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AUDITORY INTERNEURONES IN THE METATHORACIC GANGLION OF THE GRASSHOPPER *CHORTHIPPUS BIGUTTULUS*

II. PROCESSING OF TEMPORAL PATTERNS OF THE SONG OF THE MALE

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Summary

1. Auditory interneurones originating in the metathoracic ganglion of females of the grasshopper *Chorthippus biguttulus* were investigated with respect to their processing of models of the male's song. In these models two temporal configurations were varied: (i) the song pattern, consisting of 'syllables' and 'pauses', and (ii) the duration of short gaps within syllables.

2. A precise encoding of the song pattern is found only among receptors and 'thoracic' neurones (SN1, TN1), but not among ascending neurones. The only ascending neurone that reacts tonically at all intensities (AN6) encodes the song pattern much less precisely than do receptors. Other ascending neurones (AN3, AN11) encode the gross pattern of model songs, but only at low intensities.

3. One spontaneously active 'local' neurone (SN6) is tonically inhibited and encodes the pauses of a model song. A similar response, however, is not found in three ascending neurones (AN13, AN14, AN15), which are merely inhibited.

4. Among ascending neurones, AN12 is the most reliably influenced by the syllable–pause structure of the songs. Its phasic burst marks the onset of every syllable in a behaviourally attractive song. Its activity could account for the rising part of the corresponding behavioural response curve. However, no ascending neurone shows activity corresponding to the falling part of the behavioural response. Among local neurones, the phasic BSN1 neurones are most clearly influenced by varying syllable–pause combinations.

5. Gaps within the song syllables cause a complete inhibition of the activity of AN4. The response of AN4 to syllables with and without gaps is strikingly similar to the behavioural response and is maintained over the whole intensity range tested. Several local neurones, especially SN6, are strongly influenced by gaps within model songs – though only in certain intensity ranges.

6. In accordance with behavioural results, the pathways for information on

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song pattern and sound direction appear to be separated among ascending neurones. Among local interneurones, however, this separation does not appear to take place, since the most directional local neurone, BSN1, might also be suited for pattern-filtering tasks.

Introduction

Acoustic communication guides the meeting of sexual partners and prevents a hybridization between related species in many acridid grasshoppers (Perdeck, 1975; D. von Helversen and O. von Helversen, 1975, 1983, 1987). Prerequisites for this communication are species-specific song patterns, a recognition system for these signals, and the ability to localize a sound source. Since tympanic receptors show no tuning for species-specific song patterns (Ronacher and Römer, 1985), these songs are obviously detected by neuronal filtering networks within the central nervous system (CNS). This pattern recognition (as well as the pattern production) is an innate capability and represents a typical example of the ethological concept of an 'innate releasing mechanism' (IRM, Lorenz, 1943; Tinbergen, 1953).

The IRM of female *Chorthippus biguttulus* for the male's song has been the subject of many investigations. By observing the behavioural response of *C. biguttulus* females to model songs, it was possible to measure many 'filter characteristics' of this IRM and to ascertain which parameters of the male song are 'sign stimuli' for this IRM (D. von Helversen, 1972; O. von Helversen, 1979; D. von Helversen and O. von Helversen, 1983). Furthermore, the results of behavioural tests with a split song paradigm (D. von Helversen, 1984; D. von Helversen and O. von Helversen, 1990) together with results after microsurgical ablations (Ronacher *et al.* 1986) and selective heating of parts of the CNS (Bauer and von Helversen, 1987) led to a concrete concept of the routes and levels of information flow within the CNS (for a review see D. von Helversen and O. von Helversen, 1987, 1990). The first level of auditory processing is located within the metathoracic ganglion. Here information is divided among *parallel pathways*. (i) The interpretation of the *song pattern* requires a summation of the inputs from the left and the right ears within the metathoracic ganglion (D. von Helversen, 1984). The decisive steps of pattern recognition, however, are apparently made within the brain (Ronacher *et al.* 1986; Bauer and von Helversen, 1987; D. von Helversen and O. von Helversen, 1990). (ii) For the analysis of *sound direction*, a substantial amplification of left–right differences by mutual inhibition occurs in the metathoracic ganglion. This enhances the directional information, which is then converted within the head ganglia into an unequivocal decision to turn left or right.

We have investigated how these steps of processing, inferred from behavioural results, are realized in the metathoracic auditory interneurones, and what specific information is provided by the signals of interneurones ascending from the metathoracic ganglion to the brain.

The morphological and physiological properties of the metathoracic auditory interneurones of *C. biguttulus* have been described in the preceding article (Stumpner and Ronacher, 1991). These interneurones are assumed to be homologous with those of the locust (*Locusta migratoria*). A large amount of information on the properties of the locust's auditory interneurones has been accumulated (for processing of model songs, see Adam, 1969; Kalmring, 1975; Boyan and Altman, 1985; Römer and Seikowski, 1985). However, no results have been published that demonstrate acoustically guided *intraspecific* behaviour in the locust. A recent report describes negative phonotaxis to ultrasound during flight, which is interpreted as predator avoidance (Robert, 1989). For the locust, no correlations between neuronal responses and acoustic communication behaviour have been published.

This paper focuses on a direct comparison between neuronal activity and the song-recognizing behaviour of *C. biguttulus*. The metathoracic auditory interneurones are investigated with respect to their possible roles as analyzers of species-specific song parameters and as channels for the transmission of information about the song to the head ganglia. We tested the neurones using models of the conspecific song, and varied the following parameters, which turned out to be critical in behavioural tests. (i) A model song is attractive to a female if it consists of several (more than 10) syllables (white noise) separated by pauses of a certain duration (D. von Helversen, 1972; terminology after D. von Helversen and O. von Helversen, 1983). If, for example, the pauses between the syllables are either too long or too short, the model songs are ineffective (Fig. 1A). The effectiveness of pause durations depends upon syllable duration, sound intensity and temperature; longer syllables and those at lower intensities and at lower temperatures require longer pauses (D. von Helversen, 1972; O. von Helversen, 1979). (ii) At room temperature, the attractiveness of a model song is drastically reduced if gaps of

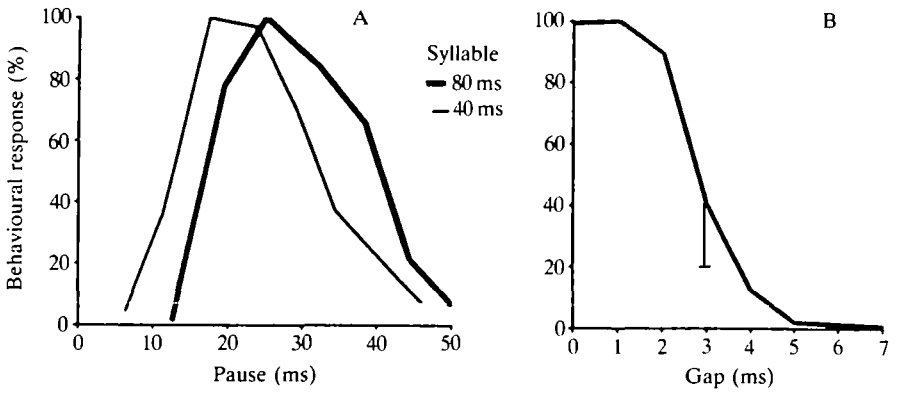

Fig. 1. Behavioural responses of *Chorthippus biguttulus* females to model songs with varying pause durations between syllables (A) or with gaps of varying durations within syllables (B). Sound intensity 72 dB SPL. Temperature during experiments 23–25 °C. Data from one female (N=130 per point) in A; from 3 females (N=160) in B, largest standard deviation indicated by vertical bar.

more than 2–3 ms are inserted in the syllables (Fig. 1B, see also Ronacher and Stumpner, 1988); at higher temperatures and at high intensities even 1–1.5 ms gaps are detected (D. von Helversen, 1972; O. von Helversen, 1979; Ronacher and Römer, 1985).

