions (saline, pH, temperature, saline with sugar or without) for at least 30 min up to 4 h. The data were amplified 1000× and stored on magnetic tape (TASCAM portastudio 424 taping).

2.2.3. Analysis of the data

The stored data of efferent ventilatory activities could be printed continuously on an 8 channel printer (UNISCRİPT digital, PICKER International Ltd).

To obtain average records of ventilatory activity, the number of ventilatory bursts were counted in subsequent 1 min intervals. Only clear bursting activity (above sometimes prevailing tonic discharge) was counted as breathing activity. Whenever at least one interval elapsed without breathing patterns, this inactivity was taken as discontinuous ventilation (DV) according to earlier studies (Lighton, 1990; Harrison et al., 1995).

The results of this analysis were visualised as sequential histograms showing the neuronal ventilatory activity against the time recorded.

For analysing the data as described above we defined some important parameters as follows:

- **Burst**: A high frequency discharge of spikes from ventilatory motoneurones lasting about 0.5–1.5 s.
- **Bursting period**: Period of time in which repetitive bursting activity occurs, separated from adjacent periods by at least 1 min without any bursting activity.
- **Pauses**: Period of at least 1 min duration without any bursting activity.
- **Counting Interval**: For our sequential histograms we used time intervals of 1 min. Within these intervals the number of bursts were counted.
- **Continuous ventilation**: Long lasting bursting periods with a wide range of frequencies (Fig. 1A).
- **Discontinuous ventilation**: Period of time in which bursting periods are separated by silent or just tonic discharge intervals of at least 1 min (Fig. 1B).

To describe more detailed the observed patterns we made pause duration — histograms based on the sequential histograms (see Section 3.2).

3. Results

3.1. Ventilatory patterns of the isolated nerve cords
3.1.1. Ventilation patterns in *Taeniopoda eques*

The spiking discharge patterns of ventilatory motor units observed in nerves of the isolated CNS show a wide range between total inactivity to continuous rhythmicity (Fig. 3C and D and 4). In order to classify within this range two basic types of ventilation are defined. The results show the two types with transitions between them. Discontinuous ventilation pattern occurs quite often.

In Fig. 3C ventilation begins with high frequencies ranging over 30 bursts/min. After 30 min discontinuous patterns develop with pauses of max. 16 min between two burst periods.

Fig. 3D shows the results of an experiment in which the nerve cord was isolated but not removed from the abdomen. As in the other examples the connectives of the nerve cord were cut in front of the prothoracic ganglion and behind the first abdominal ganglion. The connections to the tracheae were maintained supplying the ganglia with oxygen. Abdominal muscle movements of the posterior segments agitated the saline around the ganglia. During the first 113 min the ganglia performed continuous ventilation patterns, but with extreme frequency variations. A pause of several minutes initiated a period of discontinuous ventilation which lasted until the end of the recording. This long term recording (215 min) shows patterns of strong continuous ventilation rhythms as well as DV and transition between both extremes. Similar patterns were found in several other experiments. At 175 min the temperature of the saline was increased to see possible reaction in the patterns. A rise in burst per min developed — as the temperature increased — and decreased with falling temperature in the saline. The maximum ventilation rate was 30 bursts per min.

3.1.2. Ventilation patterns in *Locusta migratoria*

The ventilation patterns in the isolated nerve cord of *Locusta migratoria* show a wide range between total inactivity and continuous rhythmicity including discontinuous ventilation patterns. Discontinuous ventilation is visible in Fig. 3A. In this recording the maximum ventilation rate was 19 bursts per min. A different sequence of patterns from another isolated nerve cord of *Locusta migratoria* is shown in Fig. 3B with discontinuous ventilation and irregular oscillations occurring during a burst period. Similar patterns of *Locusta migratoria* at rest could be observed by making movement video recordings from undisturbed animals. The maximum ventilation rate was 55 bursts per min.

