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Modulation of auditory information processing in tethered flying locusts

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Summary. 1. The activity and the response to auditory stimulation of the auditory nerve, of single receptor fibres and of thoracic auditory interneurons were recorded and analysed in resting and in tethered flying locusts (*Locusta migratoria*). During the behavior in the auditory system (I) flight induced activity and (II) modulations of the auditory response to sound pulses occurred. It was analysed whether flight-induced activity and the modulations of the auditory response were due to influences affecting the auditory receptors and/or interneurons.

2. Flight evoked tonic and phasic activity in the tympanal nerve and in single auditory receptors. Receptor activity was caused by thoracic movements stimulating the auditory organ and additionally by flight noise (Figs. 1–3).

3. Auditory interneurons also received excitatory input during flight. The activity of the interneurons was either markedly reduced or totally eliminated when the auditory organ was destroyed. Thus flight activity in auditory interneurons was largely or entirely caused by the auditory receptors (Fig. 4). No evidence was found for the flight pattern generator having a discrete effect on auditory interneurons.

4. The summed nerve response to auditory stimuli (10 ms, 70 dB SPL white noise) was reduced to 19% during flight. This corresponded to a decrease in sensitivity of 20 dB. Appropriate reductions of the auditory response also occurred in single receptor fibres (Figs. 1, 3).

5. The response of interneurons to sound pulses was reduced during flight to 26–35% of the response at rest (Figs. 5, 6). If the auditory organ was destroyed and the auditory nerve was stimulated electrically, no reduction of the electrically evoked response occurred (Fig. 7). Thus the auditory response reduction during flight must be due to influences affecting the auditory organ.

6. In one auditory interneuron flight enhanced the response to auditory stimulation and to electrical stimulation of the auditory nerve due to an additional sensory excitation of the neuron (Figs. 5–7).

Introduction

The auditory pathway of locusts has been subject of intense investigations dealing with the peripheral and central elements involved in auditory information processing. Some of the analysed peripheral properties have been the biophysics of the tympanal organ (Michelsen 1971b, 1973; Stephen and Bennet-Clark 1982; Breckow and Sippel 1985) and receptor structure and physiology (Gray 1960; Michelsen 1971a; Rehbein 1976; Rehbein et al. 1974; Römer 1976, 1985; Sippel and Breckow 1984). Aspects of the central elements that have been investigated were the physiological and morphological properties of interneurons (Kalmring 1975a, b; Kalmring et al. 1972, 1978a, b; Rehbein 1976; Rehbein et al. 1974; Römer et al. 1981; Römer and Marquart 1984) and synaptic processing and connections within the system (Römer and Dronse 1982; Marquart 1985b). These investigations have given a detailed picture about many functional properties within the auditory pathway. However, in these experiments restricted immobilized preparations were used to obtain optimal acoustic and recording conditions. Thus it remains an open

Abbreviations: *AP/s* action potential(s) per second; *AP/Stim* action potential(s) per stimulus

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question whether auditory information processing during ongoing behavior is maintained as in the restricted preparation. In grasshoppers ventilatory and hindleg movements actually influence auditory receptor activity (Hedwig 1988; Hedwig et al. 1988). Further there is additional evidence in grasshoppers (Wolf and Helversen 1986; Hedwig 1986) and also in crickets (Stout and Huber 1972; Schildberger et al. 1987) that auditory responses are subject to modulatory influences which depend on the behavior of the animal. Peripheral modulations, i.e. influences on the auditory organ and its receptors, and central modulations, i.e. influences on auditory interneurons, may occur.

Although flying locusts respond to sound stimuli (Weis-Fogh 1956; Haskell 1957; Yinon et al. 1971), problems for sound processing during flight may be present. Flight behavior is accompanied by sonorous wing-beat noise when the wings hit the legs during take off and by low amplitude flight noise at normal level flight (Haskell 1957). The modulatory influence of wind and of sound with the same spectrum as wing-beat noise has been studied (Boyan 1986). But also strong thoracic movements and forces are produced by the muscle contractions. Knowing the enormous sensitivity of tympanic receptors towards vibrations (Michelsen and Larsen 1978) it seemed likely that auditory information processing must be influenced by flight. Thus the experiments presented here were designed to examine peripheral and central modulations of auditory information processing in tethered flying locusts and compare the activity and the response of auditory receptors and interneurons during rest and flight.

Materials and methods

Animals. Experiments were performed at room temperature on female mature *Locusta migratoria* aged three weeks after the imaginal moult. The animals were obtained from a colony at the University of Alberta.

Preparation. To record intracellularly from auditory interneurons a preparation was used which was similar to that developed by Wolf and Pearson (1987). The legs of the animals were amputated and the animals were waxed upside down to a holder which allowed normal movements of the wings. The ventral cuticle was removed above the meso- and metathoracic ganglia. The ganglia were supported by one metal spoon and stabilized by a second ring-like spoon gently pressed on their ventral surfaces. The metathoracic Nv6 contralateral to the stimulation and recording side was cut and the ipsilateral Nv6 lifted on a pair of hook electrodes. To abolish capacitive coupling between the microelectrode and the wing movements two thin copper plates were placed parallel to the body's length axis and so shielded the recording site from wing movements.

Recording and staining. EMG recordings were obtained from mesothoracic depressor muscles by inserting copper wires (0.1 mm in diameter) into their ventral attachment sites. These served as references for flight.

In some experiments only the summed nerve response of Nv6 was recorded with hook electrodes. The recording site was insulated with silicon grease and Nv6 was cut close to the metathoracic ganglion.

Intracellular recordings of auditory interneurons in the meso- and metathoracic ganglion were obtained with glass microelectrodes. The electrode tip was filled with 5% Lucifer Yellow and the electrode resistances were between 80–120 MΩ. Neurons were stained iontophoretically by applying a hyperpolarizing current of 2–4 nA for 3–5 min. After dye injection the ganglia were removed, processed by standard histological techniques and viewed under a fluorescence microscope.

Single cell recordings from auditory receptors were made in the frontal auditory neuropil of the metathoracic ganglion. Lucifer Yellow was used to trace their anatomy in the metathoracic ganglion and to identify them as auditory receptors.

Acoustic stimulation. Acoustic stimuli were presented using a piezoelectric high-frequency loudspeaker (Motorola type PH10). The loudspeaker was placed at a distance of 31 cm at the same level of the tympanum 45° frontal to the animal. The acoustic stimulator and the loudspeaker were calibrated for 4 kHz, 12 kHz and white noise from 50 to 85 dB SPL in 5 dB steps by means of a Brüel & Kjaer microphone (type 4133) connected to a Brüel & Kjaer measurement amplifier (type 2608). If not otherwise indicated a 70 dB SPL white noise stimulus of 10 ms duration was given at intervals of 100 ms.

Destruction of the tympanal organ. The auditory organ could be reliably abolished by destroying the tympanal membrane with a hot needle and putting a drop of hot wax in its outer cavity. The tympanal receptors were killed by this at once and auditory activity in Nv6 ceased immediately.

Electrical stimulation. After destruction of the auditory organ Nv6 was stimulated electrically via hook electrodes (Römer and Rheinlaender 1983). Stimulus trains of 10 ms duration were given at 100 ms intervals (Grass S88 stimulator). Stimulus amplitude, duration and repetition rate within a train were adjusted to elicit a response in the intracellularly recorded neuron that was similar to the acoustically elicited response. The stimuli were not recorded on tape. The times of stimulus presentation (Figs. 5–7) were determined from the stimulus artefacts.

Data evaluation. All single cell recordings were stored on tape. Action potentials were transformed into TTL pulses by a window discriminator (WPI model 121). Together with a trigger pulse, marking the beginning of the acoustic or electrical stimulus, they were fed into a computer (Dec LSI 11/23). PST histograms with bin-width of 2 ms were calculated and plotted for the time after the onset of sound or electrical stimulation. The histograms were normalized to a single stimulus, their amplitudes are thus directly comparable. The number of stimuli (N) and the number of action potentials (AP) are given in each histogram.

Synaptic responses to the stimuli were averaged for a time of 80 ms after stimulus onset using a Biomac 1000 averager. Bin-width was 7.8 μs. Summed nerve responses to the auditory stimulus were amplified 1000 fold, full wave rectified and fed on-line into the averager using the same time parameters as above. Full wave rectifying was used to evaluate positive and

negative components of the signal. The amplitude of the averaged summed nerve response and of synaptic potentials was measured with a μ80 microline system (Lab. Company Systems) as the integral of the response over time. For the auditory responses obtained during flight, only the signal component above the flight related background activity was evaluated as an auditory response.