Materials and methods

Experiments were performed with adult females of *Chorthippus biguttulus* L. (Acrididae: Gomphocerinae), caught in the field in southern Germany.

The electrophysiological methods were conventional, and they and the stimulation apparatus have been described in the preceding article (Stumpner and Ronacher, 1991). The loudspeakers were mounted 35 cm from the animal at right angles to its longitudinal axis. The stimuli were presented on the side of the lower excitatory threshold of the respective neurone. A standard stimulation programme for the general characterization of every cell started with shortened models of male songs. These models consisted of six syllables of rectangularly modulated white noise (WN, 2.5–40 kHz; Fig. 2A). Longer versions of the same models were used in the behavioural experiments (O. von Helversen, 1979).

For more detailed tests the following stimulation programmes were used. (i) Variation of syllable intervals: six syllables of constant durations (40, 85 or 110 ms) were separated by pauses which varied from 3.2 to 42.5 ms; each stimulus type was repeated eight times; the sequence of pause durations was 33.0, 16.3, 24.0, 8.8, 42.5 and 3.2 ms. (ii) Insertion of short gaps into syllables: two syllables, separated by a pause of 25 ms, formed a model. Each syllable was composed of 11 WN 'pulses', 5 or 7.5 ms in duration, with interspersed gaps, the gaps varying between 0.8 and 6.7 ms. Each stimulus type was repeated eight times; the sequence of gap durations was 5.2, 2.7, 3.9, 1.6, 6.7 and 0.8 ms. The stimulus without gaps was presented at each change of stimulus type (i.e. change of gap duration) to serve as a control for the stability of the neuronal recording during the test series.

Data evaluation

The results are presented as post-stimulus-time histograms (PSTH, bin-width 2 or 6 ms) or as diagrams showing the spike numbers of single cells. In the latter case the action potentials were either counted during the whole stimulus time or only in certain windows, depending on the syllable structure of the stimulus (e.g. for gap variation, 100 ms from the onset of each syllable, starting 1 ms before the shortest latency of the respective neurone). This procedure was intended to demonstrate those effects that might be obscured by the different durations of stimulus types, which are caused by different durations of pauses between or within syllables.

Results

Information about a time pattern can be transmitted by a single spiking neuron

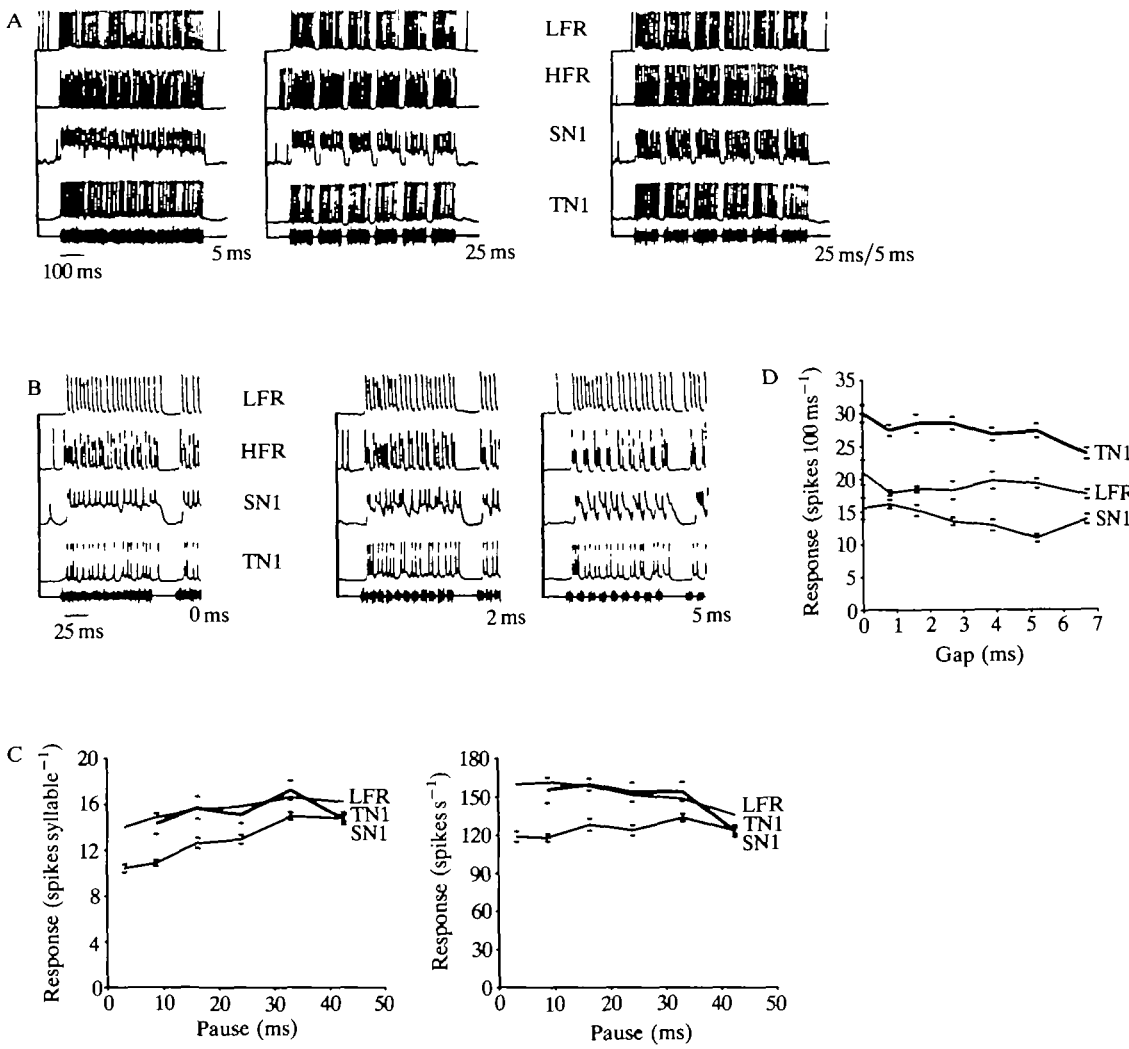

Fig. 2. Tonically active thoracic neurones. LFR and HRF are low- and high-frequency receptors, respectively, SN1 is a segmental neurone and TN1 is a T-shaped neurone (see Stumpner and Ronacher, 1991). (A,B) Sample recordings with various song patterns (at 72 dB SPL). In this figure and similar ones, the stimulus type is shown in the lowest trace, and the numbers on the lower right of each stimulus give the respective pause durations. (A) On the left, 5 ms pauses between 100 ms syllables; in the middle, 100 ms syllables and 25 ms pauses; on the right, 100 ms syllables composed of 7.5 ms pulses and 5 ms pauses, again with a 25 ms pause between syllables. (B) The first syllable of a stimulus without gaps (left) or with 2 ms (middle) or 5 ms gaps (right) is shown on a larger time scale. (C,D) Corresponding spike numbers (spikes per syllable or spikes per time period) for variations in pause duration (C) or gap duration (D) at 72 dB; N=8 for each value; standard deviations for the low-frequency receptor in C were omitted for reasons of clarity. The cells in C and D are different.

