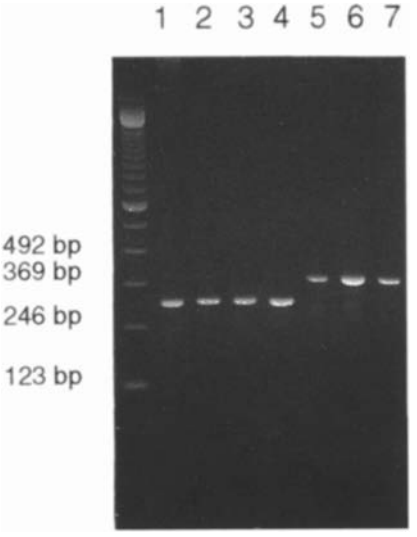

Fig. 1. Size fraction of PCR-amplified mtDNA fragments on a 2.5% agarose gel. The two primers used were C2-J-3400 (COII-specific) and TK-N-3785 ($tRNA^{lys}$-specific); for primer details see [14]. *1 Drosophila melanogaster* (Diptera: Drosophilidae), *2 Grylloblatta rothi* (Grylloblattoidea: Grylloblattidae), *3 Ruspolia nitidula* (Orthoptera, Ensifera: Tettigonioidea), *4 Cyphoderis monstrosus* (Orthoptera, Ensifera: Haglidae), *5 Locusta migratoria* (Orthoptera, Caelifera: Acrididae), *6 Oedipoda coerulescens* (Orthoptera, Caelifera: Acrididae), *7 Prosphena scudderi* (Orthoptera, Caelifera: Pyromorphidae)

es that the rearrangement has occurred within Orthoptera, unquestionably a monophyletic group with respect to the other taxa in our sample.

These two sets of observations indicate that the KD→DK rearrangement has occurred at least twice during insect evolution; once in the lineage leading to Hymenoptera, and once in the lineage leading to the orthopteran suborder Caelifera. However, the overall divergence of the *Apis* genome, including the apparently high number of tRNA rearrangements (11 with respect to *Drosophila*), indicates that the observed gene orders are probably the result of quite separate rearrangement events in the different lineages. Therefore, the observations support a previous study of mtDNA gene order evolution which stressed the importance of considering rearrangements of groups of tRNA genes rather than of individual genes [13]. This has important consequences for any future studies of insect evolution using tRNA gene orders and is, to our knowledge, the first reported case of an apparently homoplastic mtDNA gene rearrangement in metazoans. The result emphasizes the need to sample both a representative number of insects, and a large enough sample of tRNA genes in order to make a correct interpretation of the data.

Received March 1 and May 8, 1995

1. Hennig, W.: Insect Phylogeny. New York: Wiley 1981
2. Kristensen, N.P., in: Insects of Australia, p. 125 (I.D. Naumann, ed.). Ithaca, NY: Cornell Univ. Press 1991
3. Pashley, D.P., McPheron, B.A., Zimmer, E.A.: Mol. Phyl. Evol. *2*, 132 (1993)
4. Liu, H., Beckenbach, A.T.: ibid. *1*, 41 (1992)
5. Flook, P.K., Rowell, C.H.F., Gellissen, G.: J. Mol. Evol. (in press)
6. Crozier, R.H., Crozier, Y.C.: Genetics *133*, 97 (1993)
7. Beard, C.B., Mills Hamm, D., Collins, F.H.: Insect Mol. Biol. *2*, 103 (1993)
8. Mitchell, S.E., Cockburn, A.F., Seawright, J.A.: Genome *36*, 1058 (1993)
9. Clary, D.O., Wolstenholme, D.R.: J. Mol. Evol. *22*, 252 (1985)
10. Perez, M.L., Valverde, J.R., Batuecas, B., Amat, F., Marco, R., Garesse, R.: ibid. *38*, 156 (1994)
11. Jermiin, L.S., Crozier, R.H.: ibid. *38*, 282 (1994)
12. Smith, M.J., Arndt, A., Gorski, S., Fajber, E.: ibid. *36*, 545 (1993)
13. Sankoff, D., Leduc, G., Antoine, N., Paquin, B., Lang, B.F., Cedegren, R.: Proc. Nat. Acad. Sci. USA *89*, 6575 (1992)
14. Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P.: Ann. Entomol. Soc. Am. *87*, 651 (1994)

Naturwissenschaften 82, 337–340 (1995)