Terminology. The interneurons presented here have been recorded and described under different names. The neurons TN1, BSN1, AN1 (Römer and Marquart 1984) and G (Rehbein 1976) have also been named 601, 530, 531 and 714 (Robertson and Pearson 1983; Pearson and Robertson 1987). AN1 has been described as B1 neuron (Rehbein 1976) and TN1 has been described as thoracic low frequency neuron (Rehbein et al. 1974). In this paper the terminology of Römer and Marquart (1984) was used.

Results

Recordings of auditory receptors

The auditory organ of locusts is located in the pleura of the first abdominal segment. The auditory nerve (Nv6, tympanal nerve) runs from the auditory organ to the metathoracic ganglion, where the receptors make connections with auditory interneurons in the caudal and frontal auditory neuropils (Rehbein et al. 1974).

Auditory nerve recordings. In initial experiments modulations in the periphery of the auditory pathway were analysed. The tympanal nerve was cut at the metathoracic ganglion and the summed nerve respone to auditory stimulation was recorded and compared in resting and flying locusts. Because the wind stimulus used contained acoustic components (Boyan 1986) flight was always elicited in the preparations with brief wind pulses only. As soon as flight started no further wind was given and auditory stimuli were applied.

In the resting locust recordings of the tympanal nerve showed the summed nerve response to auditory stimulation (Fig. 1A). With a latency of about 4.5 ms the response started and showed four activity peaks. These activity peaks corresponded to the synchronous activity of a large number of receptors (Autrum et al. 1961; Römer 1976; Adam 1977a, b; Ronacher and Römer 1985). They were also present in the average of the response (Fig. 1B). Due to rectification (see Methods) the activity peaks revealed two peaks being separated by 1.5 ms. In addition to the auditory response there was also some non-auditory background activity, which arose from the discharge of auditory receptors in response to ventilatory movements.

Flight caused an activity pattern in the tympanal nerve (Fig. 1C) which gave in the average a tonic component with one or two broad phasic peaks after depressor activity (Fig. 1G). Simultaneous auditory stimulation evoked no obvious auditory response in the tympanal nerve (compare Fig. 1C with response in the resting animal, Fig. 1A). Only the averaged signal showed the existence of an auditory response in the activity pattern (Fig. 1D). Compared to the resting animal the response in the mean was reduced to 19% and additionally flight-evoked activity summed up to a high level of background activity. To examine more quantitatively the changes of the auditory response, pulses of 50 to 85 dB SPL white noise were given at rest and during flight. The average of the summed nerve response in each case was obtained and normalized to the response to 70 dB SPL at rest, which was set equal to 1 (Fig. 1E). During flight a reduction of the auditory response occurred which corresponded to a decrease in auditory sensitivity of 20 dB, i.e. a signal of 70 dB SPL white noise gave the same response as a 50 dB SPL white noise signal at rest (Fig. 1F), but during flight the response appeared on top of a high background activity (compare Fig. 1D and 1F). This background noise corresponded to 60 dB SPL in the average because the same activity level in Nv6 as during flight could be obtained by a tonic auditory stimulus of 60 dB SPL white noise. In different animals the flight induced activity in the tympanal nerve varied and was equivalent to a tonic acoustic stimulus of 55–65 dB SPL.

If the tympanal organ was destroyed neither the auditory response nor the flight-evoked activity could be recorded (Fig. 2A). Corresponding to this the average signal also showed no activity (Fig. 2B). Thus the reduction in the auditory response as well as the activity in Nv6 during flight had to be related to the activity of the auditory organ itself.

In these experiments Nv6 had been cut at the metathoracic ganglion, ruling out any contribution of efferent activity on the response pattern. But flight-induced activity and the reduction of the auditory response remained unchanged when the tympanal nerve was not cut at the metathoracic ganglion.

Effects of wind and muscle contractions. The flight-evoked activity in the auditory nerve could be caused by flight noise and wind, produced by the moving wings, or it could be the result of a mechanical stimulation of the auditory organ. In an attempt to reduce any self-generated auditory and

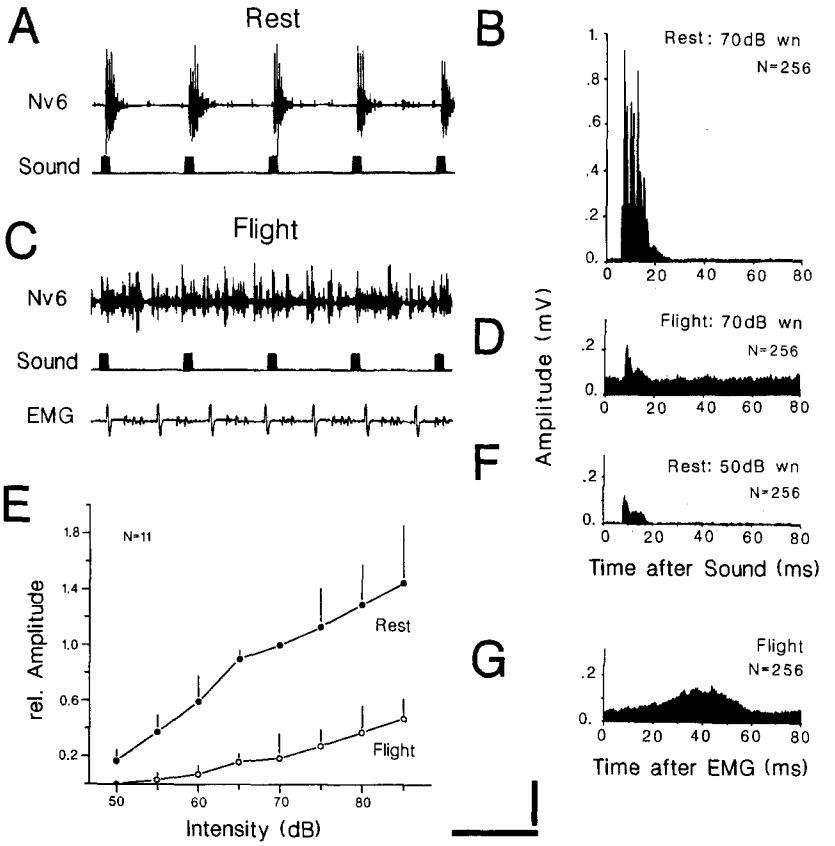

Fig. 1A–G. Activity and summed nerve response of Nv6 to acoustic stimulation in resting and flying locusts (left) and the average of the response (right). **A** Summed nerve response to auditory stimulation at rest. **B** Average of the full wave rectified summed nerve response to auditory stimulation at rest. **C** Activity and summed nerve response to auditory stimulation during flight. **D** Average of the summed nerve response to auditory stimulation during flight. **E** Relative amplitude of the summed nerve response in resting and flying locusts. According to the response amplitude the sensitivity during flight is reduced by 20 dB, i.e. the 70 dB SPL response during flight is equivalent to the 50 dB SPL response at rest. All amplitudes have been normalized to the 70 dB SPL response at rest. Vertical bars indicate half mean error, N number of animals tested. **F** Average of the summed nerve response to auditory stimulation with 50 dB SPL white noise at rest. Compare with the response to 70 dB SPL white noise at flight (**D**). The responses are equivalent, but the response during flight occurs on top of a high background activity. **G** Average of Nv6 flight activity after mesothoracic depressor activity. Traces: *Nv6*, recording of the tympanal nerve; *Sound*, auditory stimulus, 70 dB SPL white noise, 10 ms duration, 100 ms interval; *EMG*, electromyogram of mesothoracic depressor flight muscle. The average of the summed nerve response is given for 256 stimuli (**B, D, F**) or for 256 flight cycles (**G**). Scale bars: horizontal 100 ms, vertical 0.5 mV for Nv6

wind stimulation the wings were cut down to few millimeters. This was unsuccessful, however, because the wingstumps produced a sound when they hit the body. Furthermore flight performance was very variable and only short flight sequences were elicited which were difficult to compare with the intact behavior. Nevertheless, even in this situation some animals gave the same (reduced) auditory response as flying with intact wings. Thus no statements about the influence of flight noise were possible. But these experiments indicate that the wind produced by the wings was not the decisive factor for flight related receptor activity and reduced auditory responsiveness.