in two different ways: (i) by '*encoding*' – i.e. the temporal pattern of a stimulus is reflected in the spike frequency modulation, or (ii) by '*weighting*' – i.e. certain changes of parameter values elicit a change in response while others are disregarded. Of course, these two possibilities are not mutually exclusive; they represent the extremes of a continuum. Neurones that emphasize certain aspects of a stimulus could, nevertheless, encode the gross stimulus pattern (but frequently they do not, see Schildberger, 1984; Rose and Capranica, 1984; Feng *et al.* 1990).

Encoding of the song pattern

Both low-frequency receptors and high-frequency receptors respond tonically to acoustic stimuli and can be classified as neurones that encode the song pattern (Fig. 2A). Stimuli with increasing pause durations cause a fairly constant number of spikes, whether spikes per syllable (left diagram in Fig. 2C) or spikes during a certain period (Fig. 2C, right diagram) are counted. Similar responses can be found in two neurones confined to the thoracic (and adjacent abdominal) ganglia, SN1 and TN1 (a local neurone and a T-fibre respectively, see Stumpner and Ronacher, 1991). The lower limit for encoding the pause duration in a spike train depends on the interspike interval, which ranges from 3 to 5 ms, or in rare cases down to 2 ms (especially in high-frequency receptors at high intensities). Therefore, pauses of less than about 3 ms can hardly be encoded by these neurones (Fig. 2B, middle).

The number of spikes in these tonically responding neurones is hardly affected by the presence or absence of gaps in the syllables (Fig. 2D). Nevertheless, gaps larger than the respective interspike interval (see above) may have a marked effect on the *temporal* distribution of spikes. This is most clearly evident in the spike trains of high-frequency receptors, but also in SN1 (Fig. 2B, right). A more precise triggering of action potentials by every noise pulse takes place when the gap exceeds 3–5 ms. In high-frequency receptors, this critical gap width may be even lower, but it should be mentioned that these receptors have a threshold for white-noise stimuli of around 60–70 dB (Stumpner and Ronacher, 1991) and, therefore, can contribute to the filtering network only in the upper intensity range of the behavioural response (O. von Helversen, 1979; Stumpner and Ronacher, 1991).

Several ascending interneurones show tonic responses to white-noise stimuli (Fig. 3). Only one of these, AN6, shows tonic responses over the entire behaviourally relevant intensity range (Stumpner and Ronacher, 1991). The syllables and pauses, however, are not precisely represented in the spike trains, as can be seen in original recordings and a PSTH in Fig. 3A. Again, the number of spikes per syllable is almost independent of pause duration (Fig. 3B).

The tonic responses of AN3 and AN11 are quite variable and occur only up to 20 dB above threshold (Fig. 3C). At medium intensities (i.e. around 70 dB SPL), AN11 nearly ceases to spike while the spontaneously active AN3 shows weak phasic responses at the onset of each syllable (each preceded by an IPSP, Fig. 3C).

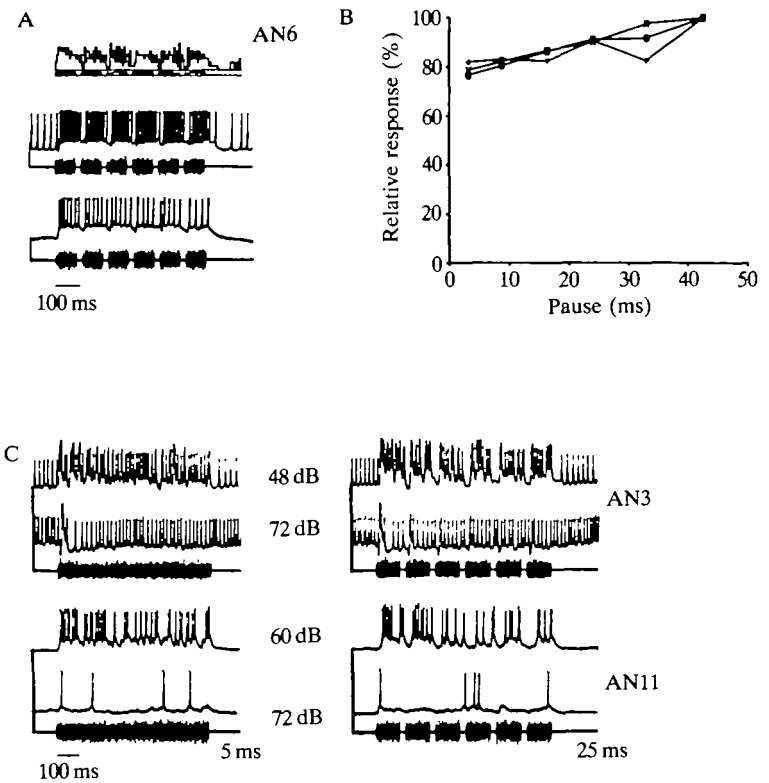

Fig. 3. Tonically active ascending neurones. (A) Sample recordings of two AN6 neurones and, above, a post-stimulus-time histogram (PSTH) (N=8, bin width 6 ms, maximum of 2 spikes bin^{-1}) for the upper of the two traces. (B) The relative spike counts of three different AN6 cells in response to model songs with different pause durations (72 dB, 6–9.3 spikes per syllable maximum). (C) Sample recordings from AN3 and AN11 for two models with 5 and 25 ms pauses at different intensities (note the strong intensity dependence).

The responses of neurones that are inhibited by acoustic stimuli might encode the pauses between syllables. A neurone that is tonically inhibited, SN6, does give a kind of 'inverted representation' of the stimulus pattern, since its spontaneous activity is suppressed during acoustic stimuli (Fig. 4A). The number of spikes produced during a model song is a monotonous function of the duration of the pauses between syllables (Fig. 4B), since longer pauses allow more spontaneous action potentials or more action potentials in a rebound burst to occur. This relationship is hardly affected by changing the duration of syllables. Therefore, SN6 may be a candidate for transmitting information about pause durations onto ascending neurones.

