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Routes and stations in the processing of auditory directional information in the CNS of a grasshopper, as revealed by surgical experiments*

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Summary. Male grasshoppers of the acridid species *Chorthippus biguttulus* respond to conspecific female song by turning abruptly toward the sound source and then singing themselves. The orienting response requires that (i) the male recognizes the female song and (ii) determines the location of its source. By observing the effects of various forms of surgical interference (cutting connectives, splitting ganglia, destruction of a tympanal organ) on the subsequent behavior, we were able to narrow down the paths of information flow necessary for these abilities and to make an estimate of the contributions of individual ganglia to the evaluation of sound direction.

1. The information from a single tympanal organ is sufficient for the *recognition* of the conspecific song. From the metathoracic ganglion (TG3) sufficient information for song recognition ascends both in the ipsilateral and in the contralateral connective chain. Thus, information for song recognition is transmitted to the opposite side within the metathoracic ganglion.

2. For a singing response to be elicited by hearing a female chirp, it is necessary and sufficient that the connectives linking TG3 and the supraesophageal ganglion (brain) of the male be intact on at least one side (as has been shown for *Gomphocerippus rufus* by Loher and Huber 1966, and Elsner and Huber 1969). Neither the three thoracic ganglia alone nor the thoracic-ganglion complex plus the subesophageal ganglion suffice to elicit either the turning or the singing response. The most probable explanation of our results is that the information paths for both responses make a loop via the brain.

3. A crucial step in sound direction processing takes place within TG3 itself. The results after connective cutting indicate a reciprocally inhibitory interaction within this ganglion by which the difference between right and left tympanal input is enhanced.

4. The information about sound direction carried in either connective alone is incomplete. Therefore, an additional 'comparator element' must exist, which receives the 'directional signals' from both connectives and converts them into an unambiguous turning command. This step in direction processing must occur either in the brain or along the pathways descending from the brain to the anterior two thoracic ganglia.

5. The anterior two thoracic ganglia, as well as the subesophageal ganglion, make either no contribution or a negligibly small contribution to the determination of sound direction.

Introduction

In many acridid species it is the male's active searching that brings the two sexes together. The male wanders about, pausing to sing at regular intervals, and when he hears a female in mating condition answer with chirps of her own, he responds in a highly stereotyped manner. He makes a very characteristic, abrupt turn toward the side from which the sound is coming, usually moves forward a short distance in the new direction, and

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* Dedicated to Prof. Franz Huber on the occasion of his 60. birthday

Abbreviations: CNS Central nervous system; *Brain* Supraesophageal ganglion; *SOG* Subesophageal ganglion; *TG1* Prothoracic ganglion; *TG2* Mesothoracic ganglion; *TG3* Metathoracic ganglion (including the first 3 abdominal ganglia, which are fused to it); *TO* Tympanal organ; *SA* Stridulation apparatus; *TA* Turning apparatus; *IRM* Innate releasing mechanism; *LS* Loudspeaker; *n* Total number of stimulus (female chirp) presentations sings again, whereupon the female again answers. This sequence of behaviors is repeated several times, until the male has reached the immediate vicinity of the female.

To achieve this end, the male must have an auditory system capable of two sensory functions: (i) it must recognize the female song as such, and (ii) it must be able to estimate the direction of the sound source. In the latter process, the animals differentiate only between right and left: that is, they decide only which of the two ears is receiving the louder signal (von Helversen and von Helversen 1983; Rheinlaender and von Helversen, in preparation).

The present experiments were designed to identify the locations, within the CNS of the male grasshopper, of the processing systems and information channels responsible for these two functions. The approach was to separate parts of the CNS from others (mainly by transecting connectives and splitting ganglia longitudinally) and then to determine by behavioral tests whether, and to what extent, the ability to recognize and locate the source of female song had been impaired. Similar surgical experiments have been performed on orthopterans already by Huber (1952, 1955, 1959, 1960), Loher and Huber (1966), Elsner and Huber (1969), Otto (1967, 1971) and Kutsch and Otto (1972), but with the aim of demarcating the motor centers that elicit courtship behavior, and the 'pattern generator' for song production. A number of results published by the above authors are confirmed by our observations. In addition, our findings bear on the flow routes of the auditory information, from its acquisition in the tympanal organs to the elements that elicit the behavioral response. They enable us to estimate the contribution of individual ganglia in the thorax and head regions to the process of determining the direction of a sound source.

Materials and methods

The animals used were males of the acridid species *Chorthippus biguttulus* (L.) which had been caught in the field (near Erlangen or Freiburg). A total of 240 grasshoppers were studied.

Operations. Three types of operation were performed, separately or in combination: deafening, connective transection, and mediosagittal hemisection of ganglia ('splitting'). For all of them, the animals were briefly anesthetized by 1–2 min exposure to CO_2, then firmly attached to a holder with transparent adhesive tape. The surgery was carried out under a binocular microscope. The animals were deafened by opening the tympanal slit and pulling out the receptor region (which is visible from outside) with fine forceps. The entire tympanal membrane was also destroyed, and the opening was occluded with wax-colophonium. This severe interference, of course, also affects the acoustic properties of the contralateral ear (see Michelsen 1971; Miller 1977). But it was necessary to accept this disadvantage, for the sake of ensuring that the damaged ear was completely nonfunctional (for controls see Results).

For the transection of thoracic connectives a small window (less than 1 × 1 mm) was cut in the ventral cuticle, and the connective was gripped with forceps and cut with a miniature iris-scissors (Weiss, London). Then the excised piece of cuticle was replaced and attached with wax-colophonium. (In the case of the cervical connectives it proved unnecessary to close the wound, because it closed itself by hemolymph coagulation.) The circumesophageal connectives are not easily accessible, at least, if the animal is meant to survive in good condition. Most animals in which the ventral surface of the neck was opened soon died, presumably because the operation had damaged the innervation of the chewing apparatus. It eventually proved most useful to cut a window in the cuticle of the frons and cut the circumesophageal connective immediately behind the tritocerebrum. The transections at each level were done sometimes on the right and sometimes on the left connective. In all cases mirror-symmetric results were obtained, so that in the presentation here it suffices to refer to intact and transected side only. The splitting of ganglia was also done through a window in the ventral cuticle, with a splinter of razor blade. During this operation some tracheae were unavoidably damaged, but evidently with no great harm to the animal (see Results).