The effects on the auditory organ of mechanical stimulation by contractions of the flight muscles without any accompanying sound and wind were tested. To do this the thoracic flight muscles in the vicinity of the auditory organ were stimulated electrically at frequencies, similar to flight frequency. The forces and movements in the thorax induced with this method were obviously smaller than those occurring during flight. Nonetheless muscle contractions in the vicinity of the tympanal organ always elicited activity in the tympanal nerve. The auditory response was only reduced half as much as during flight. It is doubtful that stronger response reductions can be obtained with this method because the vigor of the actual flight pattern could not be evoked. But when stronger movements were elicited by stimulating the metathoracic nerves 1, 3 and 4 (Fig. 2C), which contain motor

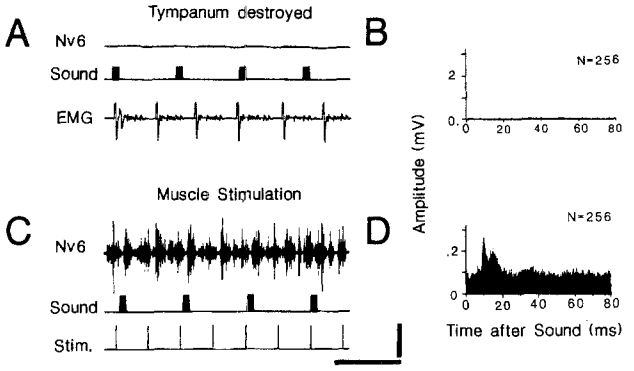

Fig. 2. A, B recording of the activity and summed nerve response of Nv6 (left) and average of the response (right) after destruction of the auditory organ. **C, D** Recording of the activity and summed nerve response of Nv6 (left) and average of the response (right) during electrical evoked muscle contractions and auditory stimulation. Auditory stimulus 70 dB SPL white noise, 10 ms duration, 100 ms interval. Traces: *Stim*, Stimulation of motor nerves at intervals of 52 ms, all other traces see Fig. 1. Scale bars: horizontal 100 ms, vertical 0.5 mV for Nv6

axons to flight muscles, the activity in the auditory nerve almost matched the activity observed during flight. Simultaneous stimulation of the motor nerves and presentation of auditory pulses reduced the response to a 70 dB SPL white noise pulse to 27% of its value at rest (Fig. 2D). This was close to the reduction in flying animals. Thus the movements and forces produced by muscle contractions are an important factor affecting the auditory organ during flight.

Single receptor recordings. The summed nerve recordings gave no information about whether single auditory receptors were influenced in the same way as the summed nerve response. Therefore a survey on auditory fibres was made by recording from the axons of single receptors. Stable recordings during flight were difficult to obtain, so that only nine fibres were examined. No systematic auditory stimulation was done to relate the fibres to any groups of auditory receptors described by Michelsen (1971a) and Römer (1976). Eight of these receptors matched the summed nerve response, data for two of them are given in Fig. 3A–F. During flight they were almost tonically active with discharge rates up to 120 AP/s (action potentials per second) (Fig. 3A) but additional phasic modulation in the flight rhythm occurred (Fig. 3D). In the resting animal they gave a clear response to the auditory stimulus (70 dB SPL, white noise) with 2–3 AP/Stim (action potentials per stimulus) (Fig. 3B, E). During flight the response to the auditory stimulus was almost completely (Fig. 3C) or distinctly reduced (Fig. 3F) and at the same time flight-induced discharges of 120 AP/s occurred in the first receptor and of 75 AP/s in the second receptor. This activity additionally masked the reduced auditory response. At 80 and 85 dB

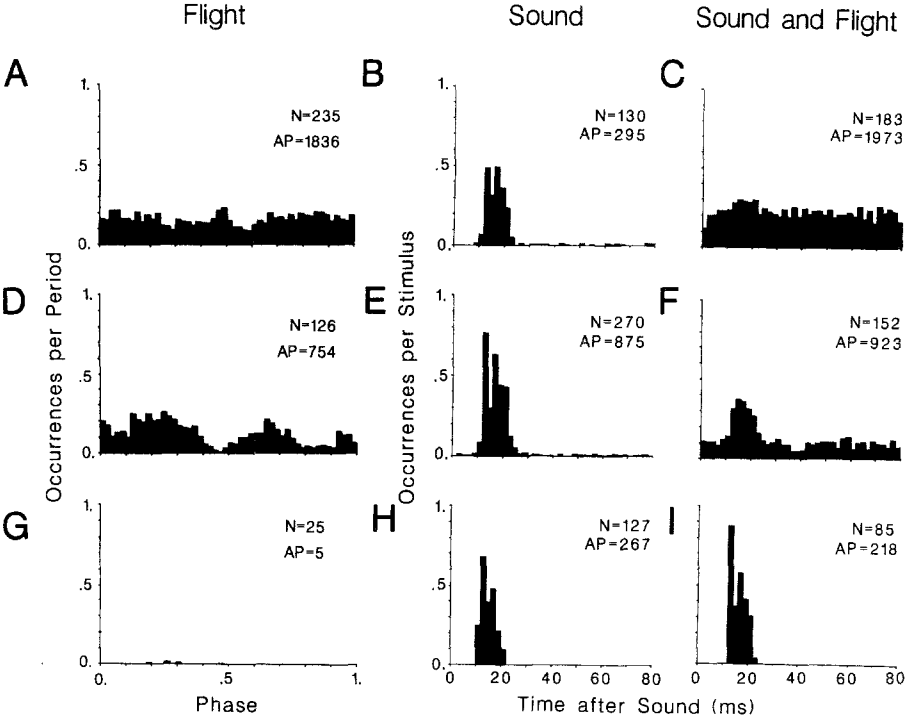

Fig. 3A–J. Responses of three different auditory receptors (horizontal rows) to flight (left), to auditory stimulation at rest (middle) and to auditory stimulation during flight (right). **A, D, G** Phase histogram of receptor activity during flight. **B, E, H** PST histogram of the auditory response at rest. **C, F, I** PST histogram of the auditory response during flight. Auditory stimulus for the first and second receptor 70 dB SPL white noise and for the third receptor 80 dB SPL white noise. Number of evaluated flight cycles or stimuli is given by *N*, number of action potentials by *AP*

SPL sound intensity the receptors generally showed an increased response to the auditory stimulus. But at least one low frequency fibre gave no auditory response at all, even at intensities of 90 dB SPL. The activity patterns of these receptors thus corresponded well to the data obtained from summed nerve recordings.

An exception was one auditory receptor (Fig. 3G–I). Its threshold was so high that at 70 dB SPL white noise it did not respond at all. It gave 2.1 AP/Stim, when the stimulus intensity was increased to 80 dB SPL. During flight its activity was only 4 AP/s and much lower than in the other receptors, also the auditory response did not decrease but increased to 2.6 AP/Stim. The fibre responded to 12 kHz sound but not to 4 kHz. The observations on this single receptor indicated that additional there may be receptors which are only slightly influenced by flight behavior.

Recordings of auditory interneurons

To analyse any central modulations in the following experiments the activity and auditory responses of auditory interneurons were recorded intracellularly and compared in resting and flying animals. In the metathoracic ganglion the neurons TN1, BSN1 and AN1 with the soma ipsilateral to the side of stimulation were recorded 3 to 5 times in the frontal auditory neuropil. All these fibres have arborizations in the auditory neuropil of the metathoracic ganglion and an ascending axon which projects up to the brain (TN1, AN1) or to the mesothoracic ganglion (BSN1) only. In the mesothoracic ganglion the ipsi- and contralateral G neuron was recorded 15 times in the main neurite of the cell. The G neuron is a T-shaped fibre, the main axon ascends to the brain and one axon branch projects back to the metathoracic ganglion. The response patterns in ipsi- and contralateral G neurons were qualitatively similar but with unilateral stimulation the contralateral G neuron usually had a lower threshold and showed a stronger response than the ipsilateral one (Rehbein 1976). Data presented were therefore based on the G neuron with the soma contralateral to the side of stimulation. First the flight related activity of the interneurons will be presented and then the influence of flight on auditory information processing will be described.