However, none of three ascending neurones that are inhibited by acoustic stimuli (AN13, AN14, AN15) receives tonic inhibition or spikes only during the pauses between syllables (Fig. 4C). In all three neurone types the interindividual

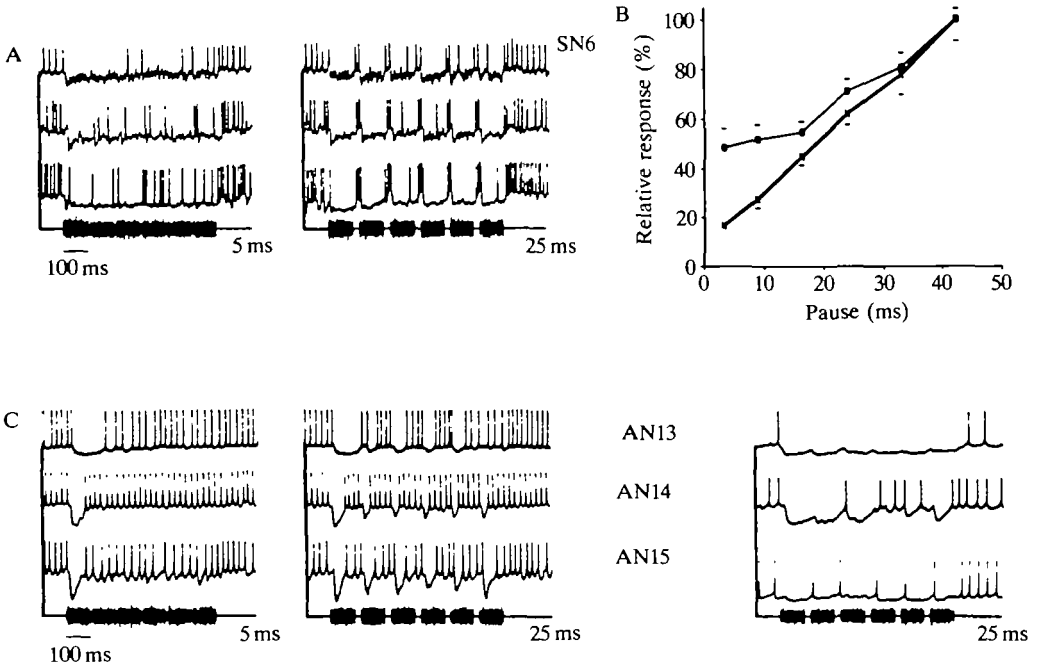

Fig. 4. Inhibited local (A,B) and ascending neurones (C). (A) Sample recordings of three different SN6 cells; (B) the relative activity and standard deviations of two cells for varied pauses between syllables (85 ms) at 72 dB (spikes are counted during the whole stimulus consisting of six syllables, the maximum spike numbers are 18.5 and 30.6; 72 dB). (C) Recordings from three ascending neurones. On the left and in the middle, the responses of a representative of each neurone type with high spontaneous activity are shown to model songs with 5 and 25 ms pauses, respectively. The response of a representative of the same neurone type with low spontaneous activity, on the right, demonstrates the interindividual variability.

variability is high, and the correlation between their spiking pattern and the stimulus type is usually weak. Evidently, the variability in spontaneous firing rates of the inhibited cells sets limits to how precisely the pauses can be encoded (Fig. 4C).

In short, some neurones confined to the thoracic ganglia encode at least the gross pattern of a model song. Among ascending neurones, however, there is no *precise* encoding of song patterns (it has to be emphasized that the ascending axon of TN1 does not reach the brain). Thus, for transmission of information to the head ganglia (see Introduction), neurones that *filter* certain features of a song appear to be more important.

Detection of the onset of syllables in model songs

AN12 is a neurone with a phasic reaction, which is maintained over a broad intensity range (Stumpner and Ronacher, 1991). The phasic burst in AN12 occurs reliably at the onset of syllables, provided that the pauses between syllables exceed

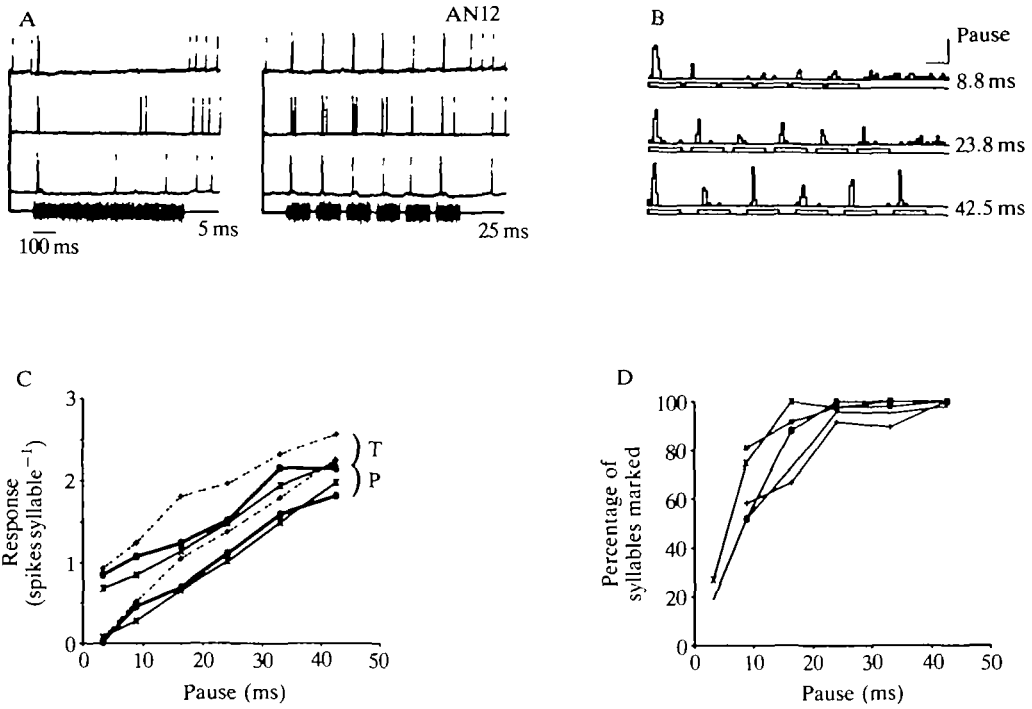

Fig. 5. Phasic activity in AN12. (A) Sample recordings from three different AN12 cells at 72 dB. The right-hand model song would be behaviourally effective, but not the left-hand one. (B) PSTHs and corresponding stimulus patterns with syllables of 85 ms at 72 dB; the stimulus in the middle would be attractive to females. (C) The respective spike counts of all spikes during a syllable (T=total) or of spikes in a 20 ms window after the onset of each syllable (P=phasic) shown with syllable durations of 40 ms (×), 85 ms (●, thick curve) and 110 ms (+, dashed curve). (In the latter case the reaction to the first syllable was omitted for reasons of clarity. This can be done since the start of any model song evokes a burst in AN12, irrespective of the subsequent time pattern.) (D) The percentage of syllables (in six-syllable models, 85 ms syllable duration, 72 dB) that elict a phasic burst in AN12 (25 ms window). Five different cells, $N=8$ in all cases; bars in B, 50 ms and 2 spikes per 6-ms bin.

a certain length (Fig. 5A,B). The number of spikes per syllable increases linearly with increasing pause duration. This can be attributed entirely to the phasic component: the upper three curves (T in Fig. 5C) show the number of spikes counted during the whole syllable, while the lower three curves (P in Fig. 5C) show the spikes counted only in a 'window of phasic response'. The response curves of different AN12 cells for variations in pause length are similar (Fig. 5A,D). In a test series with a constant syllable duration of 85 ms and increasing pause duration (see Fig. 5B), the first stimulus type in which every beginning of a syllable causes at least one spike is a model with a pause between 16 and 24 ms (Fig. 5D). However, as can be seen from Fig. 5C, the number of spikes continues to increase with longer pauses, in contrast to the peak behavioural