Usually the animals were capable of turning and singing in response to female song only a few hours after the operation, though in some cases recovery took 3–5 days. Animals with split TG2 or unilateral connective transections, when studied over long periods, responded in the same way a few days postoperatively as they did weeks later. Eventual compensation of sensory deficits (see Figs. 1, 2) was never observed, even though some animals survived as long as 5 weeks. The great majority of the results (except those that were negative) were obtained during the first week after the operation, within which time regeneration of nerve tissue can be ruled out (Campos, personal communication). The anatomical effects of all operations were checked by inspection after the behavioral tests were concluded. The CNS region involved was exposed by dissection and then fixed with Bouin's solution in situ. In general, examination of tissue so fixed revealed conclusively whether a transection or split was complete and whether a split was symmetrically placed. In the few cases of doubt the data for that animal were discarded.

Behavioral tests. The experiments were done in a reflection-attenuating room, lined with foam prisms. The temperature was between 29 ° and 32 °C. The male grasshopper rested on a round, cloth-covered foam surface (ca. 70 cm in diameter). Whenever it began to sing spontaneously, it was 'answered' by playing back a synthetic female chirp stored on magnetic tape (Racal 4D) by way of a loudspeaker (LS) positioned at 90° to the male's long axis. As a rule, the male then turned toward the loudspeaker with a jerk (turning angles usually ranged from 30° to 90°), moved forward a short distance, and sang again. The LS was movable, so that it could be repositioned with respect to the male and immediately 'answer' again. This sequence was repeated until the male no longer responded, which usually occurred after 10–15 exchanges. Ordinarily the male then resumed singing after about 1 min. In most cases the intensity of the female chirp was 52–53 dB SPL, but sometimes it was 62 or 67 dB SPL (RMS fast). Intensity was calibrated with a Brüel & Kjaer system (microphone 4133, measurement amplifier 2606).

In most tests the sound was presented from only one LS, the position of which was changed at irregular intervals from the intact to the affected side and conversely (see Figs. 1–3). In the case of animals with split ganglia, the purpose of the test was to evaluate accuracy with which the lateral discrimination was made (Fig. 7). Here two loudspeakers were fixed to a U-shaped frame placed such that the animal was exactly midway between the two speakers, with its long axis perpendicular to the line joining them. Identical stimuli were presented simultaneously over the two speakers, the intensity of each being independently variable in 1 dB steps (for details see D. and O. von Helversen 1983; Rheinlaender and von Helversen, in preparation).

All behavior of the animal during the test – walking, turning, jumping, standing still, each with or without response singing – was noted by the observer. However, only the following forms of behavior were evaluated as responses, because only they were relevant to the task being considered: turning to left or right with subsequent response singing, and response singing without previous turning. (In fact, other forms of behavior occurred much more rarely than these.)

Results

Behavior of intact animals

When the song of an intact animal was answered by a female chirp from a loudspeaker on one side of the animal, turning was always toward that side, never toward the opposite side (white bars in Fig. 1a). On about 10–15% of stimulus presentations the animal did not turn but responded only by singing in the original position (black bars in Fig. 1a).

Behavior of unilaterally deafened animals

Animals that were deaf on one side, when they turned in response to sound, always turned toward the side with the intact ear, regardless of the position of the sound source (Fig. 1b, white bars, left and right). This result was expected, inasmuch as in unilaterally deaf animals an intensity comparison of the left and right tympanal inputs will always show the sound to be louder on the intact side.

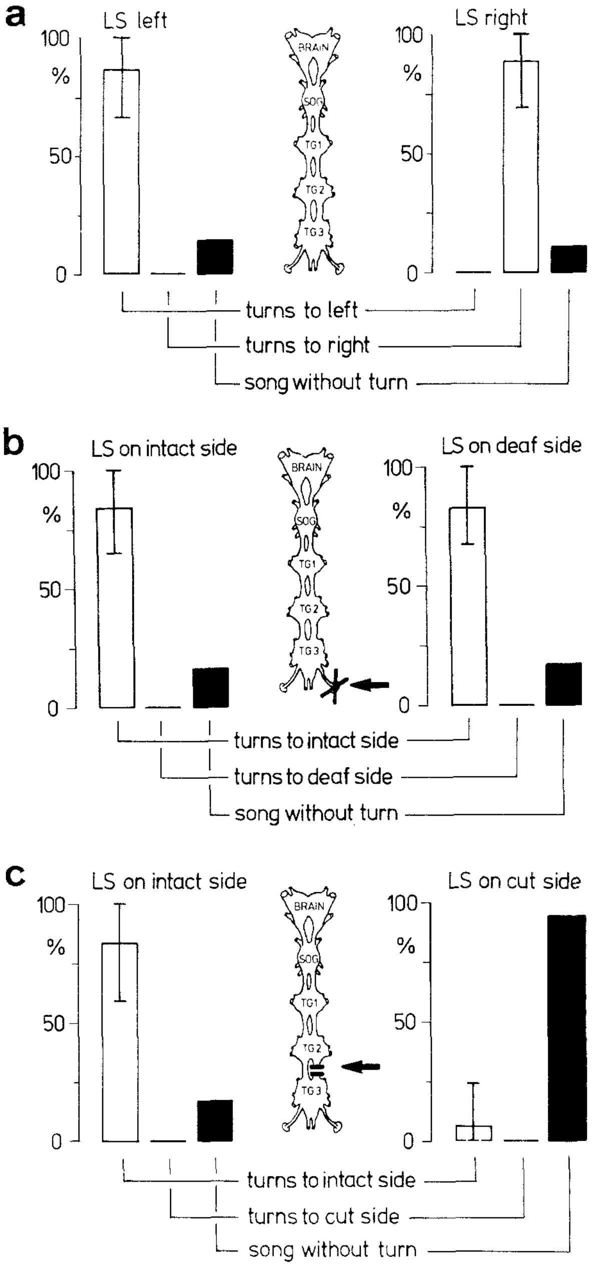


Fig. 1a–c. Responses of male *C. biguttulus* to female chirps presented by a loudspeaker on one or the other side of the animal. The responses of intact animals (**a**) are shown for comparison with animals unilaterally deafened (**b**) or with the connective TG2/TG3 transected on one side (**c**). Ordinates: percentage of the total reactions in each of the categories indicated on the abscissa. (The turns – left and middle category in each diagram – were always followed by a singing response.) Vertical lines show range of interindividual variation. From a to c, $n=$ 780 (10 animals), 727 (14 animals), 1027 (9 animals)