Flight-evoked activity. Flight caused excitatory activity in the interneurons TN1, BSN1, G and AN1 which was strongest in TN1 and BSN1. These neurons showed discharge rates of 95 AP/s and 55 AP/s, respectively (Fig. 4A, C). In addition to EPSPs IPSPs of 2 mV also occurred in BSN1 (Fig. 4C arrows). In G flight-induced activity varied in different animals. Numerous EPSPs were always evoked. These compound EPSPs could be 5–7 mV in amplitude and suprathreshold. In some animals even spike activity at rates up to 30 AP/s was elicited, but generally the excitation remained subthreshold (Fig. 4E). In these three neurons spikes and EPSPs were in part phasically coupled to the flight rhythm. In BSN1 and G the greatest compound EPSPs occurred in phase with depressor activity and in TN1 the discharge rate was transiently higher after depressor activity. AN1 discharged approximately 7 AP/s during flight but the activity was not obviously in phase with the flight rhythm (Fig. 4G).

After destruction of the auditory organ almost all spike activity ceased in the neurons (Fig. 4B, D, F, H). In TN1 flight still elicited excitation and EPSPs were recorded, but these were almost always subthreshold and spikes were only elicited at a rate of about 1 AP/s. Spike activity was absent in BSN1 and G (Fig. 4D, F). In BSN1 only minor EPSPs and no IPSPs were recorded whereas in G a constant input of EPSPs with 2–3 mV amplitude remained. Spikes still occurred in AN1 (Fig. 4H) but the discharge rate decreased to about 3 AP/s. These findings indicate that most of the synaptic input to these neurons occurring during flight was mediated by the auditory organ. There was no indication of a direct input from the flight central pattern generator to these neurons. The remaining excitation in TN1 and G was probably due to the posterior chordotonal organ in the joint of the hindwing. This organ is activated during flight and makes excitatory monosynaptic connections to TN1 and polysynaptic connections to G (Pearson and Hedwig, unpublished).

Auditory responses at rest. Although characteristics of these interneurons to auditory stimulation have been described previously (Rehbein et al. 1974; Kalmring et al. 1978a, b; Römer and Marquart 1984) their response patterns will briefly be described to allow a comparison in quiescent and flying animals. In general in the resting animal all interneurons showed a regular and constant response to auditory stimulation and only little background excitation. The neurons TN1, BSN1 and G gave an excitatory response whereas AN1 showed an inhibitory response to the auditory stimulus.

Interneuron TN1 could easily be recognized during the recordings because of its unique re-

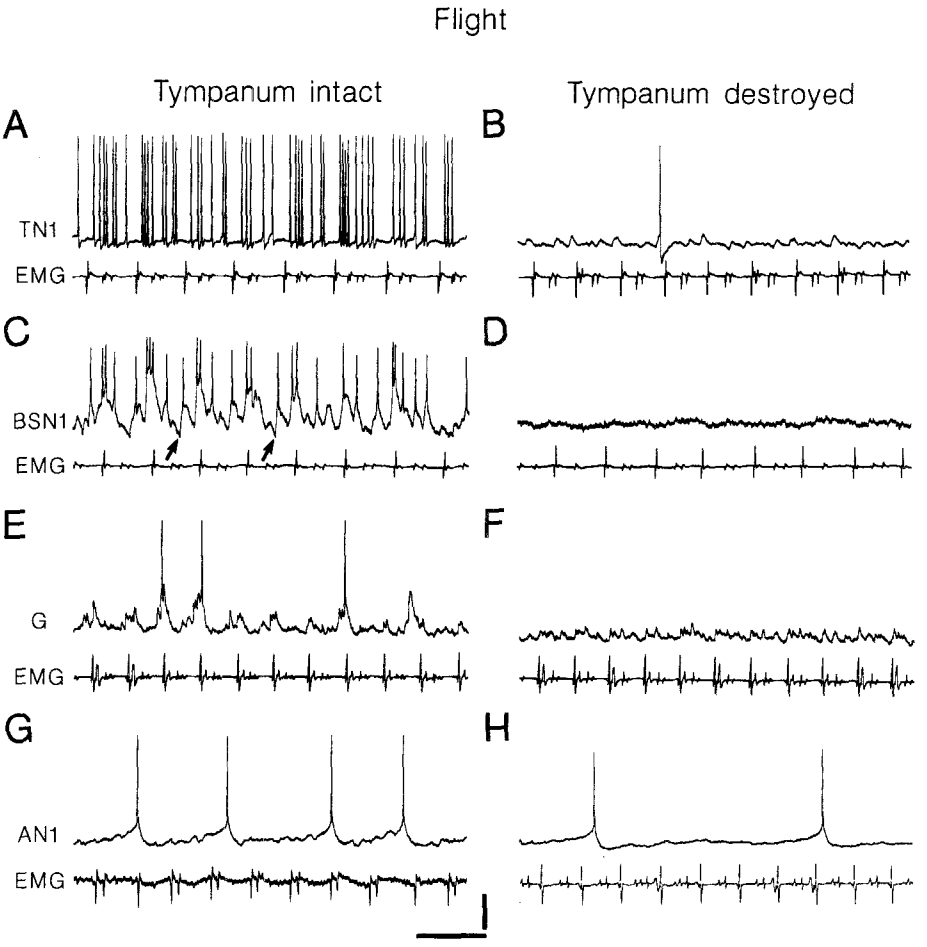

Fig. 4A–H. Intracellularly recorded activity of auditory interneurons in flying locusts with intact tympanum (left) and after the tympanum has been destroyed (right). Note the decrease in activity after destruction of the auditory organ. **A, B** Interneuron TN1. **C, D** Interneuron BSN1. **E, F** Interneuron G. **G, H** Interneuron AN1. Scale bars: horizontal 100 ms, vertical for intracellular recordings 20 mV (**A, B**), 10 mV (**E–H**) and 5 mV (**C, D**)

sponse pattern of large spikes and small EPSPs. Because TN1 is most sensitive to low frequency sound (Rehbein et al. 1974) 4 kHz pulses instead of white noise were used for stimulation. TN1 responded with 3.0 AP/Stim to a 75 dB SPL pulse. This response occurred from 10 to 20 ms after the stimulus and gave a clear peak in the PST histogram (Fig. 5A, B).

For all other interneurons a 70 dB SPL white noise stimulus of 10 ms duration and 100 ms interval was used. In BSN1 stimulation evoked a small IPSP which was followed by a large prominent EPSP (Fig. 5C). The EPSP had an initial peak and a more tonic component thereafter. The stimulus elicited on average 7.8 AP/Stim with a PST histogram shown in Fig. 5D. The histogram reflects the two phases of the synaptic potential in the number of AP/bin and shows a maximum followed by a more tonic level of activity. Auditory stimulation evoked compound EPSPs in G which started with a latency of 15 ms and lasted for about 30 ms. The mean number of spikes they elicited was almost constant in every animal, but it varied in different animals from 0.8 to 2.1 AP/Stim. An example with 1.3 AP/Stim is shown in Fig. 5E. The compound EPSP had two peaks, the first was usually subthreshold and the second was above threshold. Thus the initiation of spike activity was delayed with respect to the onset of the synaptic potential. The maximum of the response revealed by the PST histogram occurred with a latency of 36 ms (Fig. 5F). Unilateral stimulation ipsilateral to the soma site elicited in AN1 an inhibitory response (Marquart 1985a). The IPSP had an amplitude of up to 9 mV and was biphasic with a second component in the rising phase of the potential (Fig. 5G). There was no stimulus related excitation, the spikes in the recording were due to spontaneous activity. The average maximal amplitude of the IPSP was 7.5 mV (Fig. 5H), the average also showed the biphasic inhibition.