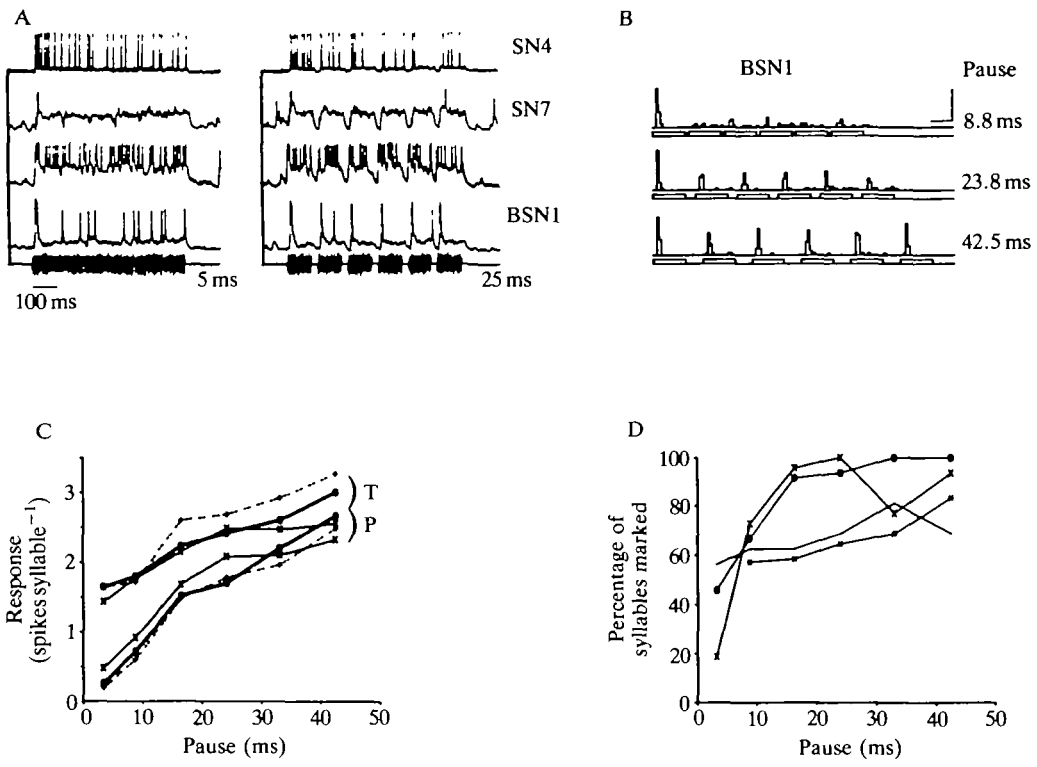

Fig. 6. Representation of the syllable–pause structure in the activity of local neurones. (A) Sample recordings from SN4, SN7 and two differently responding BSN1 cells. (B,C) The responses of a phasically active BSN1; (D) the percentage of syllables in a six-syllable model song eliciting a phasic burst in four differently responding BSN1 cells; 72 dB; other details as in Fig. 5.

response (Fig. 1A). Additionally, the reaction of AN12 does not depend clearly upon syllable duration, at least not in the range of behaviourally effective syllables (Fig. 5C; cf. Fig. 1A).

Among local cells, BSN1 neurones show response curves similar to that of AN12 (compare Figs 5 and 6). BSN1 cells respond phasically with a variable tonic component (Fig. 6A). They are clearly influenced by sound direction and sound intensity (Stumpner and Ronacher, 1991). In BSN1 neurones with predominantly phasic reactions (20 of 36 recorded cells), the phasic component mainly determines the observed increase in spikes per stimulus (cf. Fig. 6C, T and P); different syllable durations (40, 85 and 110 ms in Fig. 6C) do not change either the slope or the threshold of the response curves. The spiking patterns of BSN1 neurones in response to model songs with varying pause durations, like the response curves, differ much more between individuals than in AN12 (compare Figs 5D and 6D).

The spiking of SN4 and SN7 may also be phasic (Fig. 6A), but strongly depends on sound intensity. The response depends on the duration of pauses in model

songs – if at all – at certain intensities only. A response somewhat similar to that of AN12 is exhibited by another ascending neurone, AN4. Owing to its phasic-tonic activity (see Fig. 7A), the number of spikes increases with increasing pause duration. However, its spiking pattern is less reliable than that of AN12, and depends more on the sound intensity and the adaptational state.

Detection of gaps within syllables

Single auditory receptors are not able to encode gaps of 2–4 ms within model songs (Ronacher and Römer, 1985). Ascending neurones encode temporal patterns even less precisely than do receptors (see above). Therefore, filtering elements must be involved in gap detection.

AN4 is an excellent candidate for determining the gap-detecting ability of the IRM. Its activity is suppressed by syllables that contain gaps (Fig. 7A,C). The temporal characteristics of this response closely resemble the behavioural results (Ronacher and Stumpner, 1988). The suppression of the activity of AN4 by gaps is caused by an IPSP which precedes the excitation; this IPSP is retriggered by each sound pulse following a gap (Fig. 7B). The importance of AN4 for gap-detection is further corroborated by a test in which gaps were inserted either at the beginning

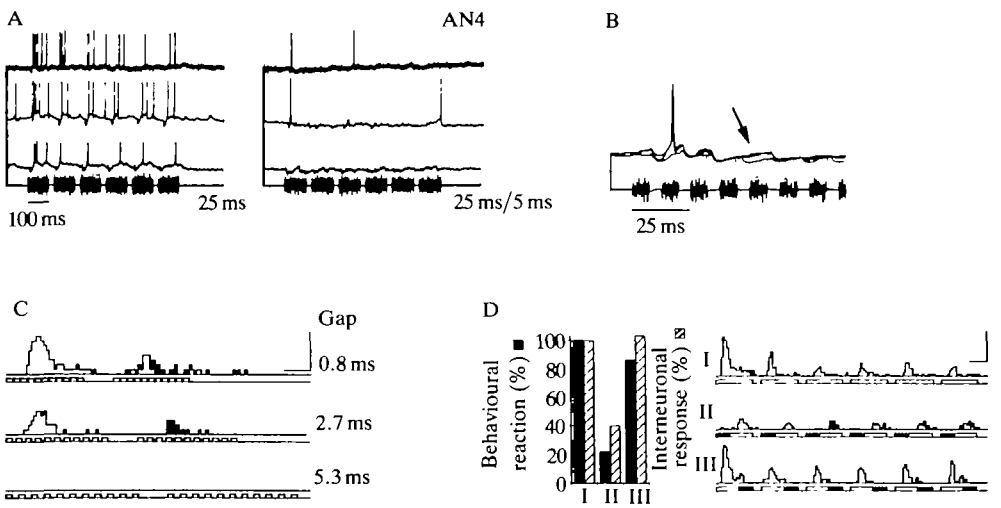

Fig. 7. The gap-detecting neurone AN4. (A) Sample recordings of the responses of three AN4 cells to model songs with gaps (right) or without gaps (left), at 72 dB. (B) The summation of IPSPs in response to a pulse train (three sweeps superimposed, pulses 7.5 ms, pauses 5.3 ms); the arrow indicates two cases in which no IPSP was evoked. (C) PSTHs and corresponding stimulus patterns in response to three model songs with increasing gap durations at 72 dB (N=8; bars, 20 ms and 1 spike per 2-ms bin). (D) A PSTH for stimuli with gaps in the first or second half of syllables (II, III) and from a control stimulus (I). On the left, the interneuronal response (hatched bars, mean of two intensities) to the same stimuli is compared with behavioural responses to corresponding stimuli (filled bars, mean of three females, tested at a higher temperature).