Behavior of animals with a single connective transected

Transection of one of the connectives between TG2 and TG3. When the female chirp was presented on the intact, the 'control' side, males with a cut connective TG2/TG3 behaved not differently from intact males (cf. Fig. 1c and a). Presentation of the female chirp on the other side elicited very different behavior. Now the males failed to turn on about 90% of stimulus presentations, producing their response song while still in the original position (black bar in Fig. 1c, right). The singing response demonstrates that in this situation they were still able to identify the conspecific song. On the rare occasions when an animal stimulated on the side with the transection did turn, it always turned toward the intact side, away from the sound source (white bar in Fig. 1c, right).

In interpreting the results of such surgery, of course, one encounters the problem of distinguishing effects due to the interruption of sensory pathways from motor deficits that may also be produced. Failure to turn toward the side with the transection (Fig. 1c, right) could be explained by (i) the cutting of essential 'command fibers' of the motor system such that the animal is unable to turn in this direction, or (ii) the absence of a command to turn in this direction because the appropriate sensory information is not received, although the ability to initiate and execute a turning movement is retained.

The following control experiment shows that failure to turn toward the side with the cut did *not* result from a motor deficit. Animals with unilaterally transected TG2/TG3 connective were presented with sound on the side of the cut, a stimulus that in itself usually elicits singing without turning but occasionally, turning *away* toward the intact side (Fig. 1c, right). If simultaneously with the acoustic stimulus a female glued to a small stick was also moved on the side with the cut, 3–5 cm away from the male, this additional visual stimulus induced the male to turn toward it – that is, toward the side with the transected connective. Furthermore, the movement was the abrupt turn characteristic of auditory orientation. (Of 166 stimulus presentations to three animals, 29 (17.5%) elicited turning toward the side of the operation. The visual stimulus given alone elicited no turns.)

The comparison with unilaterally deafened animals (Fig. 1c and b) indicates that one step in ascertaining 'which is the louder side', at least, takes place in the metathoracic ganglion. If the role of TG3 in this context were only as a channel for transmission of the auditory input to higher centers, animals with one connective cut would behave like unilaterally deafened animals.

Transection of one cervical connective (SOG/TG1). Information about the location of the sound source – and hence about the turning direction – must be transmitted to centers anterior to the metathoracic ganglion, because turning is accomplished by the forelegs and middle legs. To test whether the three thoracic ganglia *alone* suffice for a turning response in both directions, one of the cervical connectives was cut.

The behavior of animals following this operation was indistinguishable from that of animals with a transected TG2/TG3 connective (cf. Fig. 2b with c). Provided that no unspecific asymmetry was induced by the cut (see Discussion), this result shows that the thoracic ganglia are not sufficient, in themselves, to determine the side on which the sound source is located *and* carry out a turn based on this information; if they were, an animal with one cervical connective cut would behave normally, turning in both directions.

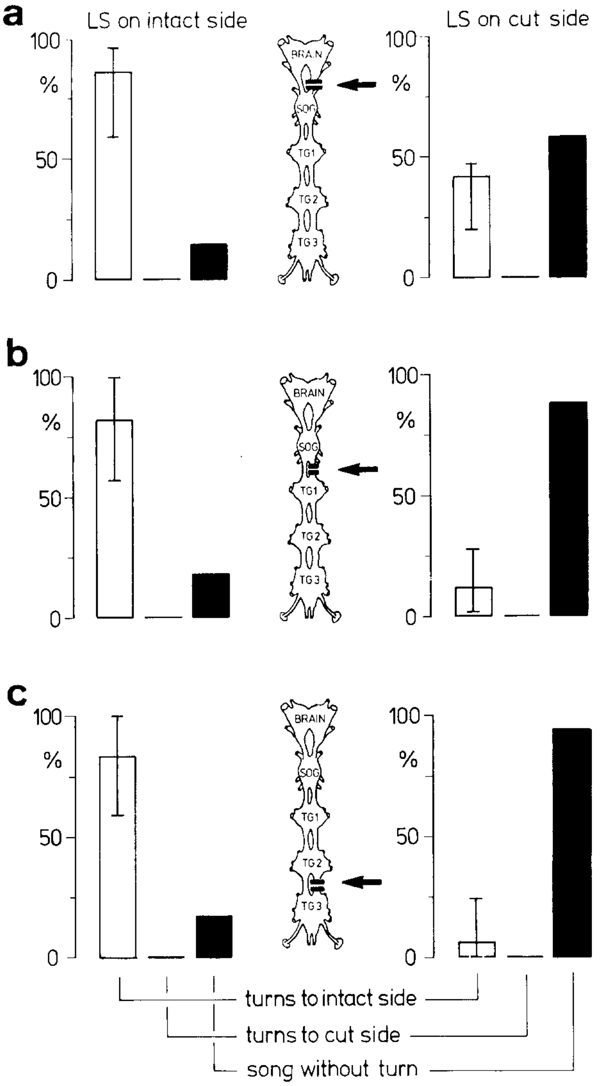


Fig. 2a–c. Responses of males with unilaterally transected connectives. **a** Circumesophageal connective cut, **b** cervical connective cut, **c** TG2/TG3 connective cut. Diagrams as in Fig. 1c. Means in the respective columns of (**b**) and (**c**) do not differ significantly, whereas they both differ from that of (**a**) with $P<0.01$. $n=437$ (5 animals), 686 (7 animals), 1027 (9 animals)

Transection of one circumesophageal connective (brain/SOG). The results of this operation were similar to those of transection at more caudal levels. Stimuli presented on the side of the operation never elicited turning toward that side; turns were always directed toward the intact side (Fig. 2a).