Auditory responses during flight. During flight the same auditory stimuli were presented as in the resting animal (Fig. 6). Because of the strong flight activity in TN1, any auditory responses were al-

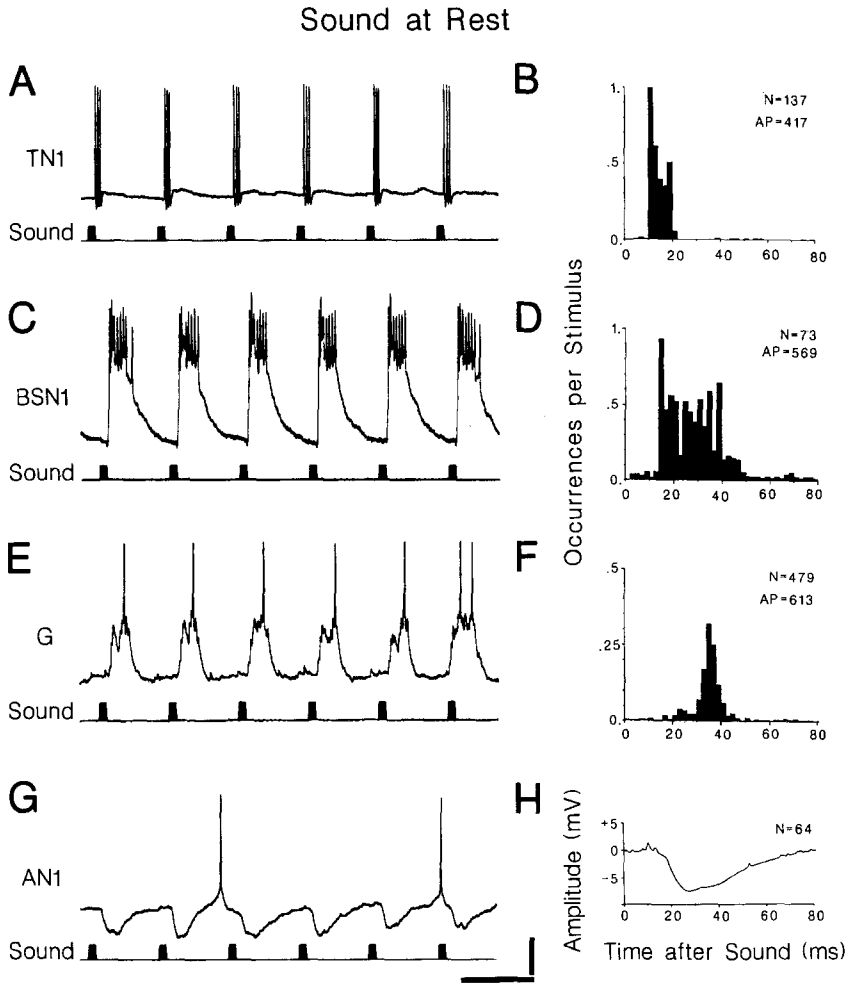

Fig. 5A–H. Intracellularly recorded responses of auditory interneurons to auditory stimulation in resting locusts (left) and PST histograms or average synaptic potential of the response (right). **A, B** Interneuron TN1. **C, D** Interneuron BSN1 hyperpolarized by 1.5 nA. **E, F** Interneuron G. **G, H** Interneuron AN1. Sound stimulus: 75 dB SPL 4 kHz in **A** else 70 dB SPL white noise, 10 ms duration, 100 ms interval. Scale bars: horizontal 100 ms, vertical for intracellular recordings 20 mV (**A**), 10 mV (**E, G**) and 5 mV (**C**)

most totally masked (Fig. 6A). Only the PST histogram revealed that there was still an auditory reponse of 0.8 AP/Stim as calculated from the histogram (Fig. 6B). This is 26% of the response in the quiescent animal. A similar change occurred in BSN1. During flight the auditory-evoked EPSP was reduced in amplitude and duration and generated only 2.8 AP/Stim (Fig. 6C). This is 30% of the response in the resting animal. As the PST histogram revealed (Fig. 6D) the initial maximum as well as the tonic response component were both decreased. Furthermore IPSPs occurred at all phases of the recording rather than only after the onset of auditory stimulation (Fig. 6C arrow).

In G the auditory-evoked EPSP always showed a much greater variability than in the resting animal (Fig. 6E, F). Usually also the first peak of the EPSP elicited spikes but sometimes the first or the second component were missing, leading to a synaptic potential of only 20 ms duration (Fig. 6E arrow). The EPSPs could also be considerably reduced (Fig. 6E left) or sometimes generate up to three spikes and additional flight-evoked EPSPs occurred. Corresponding to this increased variability of the synaptic response during flight the standard deviation for the number AP/Stim was 0.8, what was double the deviation seen at rest. The PST histogram showed a shorter latency of only 22 ms for the suprathreshold response and reflected the two peaks of the EPSP in two maxima of spike activity. In contrast to TN1 and BSN1 the mean number of AP/Stim was enhanced from 1.3 to 1.5 AP/Stim. The response increase was especially clear when 50 ms pulses were given because then the number of AP/Stim increased by 60% from 3.2 to 5.3 AP/Stim. An increase in the auditory response could be observed in most G neurons with subthreshold flight activity. But in one example, where flight evoked spike activity of 30 AP/s in G, the auditory response was reduced by 50%.

In AN1 the response to auditory stimulation also became very variable (Fig. 6G). Obviously the IPSP sometimes failed totally (Fig. 6G left) and

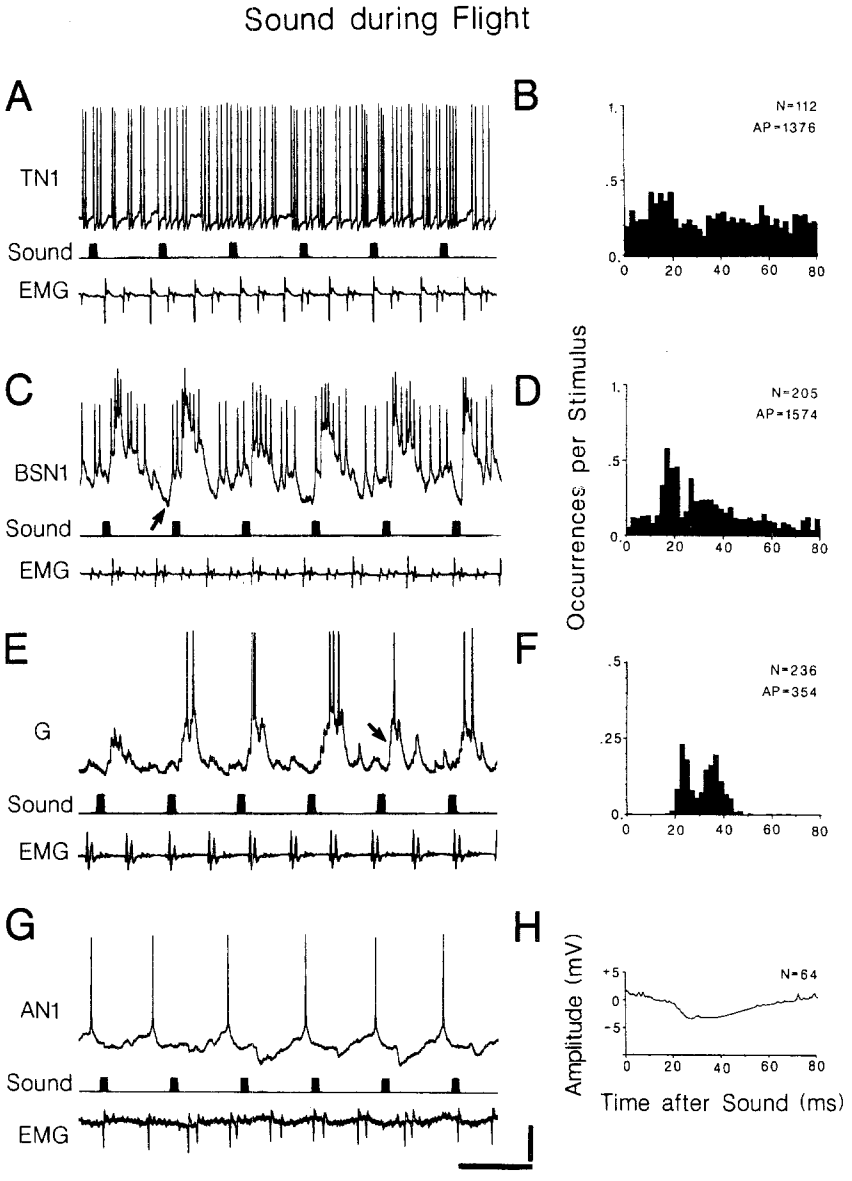

Fig. 6A–H. Intracellularly recorded responses of auditory interneurons to auditory stimulation in flying locusts (left) and PST histograms or average synaptic potential of the response (right). **A, B** Interneuron TN1. **C, D** Interneuron BSN1 hyperpolarized by 1.5 nA. **E, F** Interneuron G. **G, H** Interneuron AN1. Sound stimulus: 75 dB SPL 4 kHz in **A**, else 70 dB SPL white noise, 10 ms duration, 100 ms interval. Scale bars: horizontal 100 ms, vertical for intracellular recordings 20 mV (**A**), 10 mV (**E, G**) and 5 mV (**C**)

it never was as high as in the resting animal, but its biphasic nature was still present. The average amplitude of the IPSP was 2.8 mV (Fig. 6H) and the total response was 37% of the response in the resting animal.