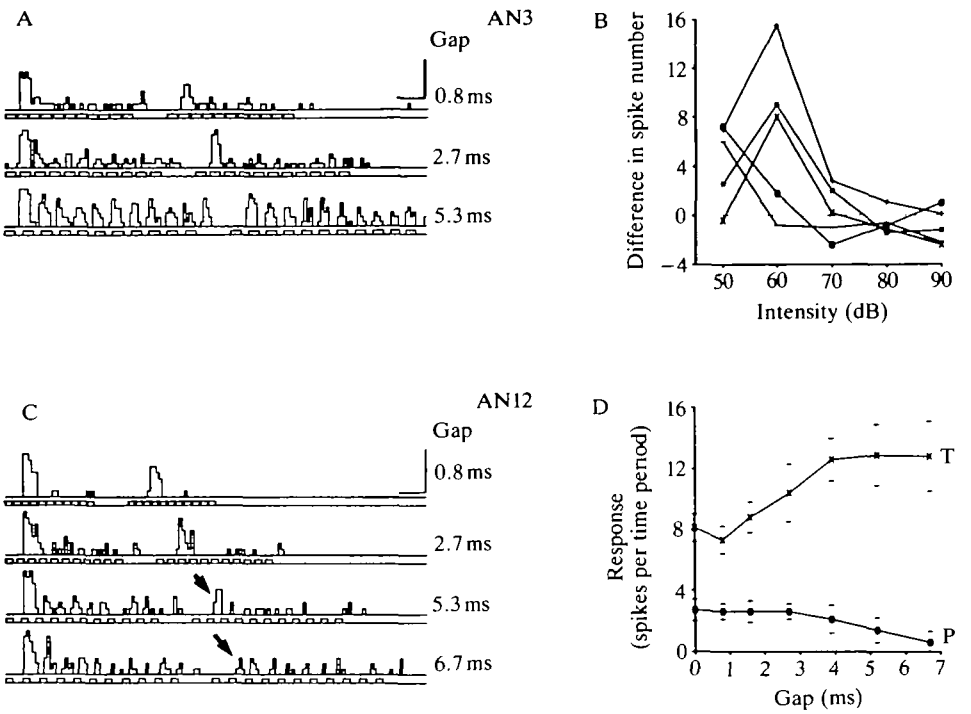

Fig. 8. Gap-detection by AN3 and AN12. (A,C) PSTHs and corresponding stimulus patterns for AN3 (7.5 ms pulses at 54 dB) and AN12 (5 ms pulses at 72 dB), respectively. The arrows in C denote the decreasing spike number in the range of the phasic response, which can also be seen in the respective spike counts in a 15 ms window (P, starting at the shortest latency) in D. The upper curve (T) in D gives the spike number in a 100 ms window after each syllable onset. (B) The differences in spike numbers in response to a pulsed and an unpulsed syllable at different intensities are shown for five AN3 cells; the zero line has been inserted. N=8 (A,C,D) or 5 (B); bars in A and C: 20 ms and 1 spike per 2-ms bin.

or at the end of syllables. Again, the response of AN4 is in accordance with the behavioural results (Fig. 7D).

The activity of AN3 may be significantly influenced by gaps, too (Fig. 8A). 22 out of 46 AN3 cells showed a stronger response to syllables containing gaps than to those not containing gaps. This is due to a repeated triggering of the phasic onset by every pulse, which begins in a similar temporal range to the behavioural response (PSTH in Fig. 8A). This response, however, was nearly always restricted to the range of more tonic discharges up to 20 dB above threshold (Fig. 8B); in no case were differences in the spike numbers found over the same intensity range as for AN4 (see Ronacher and Stumpner, 1988, and Fig. 10A).

The response of AN12 neurones is influenced by gaps within syllables in a similar way to the response of AN3. Stimuli with longer gaps evoke more spikes (Fig. 8C), although the differences are not large and the activity becomes more

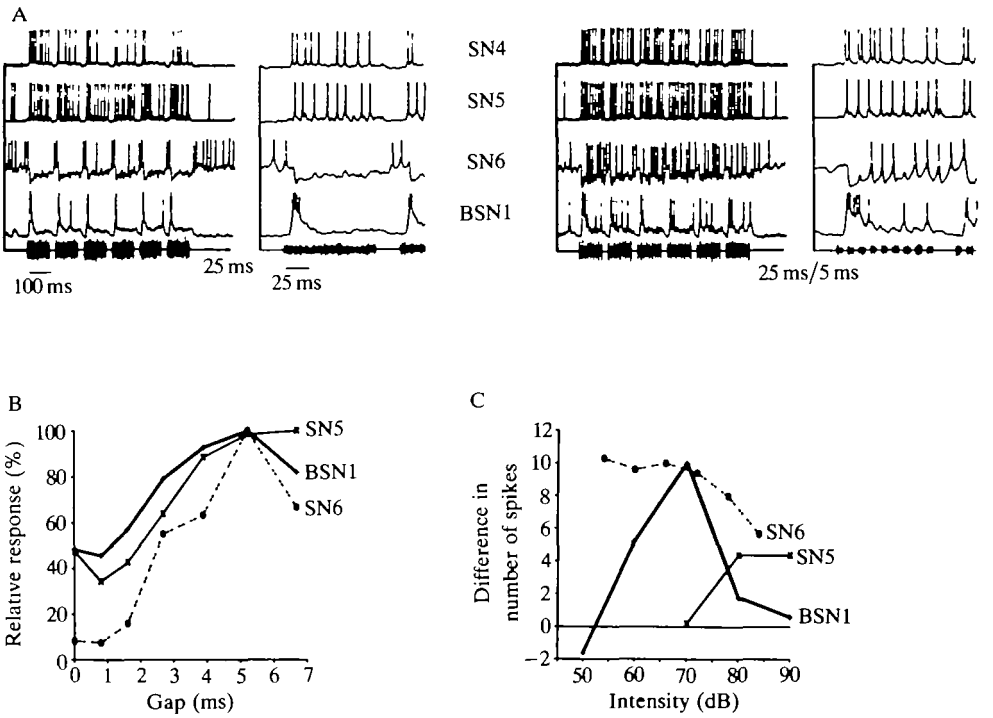

Fig. 9. Gap-detection by local neurones. (A) Sample recordings in response to a model song with gaps (right) or without gaps (left) at 72 dB. The response to the first syllable is also shown on an expanded time scale. The most marked difference is found in SN6. (B) The number of spikes evoked in response to increasing gap duration at 72 dB (SN6, BSN1) or 78 dB (SN5); maximum spike numbers are 6.1 (SN5), 6.0 (SN6) and 12.4 per 100 ms (BSN1). (C) The intensity dependence of the difference in spiking response to syllables with and without gaps for a representative SN5 cell (threshold between 60 and 70 dB), SN6 and BSN1. N=8 (B) or 5 (C).

variable with longer gap durations (see standard deviations in upper curve of Fig. 8D). An additional effect of gaps is visible in the spiking pattern: as the gaps become longer, the phasic burst at the onset of a syllable is diminished (see arrows in PSTH, Fig. 8C). This can also be seen in the decrease in the spike number in a 15 ms window – which is the range of the phasic burst (see Fig. 8D, lower curve). In other words, the regular activity of AN12 elicited by model songs is lost if larger gaps are inserted into syllables.