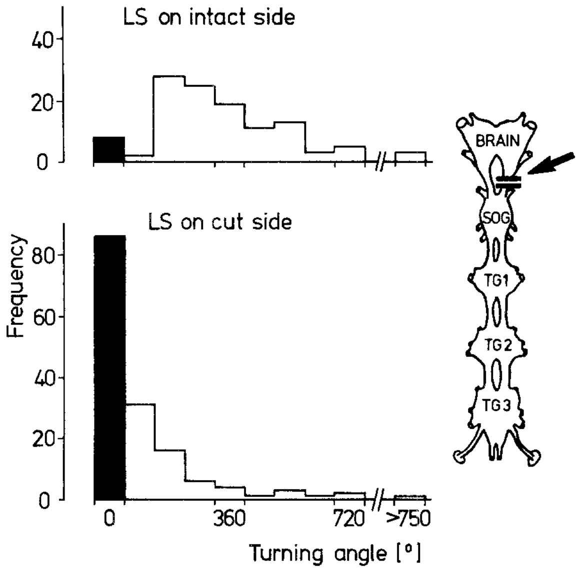


Fig. 3. Turning angles of animals with a cut circumesophageal connective, in response to stimuli on the intact (above) or transected (below) side. Abscissa: turning angle (in categories of 90°). Black bars: singing without turning. $n=278$ (2 animals)

Evidently even the intact complex of thoracic ganglia plus SOG is insufficient to ensure a normal turning response in both directions.

The responses of these animals were somewhat unusual in that stimuli on the affected side elicited relatively frequently turns (cf. Fig. 2a with b and c, right diagrams). Moreover, the turns themselves were unusual; as a rule, there was a rapid initial turn that was, sometimes after a brief stop, continued until, in some cases, several complete circles had been made. This performance gave the impression that a 'brake was missing' (cf. Huber 1955, 1959). Especially when stimuli were presented on the intact side, the turning angles in most cases were considerably larger than those of intact animals or animals with a transected SOG/TG1 or TG2/TG3 connective. When the stimulus came from the side of the operation, the turning angles were distinctly smaller on average (Fig. 3).

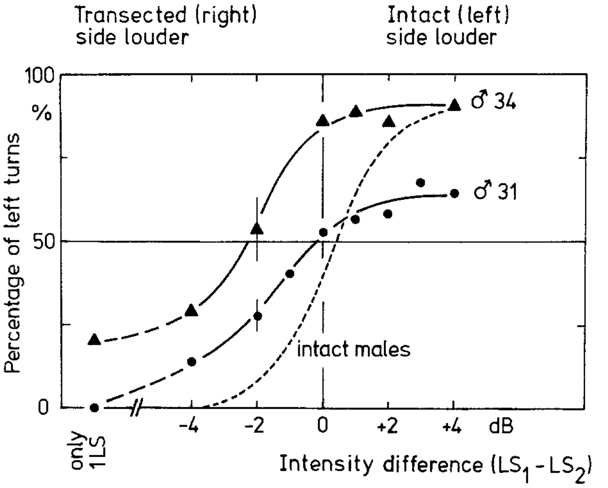


Fig. 4. Accuracy of lateral discrimination. Abscissa: difference in intensity of otherwise identical sounds from two loudspeakers positioned at an equal distance, 20 cm from each side of the animal. Ordinate: percentage of total responses that comprised left turns. For the males with right connective TG2/TG3 transected (M34, M31) the region above the curve corresponds to the percentage of responses without turning. Intact animals turn toward both sides; for comparison with the cut-connective animals, here the curve for intact animals (dashed) gives only the percentage of left turns. Therefore the region above the curve for intact animals corresponds to the percentage of responses with no turning plus those with right turns (the latter depends on the intensity difference). The fact that the curve for intact animals does not reach 50% at 0 dB thus results not from asymmetry in the animals but rather from the occurrence of responses without turning. $n=769$ (op.), 1687 (int.)

Accuracy of sound direction processing in TG3. Males with one TG2/TG3 connective transected were tested to determine the accuracy of lateral discrimination as a function of the intensity difference between two LS. The results of two males with the right TG2/TG3 connective transected are shown in Fig. 4. (The animals turned only toward the intact (left) side – cf. Fig. 2.) Though the two animals differed considerably in their tendencies to sing without turning the curves are basically the same in shape: If the transected side is less loud the frequency of turns rises as the intensity difference of the two speakers decreases. When the inputs from both sides are of the same size the curves saturate. In the left half of the diagram the curves for the two test animals are well above that for intact animals (dashed line) which turn without any mistake with intensity differences greater than 2–3 dB! Similarly, when the sound was equally loud on the two sides (0 dB) the animals with right connective cut were more likely to turn left than the intact animals.

Behavior of animals with lesions of both connectives

The experiments of Huber (1955, 1960) on crickets and of Loher and Huber (1966), Elsner and Huber (1969) and Elsner (1973) on grasshoppers showed that for information about the detection of conspecific song to be carried to the brain and for the subsequent initiation of coordinated singing, the connectives need be intact on only one side, and furthermore that intact connectives on at least one side are necessary for the behavior to be triggered by song stimuli (but see Kutsch and Otto 1972).