Electrically evoked responses during flight. The activity of the interneurons revealed basically the same features as the auditory receptors. During flight they were activated and showed a decrease in their auditory response. Thus the interneurons reflected the receptor activity, this was already shown for the flight-evoked activity (see Fig. 4). To examine the reason for the auditory response reduction in interneurons any modulations of the signal by the auditory organ had to be prevented. Therefore the auditory organ was destroyed and auditory stimulation was replaced by electrical stimulation of Nv6 (Römer and Rheinlaender 1983). Trains of 10 ms with several pulses were used to elicit a response in the interneurons which was similar to the auditory response. Electrical stimulation evoked very constant responses in the resting animals. These responses remained unchanged during flight (Fig. 7). As described above (Fig. 4) little flight-evoked activity remained in the interneurons, but this had no effect on the electrically-evoked responses in TN1, BSN1 and AN1. Electrical stimulation elicited 2–3 spikes in TN1 (Fig. 7A, B), an EPSP with 9–10 spikes in BSN1 (Fig. 7C, D) and an IPSP in AN1 (Fig. 7G, H). These responses did not change during flight. Nei-

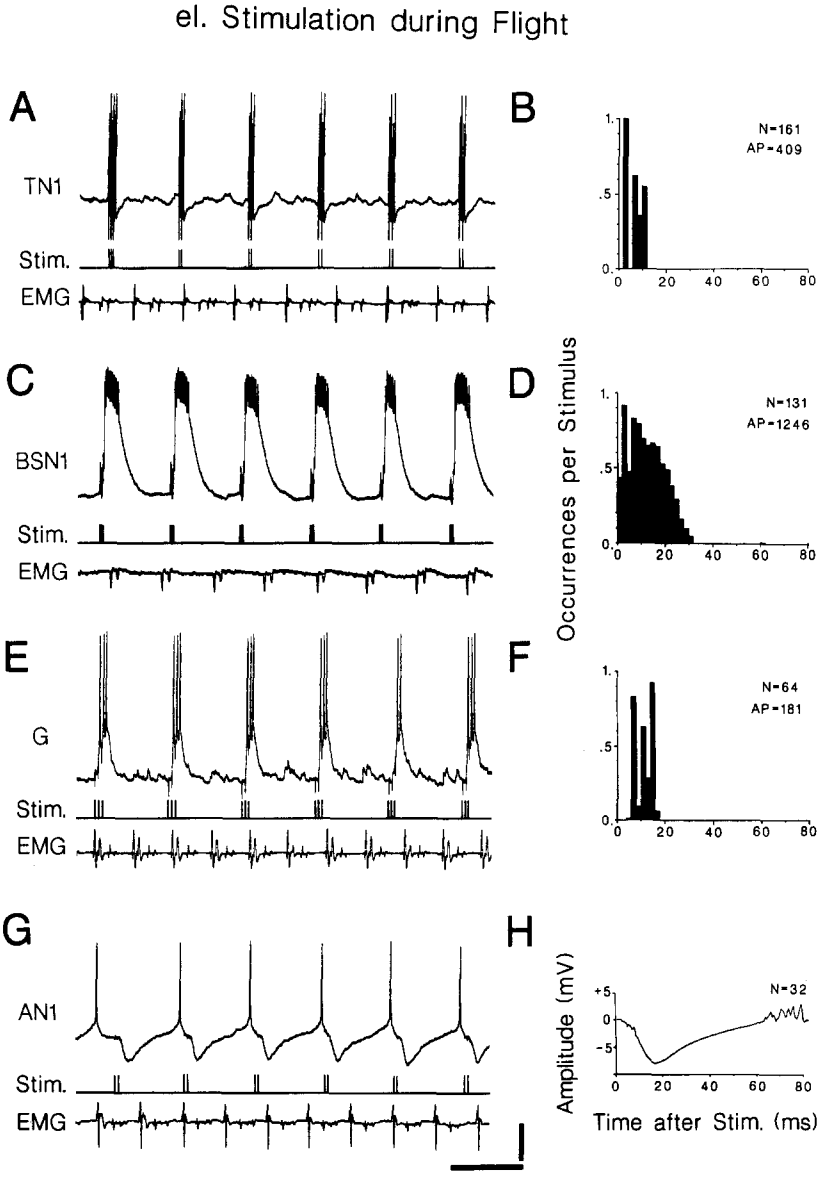

Fig. 7A–H. Intracellularly recorded responses of auditory interneurons to electrical stimulation of Nv6 in flying locusts (left) and PST histograms or average synaptic potential of the response (right). **A, B** Interneuron TN1. **C, D** Interneuron BSN1 hyperpolarized by 1.5 nA. Events in the first three bins of the histogram are due to stimulus artefacts. **E, F** Interneuron G. **G, H** Interneuron AN1. Traces: Stim, electrical stimulation of Nv6, train duration 10 ms, intervals 100 ms. Scale bars: horizontal 100 ms, vertical for intracellular recordings 20 mV (**A**), 10 mV (**E, G**) and 5 mV (**C**)

ther were the electrically-evoked responses reduced, nor were they masked by flight activity. The responses were very similar to the auditory responses of the neurons at rest and they were quite different to the auditory responses during flight (compare Fig. 7 with Figs. 5 and 6). In G electrical stimulation elicited an EPSP with mostly 2 spikes in the quiescent animal. During flight some flight-evoked excitation remained after destruction of the auditory organ (see Fig. 4). As with auditory stimulation, this excitation enhanced the electrically-evoked response by 22% from 2.3 AP/Stim in the resting animal to 2.8 AP/Stim during flight (Fig. 7E, F). When stimulus trains of 30 ms duration were used, the increase in the number of AP/Stim even reached 71%. However, the standard deviation for the mean number of AP/Stim and the latency of the response remained the same at rest and flight, indicating that the response occurred with the same regularity.

Thus the reduced response to auditory stimulation during flight in interneurons TN1, BSN1 and AN1 and the increased variability of the response in interneuron G had to be due to influences on the auditory organ only.

Discussion

The purpose of the experiments was to examine auditory information processing during flight and to analyse activity and modulations of auditory responses occurring in peripheral and central ele-

ments of the auditory system, i.e., the auditory organ with the receptors and auditory interneurons, respectively. Two phenomena in receptors and interneurons were obvious. (I) During flight auditory receptors and interneurons were activated, and (II) auditory responses were generally reduced or at least more variable. In the following section the flight activity in auditory receptors and interneurons will first be considered followed by a discussion of the reduction of the auditory response.

Flight activity in auditory receptors and interneurons

Summed nerve recordings, single receptor recordings and intracellular recordings of auditory interneurons revealed flight-induced activity in the auditory system. The activity of auditory receptors during flight could have two sources. One was the flight noise and the other was a mechanical stimulation of the auditory organ by movements and forces in the thorax.

Flight noise was not measured in the experiments and its effect on the auditory system could not be directly analysed. However, flight noise in locusts has been analysed by Haskell (1957). He distinguished the wing-beat noise in tethered flying locusts, when the wings hit the trailing legs, from the noise produced during normal level flight. The former he determined to be 67 dB SPL, the latter was much lower and was not detectable with his devices which had a threshold of 51 dB SPL. The influence of wing-beat noise and its modulatory effects on the auditory system has been analysed with two-tone experiments (Boyan 1986). In the present experiments the hindlegs of the animals had been removed. Any contact between the moving wings and the hindlegs can be excluded and therefore the noise generated by the animals must be the low amplitude noise generated in normal level flight. Nevertheless whether this is a significant factor contributing to the flight-induced activity in the auditory organ and its receptors remains unclear.