Responses of Collicular Neurons to Acoustic Motion in the Horseshoe Bat *Rhinolophus rouxi*

A. Kleiser, G. Schuller

Zoologisches Institut der Universität, D-80333 München, Germany

The auditory world of an animal is constantly subject to spatial and temporal modulations. Several studies in mammals [1–4] and the barn owl [5, 6] demonstrated the existence of motion direction- as well as velocity-sensitive neurons in the ascending auditory system. In contrast to studies in mammals, in the barn owl free-field stimuli were used to investigate the underlying mechanisms for the processing of acoustic motion. Collicular neurons in the barn owl have sharply tuned spatial receptive fields and are organized in space maps [7], two features that could greatly enhance motion detection. Systematically organized space maps have not been found in the inferior colliculus of mammals; therefore, it may be that the spatial and temporal information of acoustic motion is processed differently in the mammalian auditory system as compared to the owl. In this study the neuronal processing of acoustic motion was investigated in the echolocating bat *Rhinolophus rouxi* using free-field stimuli. The presentation of spatially and temporally separated stimuli to simulate acoustic motion mimicks the natural hunting condition in which the bat scans the environment with echolocation pulses.

The experiments were performed on five rufous horseshoe bats, *Rhinolophus rouxi*. The standard methods for anesthesia, surgery, and stereotaxic procedures were applied as described earlier [8]. Extracellular recordings from the central nucleus of the inferior colliculus (ICc) were carried out in awake animals with mechanically stabilized pinnae. Stimuli were presented with a semicircular array of 24 speakers in the horizontal plane, 40 cm from the animal. Speakers were separated by 7.5° (Fig. 1). A sound source moving in azimuth from 90° on one side of the bat to 90° on the other side was simulated by activating the speakers sequentially. Tone pulses of 20 ms duration with 2 ms

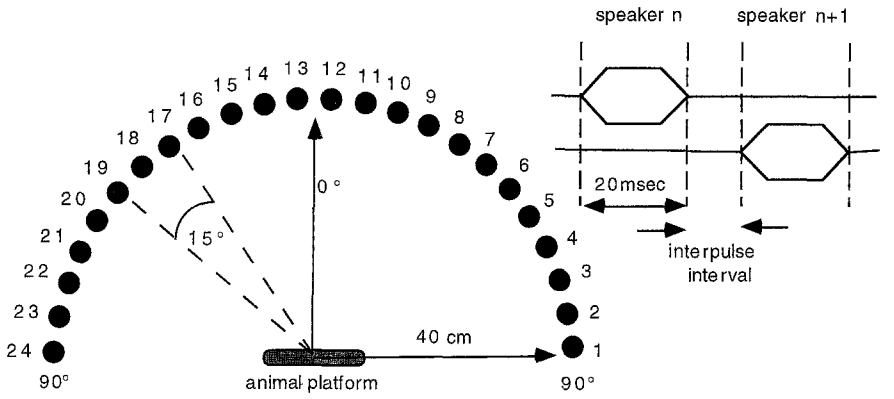

Fig. 1. Schematic top view of the experimental setup (● loudspeaker positions) and stimuli presentation (*inset*)

rise/fall time at the neuron's best frequency and 20 dB above threshold were used. Different velocities of acoustic motion were generated by changing the interpulse interval (IPI) of the spatially separated stimuli. Each velocity was generated twice, at 7.5° and 15° speaker separation (Table 1). The responses to moving stimuli were compared to stationary stimuli presented repetitively from each speaker at a constant repetition rate (F_{rep} 10/s).

The speakers were controlled by a computer that also collected and displayed the extracellulary recorded action potentials from single units. The results were averaged over five presentations.

The best azimuthal direction of the neuron's horizontal response range was characterized by a mean vector calculated from the number of action potentials (vector length) elicited at each speaker position (angle).

The results are based on 43 units having their best frequencies mostly within the frequency range of the echolocation calls (60–80 kHz, 80% of the neurons). About 2/3 of the neurons exhibited phasic responses.

Table 1. Stimulus parameters. Each velocity was generated at 7.5° and 15° speaker separation using different IPIs

Velocity	Repetition rate $T = 20$ ms		Interpulse interval	
	7.5°	15°	7.5°	15°
15°/s	2/s	–	480 ms	–
75°/s	10/s	5/s	80 ms	180 ms
150°/s	20/s	10/s	30 ms	80 ms
225°/s	30/s	15/s	13 ms	47 ms

Only two units activated by stationary stimulation were completely silent when acoustic motion was introduced.