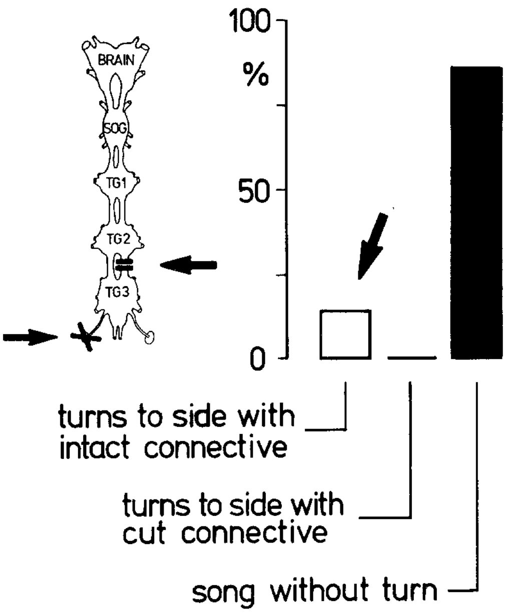


Fig. 5. Responses of males with one deaf ear and the contralateral connective TG2/TG3 transected. Diagram as in Fig. 2. In general the stimulus was on the side with the intact ear; control stimuli on the other side gave the same results (cf. with Fig. 1b). This diagram includes responses of 7 animals, each of which occasionally gave turning as well as singing responses. $n = 576$

These results are corroborated by the present findings in *C. biguttulus* (see Figs. 2 and 6a); when connectives were cut on both sides, at different levels, on more than 15,000 stimulus presentations neither response singing or any other song production was observed, even when the animals tested were in the presence of females ready for mating. Nor was a turning response to female song ever observed, even though the animals were quite capable of turning to the right and left during locomotion (cf. Elsner and Huber 1969; Graham 1979; Kien 1983).

Destruction of one tympanal organ (TO) and one connective TG2/TG3

The first step here was to destroy the TO (see Methods) and then check on the success of the operation by behavioral tests. When it had been established that the animal was unilaterally deaf (i.e., behaved as illustrated in Fig. 1b), in a second operation either the ipsilateral or the contralateral connective between TG2 and TG3 was transected.

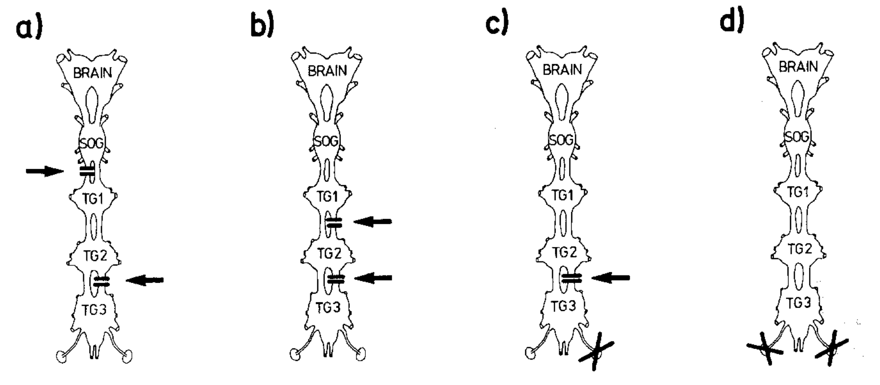
Animals with the *ipsilateral* connective transected (Fig. 6c) behaved like animals in which only the TO was destroyed (cf. Fig. 1b).

Of the 19 males in which the connective *contralateral* to the destroyed TO was cut, 11 sang in response to female chirps and thereby showed that they could still recognize the conspecific song (8 animals did not respond at all). Surprisingly, of these 11 males, 7 actually exhibited turning responses as well – these turns were always toward the side with intact connective (Fig. 5).

To rule out the possibility that the destroyed TO or other mechanosensory inputs (cf. Regen 1914; Autrum 1936; Busnel and Burkhardt 1962) might somehow be supplying the CNS with enough information to trigger response singing or turning, the following control experiment was done. In 11 males both TOs were destroyed, by the same method as previously (Fig. 6d). If this procedure had not completely eliminated the animals' hearing, they should still have sung or perhaps turned in response to (loud) stimuli. But on ca. 9,000 stimulus presentations neither response was ever observed, although the animals were of course still able to sing spontaneously (see Elsner and Huber 1969). Spontaneous songs were often produced, but never the characteristic singing in alternation with the stimulated female chirp.

Mediosagittal splitting of single ganglia

This type of operation was done primarily to learn what contributions the individual ganglia make to the process of locating a sound source.



a) No response singing
No spontaneous singing
No turning behavior

b) Behavior as in Fig. 1c

c) Behavior as in Fig. 1b

d) No response singing
Spontaneous singing possible

Fig. 6a–d. Results of operations at two sites.
a Crosswise connective transections (27 animals, $n \geq 15{,}000$).
b Transection of 2 connectives on the same side (2 animals, $n = 101$).
c Unilateral deafening plus ipsilateral transection of connective TG2/TG3 (2 animals, $n = 134$).
d Bilaterally destroyed tympanal organs (11 animals, $n = 9{,}000$)