As a reference a long term recording that lasted more than 400 min was made (Fig. 4). The tracheal supply of the isolated nerve cord was maintained. A clear and stable DV-rhythm was visible. At the end of the recording the pauses between the bursting periods showed a regular duration of 15–20 min. Also the length of the bursting periods was nearly regular at 5–6 min. The frequency of 10–15 bursts per min was equal for the last bursting periods. Similar rates were found by Miller (1960) at temperatures between 18–20°C. The temperature in saline was 18–19°C constantly.

3.2. Pause duration histograms

The typical range of pause duration between bursts (only for experiments with trachea removed) of ventilatory patterns in recordings of *Taeniopoda eques* (Fig. 5) lay between 1 or 2 min. In *Locusta migratoria* (Fig. 5) the majority of the pauses lasted about 1 min. There was a preference for short pauses similar to the distribution of pause length in *Taeniopoda eques*.

Miller (1960) made continuous recordings from preparations of *Schistocerca gregaria*. The bursting periods were interrupted by 1 min pauses, as for *Locusta migratoria*.

4. Discussion

The results of this study show that — just as in intact animals — in an isolated nerve cord a wide range of neural ventilatory patterns are generated. We can distinguish two basic types: continuous ventilation and discontinuous ventilation. These definitions describe the opposite ends of a scale with many transitional forms of ventilatory patterns in between, occurring both in intact insects and in the isolated CNS.

All the patterns of fictive ventilation found in *Locusta migratoria* (Figs. 3A and B and 4) are comparable to those observed in *Taeniopoda eques* (Fig. 3C and D), suggesting dominant intrinsic rhythm generation (basic CPG) in the fused metathoracic ganglion of locusts in general. We can record from elements of the CPG intracellularly and thereby study integral parts of the ventilatory system unchanged by sensory influence and compare it with the neural network of high frequency ventilatory pattern generation (Ramirez and Pearson, 1989) and with results from studies in intact locusts and even other insects.

Recent studies of ventilation patterns in a variety of insect groups focussed on specific aspects of insect ventilation. Oxygen consumption and ventilation during rest and locomotion in the cockroach *Blaberus giganteus* (Bartholomew and Lighton, 1985) follow ventilatory patterns similar to those of our study. Discontinuous ventilation patterns of emitted CO₂-volume could be recorded in intact *Taeniopoda eques* (Harrison et al., 1995). In the dragonfly *Erythemis simplicicollis* Harrison and Lighton (1998) found different patterns of CO₂-release, influenced by the oxygen content of the air. Previously Tonner (1936) described three basic patterns of ventilation in dragonfly larvae: normal ventilation,
Fig. 2. Efferent ventilatory activity recorded extracellularly from the stump of efferent nerves (metathoracic median nerve) in an isolated locust CNS (*Locusta migratoria*). (A) long term recording with variable inspiratory bursting patterns which is also demonstrated as sequential histogram in Fig. 4. The data from (A) clearly indicate discontinuous ventilation pattern. (B) Recording during high frequency bursting.

“gulping ventilation” and “chewing ventilation”. These types were confirmed by the study of Hughes and Mill (1966), although Hughes and Mill (1966) pointed out that between the three types a wide range occurs. These patterns of ventilation show similarities to the patterns that we have found in the isolated CNS of locusts.

The studies of Williams et al. (1997) in Drosophila, Lighton (1990) in ants, Lighton and Lovegrove (1990) (and other authors) in honeybees focussed on different aspects of ventilation and were performed under completely different conditions. However, they found in common basic ventilatory or gas exchange patterns comparable to those found in this study. These can be divided in the two basic patterns as defined in this study for fictive ventilatory rhythms. Irregular rhythms of ventilation seem to be a typical structure in insect gas exchange or ventilatory movements comparable to our analysis (ventilatory activity against time recorded).

In the isolated CNS of our two experimental locusts the various ventilatory patterns (Fig. 2C, e.g. for *Taeniojopoda eques*) are quite similar, but apparently the basic frequencies of ventilation differ from intact animals.