By contrast, mechanical activation of the tympanal organ obviously evoked activity in the auditory receptors and reduced the auditory response. This finding is supported by the results that the receptors are also activated by ventilatory movements and by passive movements of the hindlegs (Hedwig 1988; Hedwig et al. 1988). A major component in auditory receptor stimulation are relative movements between Müller's organ and the tympanum (Michelsen 1971b; Stephen and Bennet-Clark 1982; Breckow and Sippel 1985). Usually these movements are caused by sound stimuli. However, when the tympanal membrane and Müller's organ were driven by mechanically applied vibrations they also could be set into oscillations at frequencies much lower than 100 Hz (Michelsen 1973). These are the prerequisites that low frequency vibrations generated in the moving thorax may cause receptor stimulation. The amplitude of the low frequency vibrations caused by motor stimulation was probably much lower than during actual flight because flight motor activity could not be simulated completely. Thus the mechanical influence on the auditory organ may be even stronger during the behavior.

In interneurons TN1, BSN1, G and AN1 flight-evoked activity occurred in intact animals but it was considerably decreased and only weak excitation remained when the auditory organ was destroyed. Thus flight-evoked activity in the interneurons was caused by activity of the auditory receptors. The remaining excitation in the interneurons TN1 and G may well be due to input from other sense organs. TN1 and G are excited by the posterior chordotonal organ of the hindwing joint (Pearson and Hedwig, unpublished). There is no evidence for excitation or inhibition to these auditory interneurons mediated directly by the central flight pattern generator. This is consistent with the results Boyan (1986) obtained in a deafferented locust flight preparation.

Auditory response reduction in receptors and interneurons

The summed nerve response to auditory stimuli was strongly reduced during flight, a 70 dB SPL pulse gave the same response as a 50 dB SPL pulse at rest. Similar response reductions occurred in auditory receptors and in the interneurons TN1, BSN1 and AN1. The reduction in the auditory receptors may be attributed to biophysical and physiological mechanisms.

As the auditory organ responds to mechanically induced vibrations, any behaviourally induced vibrations acting on the organ must interfere with those induced by sound. It is conceivable that flight induced forces vibrate Müller's organ and change the compliance and dynamic properties of the tympanal membrane in such a way that higher sound intensities are needed to produce a certain displacement between Müller's organ and the tympanum or that the displacement to a given sound intensity is reduced (see Michelsen 1971c; Stephen and Bennet-Clark 1982; Hedwig 1988). Under these circumstances receptor activity would be determined by the interactions of both sources of

stimulation and the effectiveness of sound would be reduced as long as mechanical vibrations are influencing the auditory organ. This idea is supported by the reduction of receptor responses during ventilatory and passive leg movements which also exert a mechanical action on the auditory organ (Hedwig 1988; Hedwig et al. 1988). The detailed biophysical mechanisms in the organ which are responsible for this effect are yet unknown. They can only be analysed by highly sensitive measurements of the vibrations and forces interacting at the tympanum (Michelsen 1982).

Physiological properties of the receptors are an other important factor for the response reduction. Auditory receptors are saturated at discharge rates of 280 to 380 AP/s (Römer 1976). Flight induced discharge rates of up to 120 AP/s in the receptors. Thus during flight receptor cells are already active with one third of their saturation level. Under these circumstances their dynamic range to respond to auditory stimuli must definitely be decreased and the tonic activity will prevent a full amplitude response of the sense cell.

Two questions related to the reduction of auditory responses during flight have not yet been answered. Firstly the phenomenon may occur constantly during flight but it also could be that sensitive and insensitive periods for sound perception occur during the flight cycle. Such sensitive time windows to auditory stimuli exist in the stridulatory behavior of grasshoppers (Hedwig 1986; Meier et al. 1987), but time windows cannot be recognized on the bases of the PST histograms because these accumulate the data over the entire duration of the flight sequences. Secondly Gray (1960), Michelsen (1971a) and Römer (1976) describe four receptor groups in Müller's organ which respond to different sound frequencies. Indeed Boyan (1986) described a frequency dependent modulation of the auditory responsiveness by wind. The receptor cells recorded during flight motor activity showed a similar response pattern, but there was also one receptor which was quite different. In this study a frequency specific analysis was not attempted and white noise stimuli were used. Thus the recordings so far provide no clear answer as to whether the different receptor groups are equal with respect to how they are influenced by flight.

The auditory interneurons TN1, BSN1 and AN1 showed qualitatively similar response reductions as the auditory receptors. These reductions did not occur when the auditory organ was destroyed and the auditory stimulus was replaced by electrical stimulation of the tympanal nerve. Thus the reduction of the auditory response was caused by modulations in the auditory organ and was not due to influences on the interneurons themselves. Also due to modulations of the auditory organ in G the auditory-evoked EPSP was more variable during flight than the electrically-evoked EPSP. But further in G the mean spike response to sound and also to electrical stimulation increased and was thus different to TN1, BSN1, and AN1. The reason for this was probably that G still received a certain excitation after the tympanal organ was destroyed. This excitation summed with the auditory and electrical responses and generally increased the number of AP/Stim. A similar form of synaptic summation occurs in this neuron in the processing of vibratory and auditory signals (Čokl et al. 1977).

Behavior and auditory information processing

The reactions of locusts towards sound pulses are a sudden increase in the flight activity (Weis-Fogh 1956; Haskell 1957). For such a reaction even the impaired auditory system may be sufficient. There are no reports on phonotactic flight behavior in locusts and the results suggest this may be unlikely. The loss of auditory sensitivity and the increase in variability of the auditory response make it rather unlikely that the animals could produce a precisely oriented reaction towards a sound source. During flight the number of spikes per stimulus and the latency of the response of auditory interneurons probably vary too much for these parameters to be able to provide reliable information encoding the sound direction as suggested by Mörchen et al. (1978), Rheinlaender and Mörchen (1979) and Mörchen (1980). The auditory system has been analysed only with unilateral stimulation but it is possible that the effects of flight on the system may not necessarily be depressive when the information of both auditory organs is processed. However, recordings from G in animals with both tympanal organs intact showed the same variability of the auditory response during flight as observed in unilateral deafened animals. It is also conceivable that central influences on the auditory system which may improve information processing are not expressed at the level of thoracic ascending interneurons but occur in the signal processing of auditory brain neurons. The specificity with which these neurons respond to the time pattern of the species specific song indicates that complex processing mechanisms exist at this level (Schildberger 1984; Römer and Seikowski 1985).

A coupling between flight movements and auditory receptor activity also implies that the problems studied here are not limited to locust flight but will apply to any motor performance of these animals. Presumably evolutionary demands have favoured the development of auditory receptors which are highly sensitive to external stimuli but this creates the problem that they will also respond to the animal's own behavior. Thus an important question is what kind of strategies may be used to deal with this problem, particularly when there is no efferent control of the sense organ and peripheral modulations are not compensated by central neuronal mechanisms as has been demonstrated in this study. One solution may be to adapt the behavior to the sensitivity of the sense organ and to stop movements, whenever precise auditory reactions are demanded. Examples for this may be the antiphonal stridulation of acridid grasshoppers like *Chorthippus* (Faber 1933; Weih 1951), where the male performs pattern recognition and phonotaxis after it has listened motionless to the female's song (Rheinlaender 1984). Also the phonotactic walking pattern of some crickets shows movement pauses during which the animals localize the sound source (Murphey and Zaretsky 1972; Bailey and Thompson 1977). A similar strategy may also occur in mammals, where chewing behavior or breathing are at least stopped for a short time, so as not to impair auditory information processing.