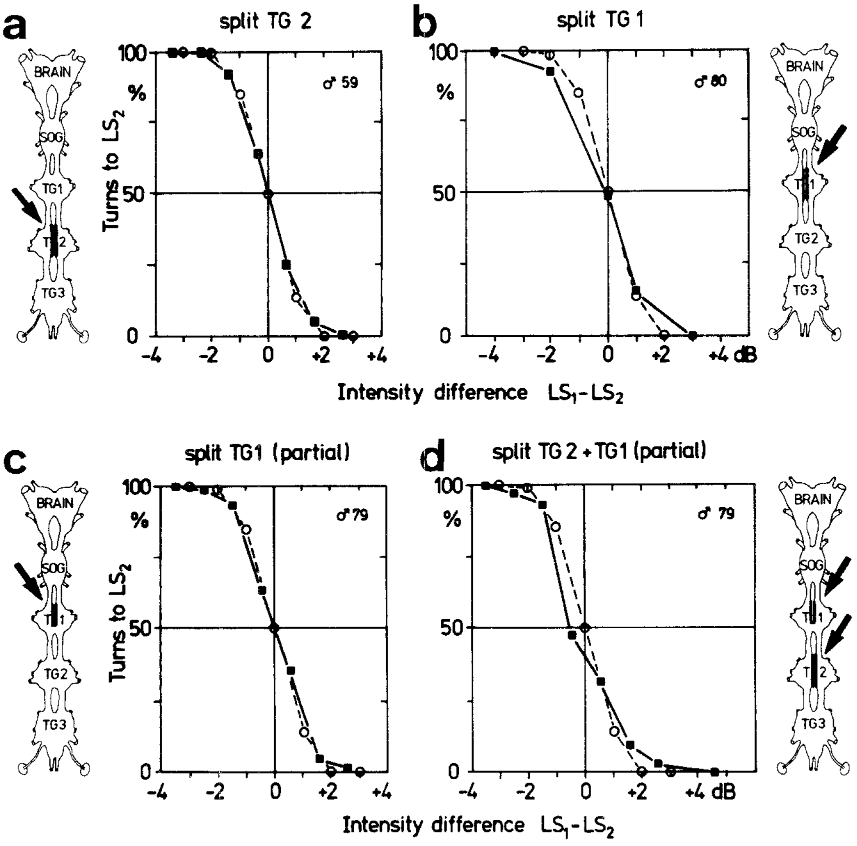


Fig. 7a–d. Accuracy of lateral discrimination of 3 animals with split ganglia (filled symbols). Abscissa: intensity difference between the two LSs (see Materials and Methods); ordinate: percentage of turns directed toward LS_2. (Unlike Fig. 4, these diagrams do not include singing responses without turns in the total.) For comparison, the mean curve for intensity discrimination by intact animals is shown (dashed) in each diagram (9 animals, $n = 821$, from von Helversen and Rheinlaender, in preparation).
a Split TG2; M59 ($n = 351$).
b Split TG1; M80 ($n = 197$).
c Responses of M79 with partially split TG1 ($n = 379$).
d In a second operation on M79, TG2 was completely split ($n = 233$)

Split TG3. Animals with metathoracic ganglion completely split (19 males) neither sang nor turned in response to female chirps, although their general vitality appeared unimpaired; most of them survived for several weeks. One of the reasons for the failure to respond was certainly a deficit in the motor system for singing, for these animals often held their hindlegs outstretched in quite unnatural positions. When TG3 was only partially split (about halfway back from the frontal edge), some animals still made singing movements (see also Loher and Huber 1966; Elsner and Huber 1969) and in some cases produced audible songs. They did not, however, respond to female chirps, nor did they give turning responses.

Split TG2. This operation had very little effect on the animals (16 males) (see also Loher and Huber 1966; Elsner and Huber 1969). Some individuals survived as long as 5 weeks. The behavior also seemed hardly to be impaired; many animals sang in response to female chirps and turned in both directions.

The reaction of one individual to small intensity differences is diagrammed in Fig. 7a. This male with split TG2 (solid curve) made the lateral discrimination as accurately as intact animals (dashed curve), with hardly any mistakes when the intensity difference between the two LS was 2 dB. Five other animals discriminated equally well after this operation. With 5 males an asymmetry was observed, 5 other animals did not respond at all.

Split TG1. The result of splitting the prothoracic ganglion was in principle the same, although less evidence could be obtained in this case. This operation had such severe effects, the nature of which we do not know, that of the 21 animals on which it was performed 16 did not survive to the next day. Only one animal (M 80) with the ganglion completely split survived long enough, with sufficient vitality, to be given the necessary tests (Fig. 7b). In three other animals TG1 was only partially split, in one case halfway from caudal to frontal and in the two others about 3/4 of the way from frontal to caudal.

All four of the animals that could be tested were capable of lateral discrimination approximately as accurate as that of intact animals and those with split TG2 (see Fig. 7b, M80 with completely split TG1). In the case of M79, with TG1 only partially split (Fig. 7c), a second operation was carried out in which TG2 was completely split. Even after such extensive separation of right and left CNS elements, the animal remained capable of accurate lateral discrimination (Fig. 7d).

The results diagrammed in Figure 7 indicate that neither the prothoracic nor the mesothoracic ganglion contributes to the determination of sound direction; if comparisons between the two sides were occurring here, splitting the ganglia should have reduced performance accuracy. This finding, together with the abolition of turning responses in animals with split TG3, shows that the two anterior thoracic ganglia are neither sufficient nor necessary for direction analysis.

Splitting the SOG was a drastic operation, which caused severe motor impairment and was usually soon followed by the death of the animal (23 males). Three males in which the SOG was only partially split (over about 2/3 of its length from the caudal or the frontal edge) remained capable of singing in response to female chirps and also, though very rarely, of turning. These turns were very different from the typical phonotaxis turns; they seemed to be made in slow-motion. Limited as they are, these observations do show that hemisection at this level does not prevent song recognition or block the command to sing in response. Because of the severe motor deficits, it was impossible to test the accuracy of lateral discrimination.

The *supraesophageal ganglion* was split in 16 males. This operation was also usually soon followed by death. None of the animals so treated has been observed to sing or turn (cf. Huber 1955, 1960).

Conclusions and Discussion

The information chain of interest here, from the detection of a female chirp to the execution of a turn toward it and the stridulatory response by the male grasshopper, comprises at least the following neural elements:

1. The *tympanal organs* (TOs), which receive the acoustic information and transmit it to the auditory neuropil regions of the thoracic ganglia (Rehbein et al. 1974; Römer 1985).

2. The *stridulation apparatus* (SA), here understood to comprise the entire mechanical apparatus, including the muscles and the neuronal network generating the stridulatory pattern. The movements of the two hindlegs are strictly coordinated, the patterns of movement on the two sides differing slightly (Elsner 1974). The fact that stridulation can be elicited by relatively unspecific stimulation applied to the brain (Huber 1955, 1960, 1967; Otto 1971; Hedwig and Elsner 1980; Wadepuhl 1983) or to the posterior stumps of the cervical connectives following their transection (Otto 1967; Elsner 1969; Bentley 1977) indicates that the network for song-pattern generation is situated in the thorax. This inference was confirmed for crickets by Kutsch and Otto (1972); in the acridid grasshopper *Omocestus viridulus* the minimal structure required to produce singing when stimulated with D.C. current is the complex of meso- and metathoracic ganglia (Hedwig 1985).