Fig. 3. Efferent ventilatory activity in long-term recordings from efferent nerves (metathoracic median nerve) in the isolated locust CNS of *Locusta migratoria* (A,B) and *Taeniojopoda eques* (C,D). Sequential histograms of bursts per min. Graphs (A) and (B) show discontinuous ventilation. In (C) and (D) temperature is indicated by diamond symbols and scale at the right side. The initial continuous ventilatory periods are followed by discontinuous ventilation patterns. The continuous parts in (D) show a characteristic irregular oscillation from high to low and again to high frequencies. In (D) a rise in frequency corresponding to a rise in saline temperature is visible. In (D) the nerve cord was not removed from the semiintact animal — in contrast to (A–C) where the nerve cord without tracheae was removed from the animal. In (D) the nerves were deafferented (connections to tracheae were maintained). The experiments were carried out at room temperature.
Ventilation rates in the isolated *Taeniopoda eques* CNS without tracheal supply (Fig. 2C) are similar to those of alert animals, but pause lengths are much lower than in *Taeniopoda eques* in rest in which pause lengths extend from 20 min to beyond 60 min (Harrison, personal communication). In long term recordings for *Taeniopoda eques* with maintained tracheal supply we found similar pause durations as in intact animals (Bustami et al., 1999). It is probable that internal stimuli like partial hypoxia influence ventilatory rhythms in ganglia with tracheae removed.

The specific patterns of DV or irregular oscillations within a bursting period have been previously called “group ventilation” (Hustert, 1974). These patterns can also be found in other insects. For example, Lighton (1990) has been able to show transitions from pure DV to fast continuous ventilation caused by activity in the desert ant *Camponotus detritus*. Transitional forms occur, corresponding to the patterns we found in our study (e.g. Fig. 3C in *Taeniopoda eques*). Earlier studies on efferent ventilation pattern from in situ nerve cords in insects have presented only regular neural output (Case, 1961; Miller, 1960; Komatsu, 1982) in short term recordings without discontinuous ventilation. A closer look at the data of Miller (1960) in resting desert locusts (*Schistocerca gregaria*) indicates the presence of discontinuous ventilation in his records of abdominal pumping movements. Recent studies even in slices of an insect ganglion (*Schistocerca gregaria*) have shown the persistence of ventilatory rhythms in isolated preparations (Ramirez et al., 1999).

For mammals it has been known for a long time that isolated brainstems in vitro are able to produce neural discharges of ventilatory activity for many hours. But in vertebrates generally only a continuous ventilatory pattern occur, except for periods of diving, slowing during hibernation, and in pathological cases (Zigmond et al., 1999). Discontinuous ventilation is not known for vertebrates to be a normal way of gaseous exchange.

---

**Fig. 4.** Efferent ventilatory activity in a long-term recording from the median nerve of the third abdominal neuromere of the metathoracic ganglion in an isolated CNS of *Locusta migratoria*. The tracheal supply was maintained Continuous sequential histograms of bursts per minute. In (A) and (B) in this recording a pronounced returning DV-rhythm is visible. In (A) most of the bursting periods last 5 to 6 min. In (B) pauses between bursting periods extend to 15–20 min. Saline at 18–19°C.
The increase of ventilation rate in the isolated nerve cord due to a rise in saline temperature (Fig. 3D) resembles a switch in intact honeybee ventilation rate and pattern caused by an increase in temperature (Lighton and Lovegrove, 1990), comparable to the increase in ventilatory activity in the grasshopper *Tmesis pulchripennis* with increasing temperature (Prange, 1990). Apparently, temperature changes affect ventilation patterns of an isolated insect nerve cord as it does in intact animals. Further studies are required for a reliable data basis on this subject.

Strong continuous ventilation patterns occur in different insects — as well as in a locust isolated nerve cord. For intact animals that we had forced to jump for 2 min (according to Krolikowski and Harrison, 1996) we found patterns similar to those high frequency ventilation patterns we found (Fig. 3C and D). The vigorous movements of jumping apparently increases the metabolic rate and therefore also the abdominal pumping.

Lighton (1988) found in *Psammodes striatus* (TokTok beetle) discontinuous gas exchange and a correlation between oxygen uptake and contractions of the abdomen. The discontinuous gas exchange is based upon DV movements generated in the CNS. These patterns of oxygen uptake in *Psammodes striatus* show similarities to the DV fictive ventilation as shown very clearly in Fig. 5. The comparison between the results from different investigations in insects and our data lead to the question of the underlying principles of rhythmogenesis of ventilation — besides many external factors described and studied by the cited authors.