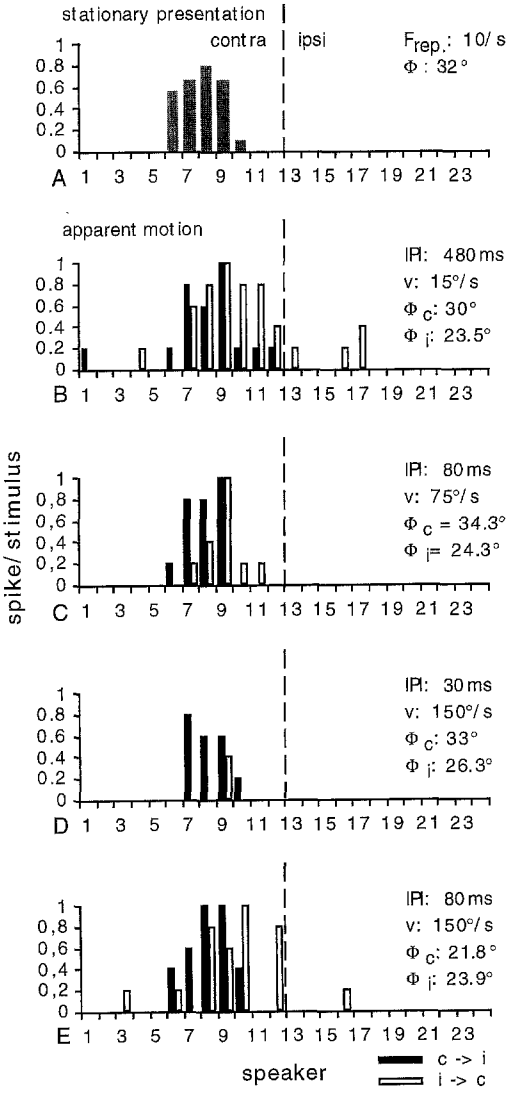

Fig. 2. Responses of an ICc unit to stationary stimuli (A) and acoustic motion in opposite directions at different interpulse intervals (B–E) and angular spacing of the active speakers. The unit responded to some spatial positions oriented towards the midline of the animal only when acoustic motion was present. The mean vector shifted towards the midline of the animal when ipsi-to-contra lalteral acoustic motion was introduced (ϕ_i). Decreasing IPI at constant angular spacing of the speakers resulted in a proceeding narrowing of the neuron's horizontal response range (B–D). Velocity was not encoded as a specific parameter. D) and E) represent the same angular velocity at different angular spacing of the speakers and IPIs. *Black bars* Contra-to-ipsi acoustic motion (speaker 1–24); *white bars* ipsi-to-contra acoustic motion (speaker 24–1); horizontal speaker separation: (B–D) 7.5° (E) 15°. $F_{rep.}$ repetition rate; *IPI* interpulse interval; v apparent velocity; ϕ_{rep}, ϕ_c; ϕ_i mean angles of the neuron's horizontal response range for stationary repetitive stimulation, sequential stimulation from contralateral to ipsilateral and vice versa

In 66% of neurons tested (27 or 41), the responses to acoustic motion stimuli differed from the stationary stimulus condition regarding the width and position of the response range, the spike distribution within the response range, or the discharge rate. The changes depended on the angular position of the successive stimuli as well as on the interpulse interval applied.

The most prominent effects of acoustic motion were changes in the horizontal response range. The histograms in Fig. 2 represent the spatial response range of a unit when tested with stationary stimuli (Fig. 2A) and with acoustic motion in both directions (Fig. 2B).

Acoustic motion enhanced responses to more medially placed speakers, expanding the azimuthal response range. Thus, speaker positions not effective during sta-