3. The *turning apparatus* (TA), which must be located in the two anterior thoracic segments. The hindlegs are not involved in turning; the body is turned by the forelegs and middle legs. Hardly anything is known about how the leg movements in this maneuver are controlled. In particular, the coordination of the two halves of the body has not been investigated. A remarkable finding here is that even with complete longitudinal hemisection of TG1 or TG2, animals were quite capable of turning in both directions – although these turns did not appear as smooth and well coordinated as those of intact animals.

Signals travelling the route TO-SA, as well as those on TO-TA, must be effectively gated by a *filter* that is selective for conspecific song. This filter is most decisive in the turning response; males will also sing in answer to the song of other males, and often they sing spontaneously, but turning is a far more specific reaction. The characteristic response turn is never performed spontaneously, but appears only as a result of the male's hearing a female chirp.

Furthermore, an appropriate turning response requires that information about the direction of the sound source be available. Hence the route TO-TA must include a direction-determining element. Behavioral experiments (von Helversen and von Helversen 1983; von Helversen 1984) gave evidence that the two processes, *evaluation of direction* and *pattern recognition*, occur in parallel and are largely independent of one another. The IRM responsible for the reaction of female *C. biguttulus* to male song is direction-independent and is activated even by a composite sound pattern, the two elements of which are each ineffective by themselves and are presented separately to the two ears. The information channel to the pattern-recognizing system 'adds', in a sense, the two tympanal inputs (von Helversen and von Helversen 1983; von Helversen 1984).

What can be inferred from the present experiments about the connections among the three elements TO, SA and TA and the steps by which processing is accomplished?

Inferences about routes of information transfer

Path from tympanal organs to stridulation apparatus (TO-SA). The results of Figs. 5 and 6c show that the auditory information for song recognition that ultimately reaches the IRM to trigger the singing response is, after arriving in TG3, available both on the ipsi- as well as on the contralateral side. Thus, the 'neuronal addition' of both tympanal inputs postulated by von Helversen (1983, 1984) most probably takes place already within the metathoracic ganglion.

For a singing response to be elicited, it is necessary and sufficient that the ascending and descending pathways be intact on only one side in the connective chain between TG3 and brain, either the side on which the auditory information originates (intact TO) or the other side (Figs. 5, 6); this confirms the results of Loher and Huber (1966) and Elsner and Huber (1969).

By far the most likely explanation of our observations, as well as those of Loher, Elsner and Huber, is of course that the information-flow route TO-SA makes a loop through the brain – perhaps because the IRM is situated there, or because it is there that the output of the IRM acts on the controlling elements that elicit singing (cf. Kien 1983; Kien and Altman 1984). Further support for this hypothesis lies in the structure of the ascending auditory neurons that have been identified; that is, all of them terminate in the brain (Rehbein 1976; Kalmring et al. 1978; Boyan 1983; Hedwig 1984, 1985; Hedwig and Elsner 1985; Boyan and Altman 1985).

Path from tympanal organs to turning apparatus (TO-TA). Two decisions must precede a turning reaction: whether a turn should be made (which involves an IRM), and if so, toward which side (determination of direction). These logically distinguishable components are evidently realized in largely independent information channels (von Helversen 1983, 1984).

The behavior of animals with one TG2/TG3 connective transected (Fig. 1c) shows that a considerable part of the process that determines 'which side is louder' is located in TG3. If TG3 were simply a passive channel for directional information, animals with a cut connective would behave like unilaterally deafened animals (but cf. Fig. 1). The direction processing involves a reciprocal inhibition between right and left inputs (see below), whereas for the recognition of the song pattern by the IRM a 'neuronal addition' of the tympanal inputs has been postulated (von Helversen 1983, 1984). The present findings therefore indicate that the channels for pattern recognition and direction analysis are already separate in TG3.

That the relevant motor facilities, in the two anterior thoracic ganglia, of these animals (Fig. 1c) were still capable of executing a turn toward the affected side was shown by the control experiment with an additional visual stimulus. Also, the frequent absence of turning reactions cannot be caused by *unspecific* asymmetry, induced by the transection of one connective. This is shown by the results of Figs. 4 and 6c. An unspecific asymmetry is not likely to produce a monotonous intensity-dependence of the reaction like that seen in Fig. 4.

For a definite decision to be made as to which of two lateral sound sources is louder, signals in both connectives are required. If complete information about the louder side were transmitted in each of the two connectives, so that one connective would carry information about a sound source on the ipsilateral as well as on the contralateral side, animals with a unilaterally transected connective would behave like intact animals, turning to both sides.

The fact that an animal with a transected circumesophageal connective – i.e. with an intact complex of thoracic ganglia plus SOG (see Fig. 2a) – is not able to perform turns in both directions indicates that the channels for directional information must take their course up to the brain.

The result illustrated in Fig. 2b (one SOG/TG1 connective cut) also argues against the following possibility. One might imagine that the directional information is provided by pathways ascending from TG3 to TG2 and TG1, the brain being required only to give a direction-unspecific 'permission' that a turn should be made (e.g., the output of the signal-recognizing filter). In this case, again, animals with cervical connective cut on one side should turn correctly to both sides.

In conclusion, our data suggest that the following route of information flow is the most probable. The patterns of excitation transmitted centrally in the two tympanal nerves are processed in two ways. On one hand, for the recognition of song pattern, they undergo 'neuronal addition' – most probably in TG3 – and then, without crossing the midline again, are conducted to the brain through the connectives on both sides. Along the way, at a site so far unknown, they are gated by a signal-recognizing filter. From the brain, descending signals are sent to the motor system for singing and to that for turning. In the second route, data from the two TOs undergo reciprocal inhibition in TG3 to determine the side of the animal on which the sound source is located. As a result of this processing step, side-specific signals are sent to the brain, each on its own side without further crossing (Figs. 6b, 7). The conversion of lateral differences in level of excitation to a decision to turn right or left will be considered more closely in the following section.