Krolikowski and Harrison (1996) suggested partial pressure of CO₂ and O₂ in the tracheae as the important parameters regulating ventilation in different kinds of insects. The change of intrathoracic partial pressures due to gas exchange or retention is supposed to have a trigger function for the performance of ventilation according to the momentary needs, e.g. in situations of discontinuous ventilation. In the isolated nerve cord no change of tracheal partial pressures exist since just “efferent ventilation” patterns are generated. So this parameter cannot be the main factors for regulation of ventilatory rhythmogenesis in the isolated nerve cord. The present results suggest intrinsic regulatory or trigger mechanisms on the level of the neural net of the central pattern generator, largely independent of sensory influences, on the level of cell metabolism of the CNS or within ventilatory interneurones.

The results seen in the long term recording shown in Fig. 4 is the clearest evidence that mechanisms within the CNS underlie the basic rhythms of ventilation. Earlier studies (Harrison, 1997) suggested a PCO₂-influenced rhythmogenesis in locusts. In this model, if a certain trigger level of CO₂ within the tracheal system is reached, the ventilatory network begins to operate in order to release the CO₂. Possible central chemoreceptors sensitive for O₂ and CO₂ inside the ganglion or inside its ganglionic tracheae have been suggested by earlier work and also by more recent studies (Miller, 1960; Harrison, 1997; Bustami et al., 1999). These could influence the CPG-output in case of misbalance (i.e. hypoxia/hyperoxia or hypercapnia) of one of those gases in the surrounding saline or in the tissue Fig. 5.

5. Conclusion

The isolated nerve cord can generate a wide range of ventilation patterns on its own. This range spans from

![Pause duration histogram](image_url)

Fig. 5. Pause duration histogram of long-term recordings from ventilatory efferent nerves (median or ventral metathoracic nerves) in isolated nerve cords from *Taeniopoda eques* (7 experiments) and *Locusta migratoria* (7 experiments) without tracheal supply. Pause lengths between two bursting periods were counted. Apparently pause duration of 1 or 2 min dominate in the preparations. Longer pauses — as occurring in the experiments with maintained tracheal supply (Fig. 4) — are rare.
discontinuous ventilation to continuous, stressed ventilation. Investigations in intact insects (Hustert, 1974; Herreid and Full, 1984; Bartholomew and Lighton, 1985; Lighton, 1990; Lighton and Lovegrove, 1990; Slama and Coquillaud, 1992; Lighton and Berrigan, 1994; Krolikowski and Harrison, 1996; Williams et al., 1997) show similarities to the patterns in the isolated locust nerve cord.

The results suggest that the ventilatory patterns are of neural or physiological origin. Furthermore we can postulate a “general principle” of ventilatory patterns which seem to have developed in different families of insects (e.g.: ants (Lighton, 1990), dragonflies (Hughes and Mill, 1966), locusts (Harrison, 1997), cockroaches (Herreid and Full, 1984; Bartholomew and Lighton, 1985) and beetles (Chown and Holter, 2000). So we find continuous as well as discontinuous ventilation patterns — with transitional pattern — throughout the different insect groups. Genetic differences as in ventilation patterns of Drosophila due to selection by imposed desiccation (Williams and Bradley, 1998) may also occur in other insects and may serve the long term adaptation of insect populations to altering environments.

The cellular mechanism of rhythmogenesis in ventilation, may be based on ionic changes in context with the bicarbonate system. This questions requires further studies.

Acknowledgements

This study has been supported by: Graduiertenkolleg “Organisation und Dynamik neuronaler Netze”, Göttingen.

References


Bustami, H.P., Harrison, J., Hustert R., 1999. Hints to an oxygen receptor in the CNS of insects which influences ventilation; Talk and abstract at: Reporting seminar at Reinhausen of the “Graduiertenkolleg: Organisation and Dynamics of Neuronal Networks” at the University of Göttingen, Germany.