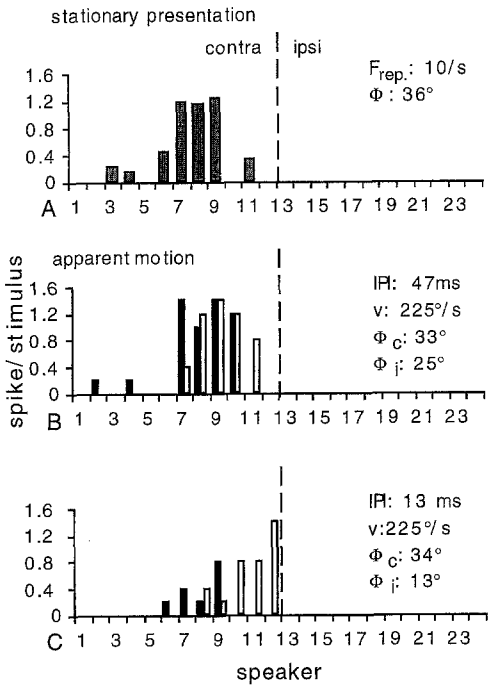

Fig. 3. Influence of the IPI; A) repetitive stationary stimulation; B) and C) acoustic motion stimuli in both motion directions. The width and the position of the neuron's response range appeared to depend strongly on the IPI as well as on the spatial speaker separation applied. *v* 225°/s; speaker separation 15° in (B) and 7.5° in (C). Symbols as in previous figures

tionary stimulation elicited responses during acoustic motion. This resulted in a shift of the mean vector on the order of 8.5° towards the midline for ipsi-to-contralateral acoustic motion.

Response range shifts towards more medial locations were found in 15 neurons, and in 12 of these neurons the shift was more prominent for ipsi-to-contralateral acoustic motion.

Decreasing interpulse intervals (IPI) at a constant angular spacing of the speakers simulates a faster velocity. We found that the width of the response range narrowed in 38% of the neurons tested with decreasing IPI (Fig. 2B–D). However, the narrowing of the response range strongly depended on the angular spacing of the speakers, as demonstrated in Fig. 2E and C, where the same interpulse interval of 80 ms was applied at 15° and 7.5° speaker separation, respectively. These two conditions represent two different angular velocities. A comparison of Fig. 2E and D reveals that, even though the apparent velocities were equal for the two conditions, the distribution of the responses, as well as the angular width of the response ranges, differed for the two stimulus constellations. The mean vector varied only little with changing IPIs for the two motion directions. The effects mentioned above for motion in the two directions are again shown in the unit in Fig. 3. Here, shortening of the IPI resulted in a nearly complete separation of the response ranges for the two directions.

In 14 of 43 ICc neurons the maximum spike rate was influenced by the interpulse interval. Decreasing interpulse intervals resulted in 12 of these units in a decrease in spike activity.

The spatial position of the speakers strongly influenced the distribution of responses. This was most apparent at large angular spacing of the speakers. With a 15° separation of successive speakers, the neuron could be stimulated using either the "odd-numbered" or the "even-numbered" speakers (see Fig. 1), providing two speaker sets with a spatial offset of 7.5°. Figure 4 shows the differences in the spike distribution within the response range for the two sets of speakers. An ipsi-to-contralateral acoustic motion using the even-numbered speaker set (Fig. 4A), evoked responses at speaker 12, 10, 8, and 6, whereas acoustic motion in the same direction, but using the odd-numbered set (Fig. 4B), was ineffective in eliciting responses at speaker positions within this range. This suggests that the marginal position of the response range most strongly influences the responses to subsequent stimuli.

Neurons in the ICc of *Rhinolophus rouxi* are sensitive to spatial and temporal components of acoustic motion. However, the effects are represented in the changes of the response range of the neurons rather than in the responses to specific spatial positions.

The graphs in Fig. 2 illustrate the different approaches used to evaluate motion sensitivity. The speaker position that evoked the maximum response during stationary stimulation was position 8 (Fig. 2A). During acoustic motion, however, position 9 evoked the maximum response at 15°/s and 75°/s (Fig. 2B, C) and position 7 at 150°/s (Fig. 2D). Taking position 8 as a reference implies that the neuron changes its directional sensitivity depending on the IPI applied. Position 9 as a reference would indicate no sensitivity to a preferred direction of acoustic motion except at short IPIs. However, for position 7 the sensitivity to different motion directions would be very strong at all IPIs. Considering the responses to only one position shows in certain cases that at 15°/s and 75°/s the unit is not directional-sensitive even though the overall changes in the response range are obvious.