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References

Adam L-J (1977a) The oscillating summed action potential of an insect's auditory nerve (*Locusta migratoria*, Acrididae), I. Its original form and time constancy. Biol Cybern 26:241–247

Adam L-J (1977b) The oscillating summed action potential of an insect's auditory nerve (*Locusta migratoria*, Acrididae). II. Underlying spike pattern and causes of spike synchronisation. Biol Cybern 28:109–119

Autrum H, Schwartzkopff J, Swoboda H (1961) Der Einfluß der Schallrichtung auf die Tympanal-Potentiale von *Locusta migratoria* L. Biol Zentralbl 80:385–402

Bailey WJ, Thompson P (1977) Acoustic orientation in the cricket *Teleogryllus oceanicus* (Le Guillou). J Exp Biol 67:61–75

Boyan GS (1986) Modulation of auditory responsiveness in the locust. J Comp Physiol A 158:813–825

Breckow J, Sippel M (1985) Mechanics of the transduction of sound in the tympanal organ of adults and larvae of locusts. J Comp Physiol A 157:619–629

Čokl A, Kalmring K, Wittig H (1977) The responses of auditory ventral-cord neurons of *Locusta migratoria* to vibrational stimuli. J Comp Physiol 120:161–172

Faber A (1933) Die Lautäußerungen der Orthopteren II. Z Morphol Ökol Tiere 26:1–93

Gray EG (1960) The fine structure of the insect ear. Philos Trans R Soc B 243:75–94

Haskell PT (1957) The influence of flight noise on behaviour in the desert locust *Schistocerca gregaria* (Forsk.). J Insect Physiol 1:52–75

Hedwig B (1986) On the role in stridulation of plurisegmental interneurons of the acridid grasshopper *Omocestus viridulus* L. II. Anatomy and physiology of ascending and T-shaped interneurons. J Comp Physiol A 158:429–444

Hedwig B (1988) Activation and modulation of auditory receptors in *Locusta migratoria* by respiratory movements. J Comp Physiol A 162:237–246

Hedwig B, Lang F, Elsner N (1988) The interference of sound and movement stimuli in tympanal receptors of *Locusta migratoria*. J Comp Physiol A 163:243–252

Kalmring K (1975a) The afferent auditory pathway in the ventral cord of *Locusta migratoria* (Acrididae), I. Synaptic connectivity and information processing among the auditory neurons of the ventral cord. J Comp Physiol 104:103–141

Kalmring K (1975) The afferent auditory pathway in the ventral cord of *Locusta migratoria* (Acrididae), II. Responses of the auditory ventral cord neurons to natural sounds. J Comp Physiol 104:143–159

Kalmring K, Kühne R, Moysich F (1978a) The auditory pathways in the ventral cord of the migratory locust (*Locusta migratoria*): Response transmission in the axons. J Comp Physiol 126:25–33

Kalmring K, Kühne R, Moysich F (1978b) The coding of sound signals in the ventral-cord auditory system of the migratory locust, *Locusta migratoria*. J Comp Physiol 128:213–226

Kalmring K, Rheinlaender J, Rehbein H (1972) Akustische Neuronen im Bauchmark der Wanderheuschrecke *Locusta migratoria*. Z Vergl Physiol 76:314–332

Marquart V (1985a) Auditorische Interneurone im thorakalen Nervensystem von Heuschrecken: Morphologie, Physiologie und synaptische Verbindungen. Dissertation, Universität Bochum

Marquart V (1985b) Local interneurons mediating excitation and inhibition onto ascending neurons in the auditory pathway of grasshoppers. Naturwissenschaften 72:42–45

Meier T, Wolf H, Helversen O von (1987) 'Auditory' window for responsiveness during stridulation of grasshoppers recorded from one of their auditory interneurons. In: Elsner N, Creutzfeld O (eds) New frontiers in brain research. Proc 15th Göttingen Neurobiol Conf. Thieme, Stuttgart New York, p 85

Michelsen A (1971a) The physiology of the locust ear. I. Frequency sensitivity of single cells in the isolated ear. J Comp Physiol 71:49–62

Michelsen A (1971b) The physiology of the locust ear, II. Frequency discrimination based upon resonances in the tympanum. J Comp Physiol 71:63–101

Michelsen A (1971c) The physiology of the locust ear, III. Acoustical properties of the intact ear. J Comp Physiol 71:102–128

Michelsen A (1973) The mechanics of the locust ear: An invertebrate frequency analyser. In: Moller A (ed) Basic mechanisms in hearing. Academic Press, New York London, pp 911–934

Michelsen A (1982) Laser techniques in studies of hearing. In: Sattelle DB, Lee WI, Ware BR (eds) Biomedical applica-

tions of laser light scattering. Elsevier Biomedical Press, Amsterdam Oxford New York, pp 357–370
Michelsen A, Larsen ON (1978) Biophysics of the ensiferan ear I. Tympanal vibrations in bushcrickets (Tettigoniidae) studied with laser vibrometry. J Comp Physiol 123:193–203
Mörchen A (1980) Spike count and response latency. Two basic parameters encoding sound direction in the CNS of insects. Naturwissenschaften 67:469–470
Mörchen A, Rheinlaender J, Schwartzkopff J (1978) Latency shift in insect auditory nerve fibres. Naturwissenschaften 65:656–657
Murphey RK, Zaretsky MD (1972) Orientation to calling song by female crickets, *Scapsipedus marginatus* (Gryllidae). J Exp Biol 56:335–352
Pearson KG, Robertson RM (1987) Structure predicts synaptic function of two classes of interneurons in the thoracic ganglia of *Locusta migratoria*. Cell Tissue Res 250:105–114
Rehbein H (1976) Auditory neurons in the ventral cord of locusts: morphological and functional properties. J Comp Physiol 110:233–250
Rehbein H, Kalmring K, Römer H (1974) Structure and function of acoustic neurons in the thoracic ventral nerve cord of *Locusta migratoria* (Acrididae). J Comp Physiol 95:263–280
Rheinlaender J (1984) Das akustische Orientierungsverhalten von Heuschrecken, Grillen und Fröschen: Eine vergleichende neuro- und verhaltensphysiologische Untersuchung. Habilitationsschrift, Universität Bochum
Rheinlaender J, Mörchen A (1979) 'Time-intensity trading' in locust auditory interneurons. Nature 281:672–674
Robertson RM, Pearson KG (1983) Interneurons in the flight system of the locust: distribution, connections, and resetting properties. J Comp Neurol 215:33–50
Ronacher B, Römer H (1985) Spike synchronisation of tympanic receptor fibres in a grasshopper (*Chorthippus biguttulus* L., Acrididae). J Comp Physiol A 157:631–642
Römer H (1976) Die Informationsverarbeitung tympanaler Rezeptorelemente von *Locusta migratoria* (Acrididae, Orthoptera). J Comp Physiol 109:101–122
Römer H (1985) Anatomical representation of frequency and intensity in the auditory system of Orthoptera. In: Kalmring K, Elsner N (eds) Acoustic and vibrational communication in insects. Parey, Hamburg, pp 25–32
Römer H, Dronse R (1982) Synaptic mechanisms of monaural and binaural processing in the locust. J Insect Physiol 28:365–370
Römer H, Marquart V (1984) Morphology and physiology of auditory interneurons in the metathoracic ganglion of the locust. J Comp Physiol A 155:249–262
Römer H, Rheinlaender J (1983) Electrical stimulation of the tympanal nerve as a tool for analysing the responses of auditory interneurons in the locust. J Comp Physiol 152:289–296
Römer H, Rheinlaender J, Dronse R (1981) Intracellular studies on auditory processing in the metathoracic ganglion of the locust. J Comp Physiol 144:305–312
Römer H, Seikowski U (1985) Responses to model songs of auditory neurons in the thoracic ganglia and brain of the locust. J Comp Physiol A 156:845–860
Schildberger K (1984) Temporal selectivity of identified auditory neurons in the cricket brain. J Comp Physiol A 155:171–185
Schildberger K, Milde JJ, Hörner M (1987) Effects of walking on auditory receptors and interneurons in crickets. In: Elsner N, Creutzfeldt O (eds) New frontiers in brain research. Proc 15th Göttingen Neurobiol Conf. Thieme, Stuttgart New York, p 82
Sippel M, Breckow J (1984) Non-monotonic response-intensity characteristics of auditory receptor cells in *Locusta migratoria*. J Comp Physiol A 155:633–638
Stephen RO, Bennet-Clark HC (1982) The anatomical and mechanical basis of stimulation and frequency analysis in the locust ear. J Exp Biol 99:279–314
Stout JF, Huber F (1972) Response of central auditory neurons of female crickets (*Gryllus campestris* L.) to calling song of the male. J Comp Physiol 76:302–313
Weih AS (1951) Untersuchungen über das Wechselsingen (Anaphonie) und über das angeborene Lautschema einiger Feldheuschrecken. Z Tierpsychol 8:1–41
Weis-Fogh T (1956) Biology and physics of locust flight. II. Flight performance of the desert locust (*Schistocerca gregaria*). Philos Trans R Soc Lond B 239:459–510
Wolf H, Helversen O von (1986) 'Switching-off' an auditory interneuron during stridulation in the acridid grasshopper *Chorthippus biguttulus* L. J Comp Physiol A 158:861–871
Wolf H, Pearson KG (1987) Intracellular recordings from interneurons and motoneurons in intact flying locusts. J Neurosci Meth 21:345–354
Yinon U, Shulov A, Tsvilich R (1971) Audition in the desert locust: behavioural and neurophysiological studies. J Exp Biol 55:713–725