Contribution of individual ganglia to sound-direction determination

Metathoracic ganglion (TG3). Given that no excitation is conducted from TG3 to the brain on the side with the cut connective the information value in this channel can be considered equivalent to '0'. If the level of excitation in the intact channel is greater than '0', or greater than some threshold, turning toward the intact side occurs. The finding that there is ordinarily no turn in response to stimuli on the affected side (see Fig. 2) shows that the level of excitation in the intact channel has not reached threshold in this situation. The frequent absence of turning when the sound source is on the affected side can be explained only by reciprocal inhibition of the two tympanal inputs in the course of direction analysis, by which the excitation level in the intact channel is reduced. A neurophysiological basis for such inhibition has been described for *Locusta migratoria* (Römer et al. 1981; Römer and Dronse 1982).

The few turns in the 'wrong' (i.e. less loud) direction (Fig. 1 c) indicate that the reciprocal inhibition in TG3 does not always completely suppress the activity on the less loud side. The slight residual excitation conducted in the intact channel could then suffice to cause turning toward the intact side, in the absence of any excitation in the transected channel. (In an intact animal, a residual excitation on the side away from the sound source would be outweighed by that on the louder side, and the turn would be made correctly.)

As the difference in intensity of the sounds reaching the two TOs decreases, turns toward the less loud side become more frequent (Fig. 4), because it becomes less likely that the input from the louder TO will completely suppress that from the other. In this sense, the curve representing turning probability as a function of lateral intensity difference can be considered as a characteristic curve for the reciprocal inhibition in TG3. From the fact that this curve for animals with cut connectives lies above the curve for intact animals (Fig. 4), as well as from the occurrence of turns toward the lower-intensity side in response to unilateral sound presentation (Fig. 2c), it follows that in intact animals the difference of excitation between right and left is further enhanced in a part of the CNS above TG3. That is, a more centrally located *comparator element* (perhaps an additional circuit for reciprocal inhibition in the brain) is required, which weighs the 'direction messages' arriving in the two connectives against one another and converts the result to an unequivocal turning command.

Turns toward the wrong, less loud side (Fig. 2) by no means imply that the 'comparator element' randomly selects a turning direction when presented with the signal '0' in both channels. If that were the case, animals with the connective TG2/TG3 transected would sometimes have turned toward the side with the cut. But turning in that direction was observed in only two out of 1,264 cases, which amounts to 0.16% – a proportion so small that one might well wonder whether some other stimulus might have caused these turns.

The result shown in Fig. 5, that animals with connective TG2/TG3 on one side cut and the contralateral ear deafened still turned in a few cases, seems surprising at first glance. Evidently the direction channel on one side can be excited by the contralateral side, as well as inhibited. The implication is that there are a number of different connections between the right and left inputs, involved in direction processing. It should be kept in mind, however, that the destruction of the ear on the side with the intact connective eliminated a number of inhibitory processes that normally might well prevent excitation of this direction channel by contralateral inputs. It is also conceivable that the inhibitory and excitatory influences exerted by the two sides on one another might be shifted in time (cf. Reichert and Wine 1983) in a way that is not discernible in the behavior of an intact animal and becomes manifest only in one that is unilaterally deaf.

Pro- and mesothoracic ganglia. From the splitting experiments (Fig. 7) it can be concluded that in the two anterior thoracic ganglia no further enhancement of the difference between the 'direction messages' occurs. Hence the receptor axons that ascend into TG2 and TG1 and all the auditory interneurons with dendritic structures in TG1 or TG2 (see Kalmring et al. 1972a, b; Kalmring 1975; Hedwig 1985) make negligible or no contribution to the determination of turning direction. The same conclusion can be drawn from the results of unilateral connective transection; an additional lateral difference enhancement in the anterior thoracic ganglia should result in less turning away from a sound source with transections of cervical or circumesophageal connectives than transection of the TG2/TG3 connective (but cf. Fig. 2). Therefore, the ultimate enhancement of the directional information must occur either in the brain or along the descending pathways from the brain to the motor systems for turning.

After TG3 had been split, the animals no longer sang or turned in response to a female chirp. The abolition of turning, in particular, indicates a severe disturbance of *sensory* processing, for the operation could hardly have affected the motor systems in TG1 and TG2. Such a disturbance seems plausible inasmuch as the cut separated the two sides of the frontal auditory neuropil in TG3 and therefore probably damaged most of the ascending auditory neurons (see Rehbein 1976; Römer and Marquart 1984; Hedwig 1985). This negative result also shows that the two anterior thoracic ganglia in themselves do not suffice to receive and process the information about song pattern and the direction of sound.

Subesophageal ganglion (SOG). After transection of a circumesophageal connective the 'errors', i.e.

turns to the less loud side, were more common than when more caudal connectives had been cut (Fig. 2). Furthermore, there were considerable differences in turning angles, depending on the position of the loudspeaker (Fig. 3), which can have resulted only from differences in the excitation level in the 'direction channel' on the intact side. This would mean that the magnitude of the sensory signal is also taken into account during processing. As in the intact animal, and also in case of connective transection between SOG/TG1, turning angles were usually in the range of 30° to 90°, the results of Fig. 3 indicate the existence of pathways which cross the midline in SOG and limit the turning movement to normal angles. Elimination of these pathways results in a higher percentage of turns toward the intact side (when stimulating from the transected side, cf. Fig. 2a, b, c), as well as in greater turning angles (Fig. 3).

The specific effects of cutting the circumesophageal connective are consistent with the role of the SOG as an important distributor of commands for the initiation of motor programs, suggested on the basis of anatomical and electrophysiological findings (Kien 1980, 1983; Kien and Altman 1979; see also Boyan and Altman 1985; Hedwig 1985). However, nothing is yet known about the way in which brain, SOG and thoracic ganglia (especially the two halves of the thoracic-ganglia) interact to trigger and coordinate the rapid turning reaction. Clarification of these interactions will require experiments with other methods (cf. Elsner 1975; Robertson and Pearson 1982, 1983; Kien 1983).

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