Several local neurones could account for the gap-dependent responses of at least some ascending neurones. The sample recordings of SN4, SN5, SN6 and BSN1 in Fig. 9A demonstrate that syllables with gaps may evoke distinctly more spikes than syllables without gaps. This is most obvious for SN6; its activity increases in a similar temporal range as does the behavioural response (Fig. 9B). In SN5 and BSN1 this response is restricted to certain intensity ranges (Fig. 9C). The activity f SN6 shown in Fig. 9C is less intensity-dependent, but interindividual variability

is great. The same is true for different BSN1 cells. Moreover, only 12 of 33 BSN1 neurones tested showed any gap-dependent response changes; twin cells were similar in this respect.

In conclusion, no single neurone described above seems to cause the specific gap-dependent inhibition of AN4. The regular shape of IPSPs in AN4, however, which sometimes appear to be cancelled totally (arrow in Fig. 7B), indicate that these IPSPs are caused by single spikes in one presynaptic neurone, which has apparently not yet been identified.

Pattern recognition and directionality

The IRM of grasshopper is not directional; in contrast, the filtering network obviously adds inputs from both ears at the thoracic level (D. von Helversen, 1984). AN6 and AN12 obviously receive nearly identical inputs from both ears (A. Stumpner, unpublished results, see also Stumpner and Ronacher, 1991). The specific gap-dependent inhibition of the spiking of AN4 is not side-dependent and not very intensity-dependent (Fig. 10A). In contrast, the most directional ascending neurones, AN1 and AN2, show quite variable and intensity-dependent spiking patterns and, therefore, are not suited to differentiate similar song patterns reliably (see Wolf, 1986). This is clearly visible in the sample recordings of Fig. 10B,C: the differences in spiking of AN2 in response to successive syllables of a model song are striking. Additionally, at medium intensities, AN1 cells and many AN2 cells are excited only slightly or not at all by contralateral stimuli (from the 'excitatory' side) (Fig. 10B,C). The inhibition by ipsilateral stimuli, however, is maintained over the whole intensity range in which phonotactic behaviour can be observed (for males, see D. von Helversen and Rheinlaender, 1988).

This separation into channels for 'orientation' and channels for 'pattern recognition' seems not to take place in the local neurones. BSN1 neurones are by far the most directional local neurones, but might simultaneously be suited for temporal filtering – although perhaps in certain intensity ranges only. Maybe the supplementary reaction of twin cells (Stumpner, 1989) or a convergence of the bilateral mirror-image partners of BSN1 onto ascending neurones compensates for these directional influences.

Discussion

This paper is mainly concerned with how thoracic auditory neurones of *C. biguttulus* process and filter the information that is necessary for the recognition of conspecific songs. We concentrate on two fundamental features of the female IRM: the recognition of the syllable–pause structure of the song and the detection of gaps within syllables (Fig. 1). The importance of gap detection for the communication system of this species has been underlined by field experiments in which females strongly preferred males with normal songs to those whose songs included gaps (Kriegbaum, 1989). Two further results are relevant for estimating the possible contribution of certain neurones (see also Introduction): th

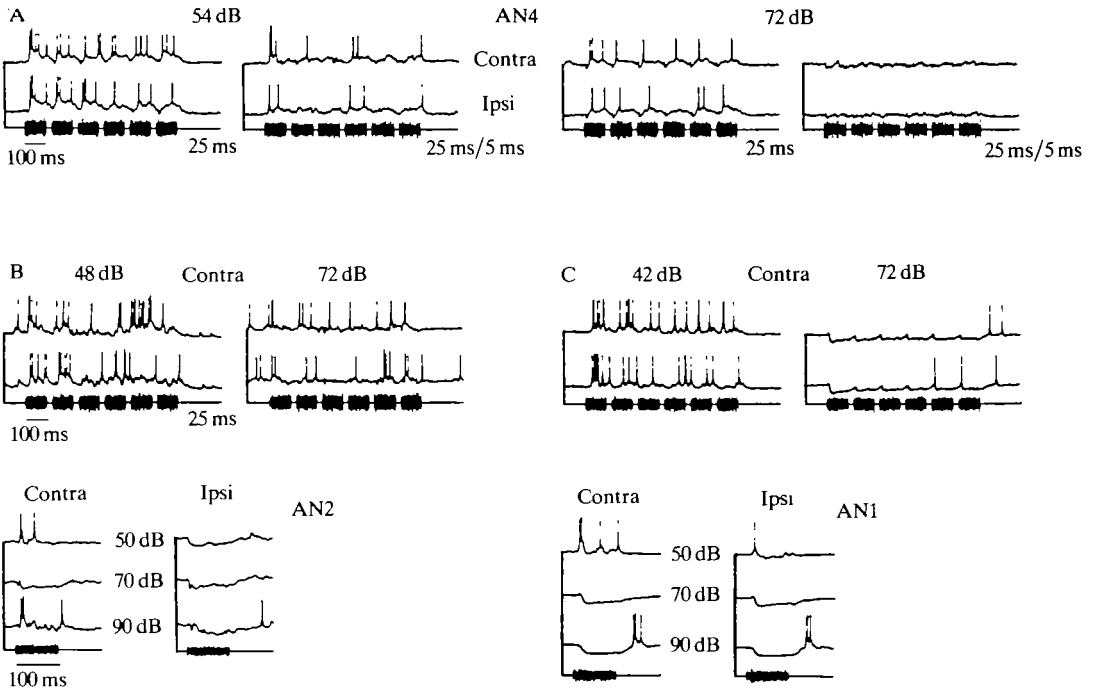

Fig. 10. The influence of sound intensity and direction on spiking patterns of ascending neurones. Sample recordings from AN4 (A) demonstrate that the gap-specific inhibition is independent of direction (ipsilateral, contralateral) and only slightly affected by intensity. The most directional neurones, AN2 (B) and AN1 (C), show highly variable spiking patterns, which are very intensity-dependent (two sweeps for the same stimulus are shown for each intensity). Moreover, ipsilateral stimuli (and at middle intensities often also contralateral stimuli) evoke complete or almost complete inhibition.

metathoracic ganglion complex is obviously an important site for processing auditory information, but information pathways up to the brain must be intact for both song recognition and sound localization. Receptor fibres and T-fibres like TN1, to our knowledge, do not ascend to the brain (see Halex *et al.* 1988), and their projections to mesothoracic and prothoracic ganglia are not necessary for song recognition (see Fig. 5 in Ronacher *et al.* 1986). Therefore, the preprocessed information about the song pattern must be transmitted to the head ganglia *via* ascending interneurones.

Processing of the syllable–pause pattern

Auditory receptors of *C. biguttulus* respond tonically to acoustic stimuli and are obviously not specialized for species-specific communication tasks (Ronacher and Römer, 1985). The first interneuronal level in the auditory pathway contains neurones confined to the thoracic (and adjacent abdominal) ganglia (local

neurones, bisegmental neurones and T-fibres). Among these, SN1, TN1 and some BSN1 cells encode the song syllables, while SN6 encodes the pauses between syllables. TN1 most probably utilizes GABA as a transmitter, and is likely to exert an intensity-dependent low-frequency inhibition on ascending neurones (Sokoliuk *et al.* 1989; see also Römer *et al.* 1981). Most probably, SN1 too is an inhibitory cell: at least one of the twins (or triplets) of SN1 has been shown to have an inhibitory influence on AN1 in the locust (Marquart, 1985*b*). The activity of SN6, which has a high spontaneous firing rate and is tonically inhibited by sound stimuli, also seems ideal for inhibiting a higher-order interneurone during the pauses between syllables. This might improve the accuracy of tonic discharges of higher-order interneurones.

The next level of auditory processing is represented by ascending neurones (Stumpner and Ronacher, 1991; see also Römer and Marquart, 1984; Römer *et al.* 1988). Only a few of them respond tonically and thus could encode the song pattern. They all encode the syllable–pause pattern much less precisely than do receptors, TN1 or even SN1. AN6, for example, does not even encode the gross song pattern precisely, because of its relatively low spiking rate, its spontaneous activity and its tendency to give responses lasting longer than the stimulus (Fig. 3A). The reactions of other ascending interneurones depend strongly upon intensity, as these neurones are all inhibited to a certain extent at medium intensities (Stumpner and Ronacher, 1991).

The precision of the behavioural reaction cannot be explained on the basis of the activity of a *single* tonically responding ascending neurone. In principle, information about the song pattern could also be transmitted to the brain *via* a set of interneurones, each 'working' in a different intensity range ('range fractionation', Cohen, 1964). This, however, is unlikely, since none of the ascending neurones precisely encodes the song pattern in the medium to high intensity range.

Accordingly, the phasically responding ascending neurone AN12 seems to be the auditory interneurone best suited to evaluate the pause durations between syllables of a model song. It responds to the syllables only if a certain pause duration is exceeded. Among local neurones, most BSN1 cells respond in a similar, though much more intensity-dependent, manner. The responses of both neurones correspond well to the rising part of the behavioural response curve: at approximately the behaviourally most effective pause durations, AN12 starts to burst at every syllable of a model song (at room temperature pauses of 20–25 ms with syllables of 85–100 ms). Neither AN12 nor BSN1, however, show a peaking response curve to the pause duration (Figs 5C,D, 6C,D), as was found for behaviour (Fig. 1A). Furthermore, in behavioural experiments, the preferred pause duration increases with increasing syllable duration (D. von Helversen, 1972; O. von Helversen, 1979), but none of the tested thoracic interneurones shows a comparable response (cf. Figs 5C, 6C).

No ascending interneurone was found with a phasic on–off response at the beginning and end of syllables or at least an off response at the end of syllables (i.e. the beginning of pauses). Therefore, the syllable duration, which is still rep-

resented in the spike trains of receptors and some local neurones, such as TN1, seems not to be transmitted in a precise manner up to the brain.

Gap detection

Model songs with gaps of more than 2 ms are no longer attractive to female *C. biguttulus*. Interspike intervals of tonically responding cells are usually longer than 2 ms. At the receptor level, therefore, it is unlikely that a single cell transfers enough information about gaps of that magnitude; it is more likely that a synchronization of spikes in *parallel* fibres allows the detection of gaps in the millisecond range (Ronacher and Römer, 1985; see also Surlykke *et al.* 1988).

The ascending neurone AN4 has filtering properties that closely resemble those of gap-detection in the behaving female (see Fig. 7, and Ronacher and Stumpner, 1988). The gap-dependent suppression of the activity of AN4 results from an IPSP that is retriggered by each pulse in a syllable when the gaps are greater than 2–3 ms (see Fig. 7B). The activity of this neurone, therefore, reliably signals whether a syllable contains gaps. Considering the congruence with behavioural data, it is likely that this neurone determines the filtering characteristics of the behavioural reaction, although the activity of other ascending neurones is also affected by gaps. The representation of syllables in a phasic burst in AN12, for example, becomes less reliable or even disappears if gaps longer than 5 ms are inserted in syllables (Fig. 8C,D). This effect possibly reduces the detection of the syllable–pause structure and should also diminish the behavioural response.

What is left for the brain to do?

All auditory systems accomplish a certain degree of 'peripheral filtering' at the receptor level. However, in crickets and frogs, auditory neurones with filtering properties related to species-specific behavioural performances and neurones with band-pass characteristics have been found only in higher centres of the CNS (Schildberger, 1984; Rose and Capranica, 1984; Walkowiak, 1984, 1988; Feng *et al.* 1990). In *C. biguttulus*, filtering of several song parameters (the syllable–pause pattern, the presence of gaps in syllables) clearly starts at the first level of interneuronal processing in the metathoracic ganglion. As mentioned above, however, the responses of local and ascending neurones were not correlated to two basic features of the IRM of *C. biguttulus*, namely the peak characteristic (band-pass) for syllable–pause variation and the influence of the syllable duration on the optimal effective pause duration (Fig. 1A). Neurones contributing to these unexplained features might have been overlooked. There are ascending neurones described in the locust (AN5, AN8, AN9; Marquart, 1985*a*), but not yet recorded in *C. biguttulus*. However, there is some evidence from behavioural experiments that the rising and the descending slopes of the peak characteristic for syllable–pause variations are generated by different neurones or at different levels of processing: the rising part of the behavioural response curve shows a distinctly smaller interindividual variability than the falling part (O. von Helversen, unpublished results). Thus, the rising part of the curve, with small interindividual

variability, could be based on the reaction of AN12, whereas the descending part could be brought about by brain interneurones. A conceivable mechanism would be the summation of EPSPs (evoked by AN12 bursts) that remain subthreshold when pauses between syllables are too long (a similar mechanism has been described for auditory band-pass neurones in the cricket brain; Schildberger, 1984).

A probable task of the grasshopper's brain is to connect pattern recognition and directional analysis. Neurones ascending from the metathoracic ganglion seem to be specialized for transmitting information either about sound direction or about song patterns. This appears to be different in crickets (Schildberger and Hörner, 1988; Stabel *et al.* 1989). As for pattern recognition by *C. biguttulus*, the activity of AN12-type neurones representing the syllable–pause structure is only slightly directional. Similarly, AN4, as a gap-detecting neurone, responds in a similar way to ipsilateral and contralateral stimulation (Fig. 10A). As for directional analysis, the most directional neurones, AN2 and AN1, exhibit extremely variable responses with a poor correspondence between their spike trains and the stimulus patterns (Fig. 10B,C; for AN1, see also Wolf, 1986). The more-or-less complete inhibition of AN1 and AN2 by ipsilateral stimuli, however, is reliably repeatable and corresponds well to behavioural results, which provided strong evidence for an amplification of left–right differences within the metathoracic ganglion (Ronacher *et al.* 1986). For phonotaxis (which can be performed by males and females) a synthesis (logical ANDing) of the information about song pattern and song direction is necessary – and this seems to take place not in the thoracic ganglia but in the brain.

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