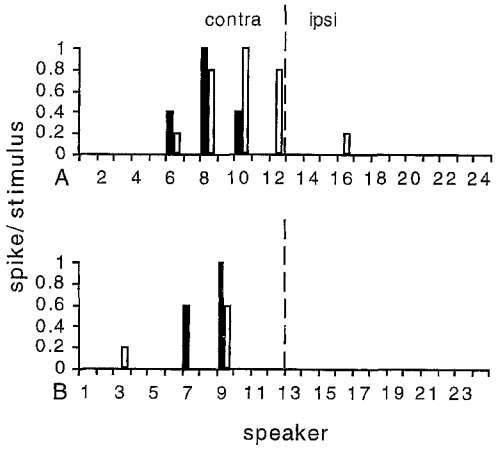

Fig. 4. Influence of the locations of acoustic motion stimuli. IPI (80 ms; speaker separation 15°. A) and B) differ in spatial position of the active speaker with "even-numbered" speakers in (A) and "uneven-numbered" speakers in (B). An angular offset of 7.5° of two successive stimuli was sufficient to alter the responses of the unit considerably. The distribution of the responses within the neuron's response range strongly depended on the specific horizontal position of the successive stimuli. Symbols as in previous figures

Using the stationary response range as a reference, the mean vector was situated at 32° lateral (Fig. 2A). It was hardly changed by contra-to-ipsilateral acoustic motion (Fig. 2A–D). However, ipsi-to-contralateral motion resulted in a medial shift of the mean vector of about 8° (ϕ_i) at all interpulse intervals applied.

The shift of the response range characterized by the mean vector seems to be more comprehensive to describe the influences of acoustic motion than spike count at a preferred angular position. The mean vector alone, however, does not describe the changes in the width of the response range, which was strongly influenced by the interpulse interval. The narrowing of the response range cannot be directly related to a velocity sensitivity of ICc neurons but indicates that the neurons responded differentially to parameters such as angular spacing of the speakers or interpulse interval (Fig. 2C–E). Moreover, it was demonstrated that specific spatial locations of stimuli influenced the responses of the neurons (e.g., Fig. 3). The response depended critically on the position and the angular spacing of the speakers as well as on the interval between the stimuli. These parameters determined the response range in the horizontal plane as well as the distribution of the responses within this range.

The observation that the horizontal response range of IC neurons generally narrowed with decreasing interpulse interval might be the result of a suppression of the lagging pulse by the leading pulse. The suppressive interaction between separated azimuthal positions is dependent on both the angular and the temporal distance. By shortening the interpulse interval, stimuli at increasing angular distance are affected by the suppression. The narrowing indicates that the balance of excitatory and inhibitory influence of moving stimuli at a given time may crucially depend on their spatial separation. Suppression effects or forward masking are commonly demonstrated features of the ascending subcollicular pathway. Auditory nerve fibers, for example, exhibit forward masking up to 40 ms after the leading stimulus [9] and a temporal interaction of spatially separated clicks in the cat IC exhibited effects up to 100 ms [10].

The shift of the response range from the lateral towards the midsagittal plane, involving stimulus locations which were not effetive during stationary repetitive stimulation, could be interpreted as an absence of inhibition or as a disinhibition of the neuron. Ineffectiveness of inhibition could be attributed to the transient characteristics of the stimuli, whereas the disinhibition would involve an active mechanism. Similar response range shifts were also found in the IC of the mustache bat using free-field acoustic motion [11] and in IC neurons of cats and gerbils using earphones [12].

Different mechanisms of inhibition seem to influence the responsiveness of an ICc neuron, resulting in a narrowing and a spatial shift of the horizontal response range during acoustic motion, and the mechanisms appear to operate at different time constants.

The encoding of parameters of moving sound sources, like the spatial and temporal constellation of successive stimuli, may serve as the basis for acoustic motion detection at higher levels in the mammalian brain.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 204 Gehör) and the Claussen-Stiftung im Stifterverband für die Deutsche Wissenschaft. We thank J.C. Dye, B. Grothe, T.J. Park, and G. Neuweiler for their helpful comments on this manuscript as well as H. Prechtl for his technical support.

The experiments comply with the *Principles of Animal Care* and the *Deutsches Tierschutzgesetz.*

Received May 11, 1995

1. Rauschecker, J.P., Harris, L.R.: Brain Res. *490*, 56 (1989)
2. Schlegel P., in: Animal Sonar Systems, p. 973 (R.-G. Busnel, J.F. Fish, eds.). New York: Plenum 1980
3. Spitzer, M.W., Semple, M.N.: Science *254*, 721 (1991)
4. Yin, T.C., Kuwada, S.: J. Neurophysiol. *50*, 1000 (1983)
5. Wagner, H.: Naturwissenschaften *77*, 439 (1990)
6. Wagner, H., Takahashi, T.: J. Neurophysiol. *68*, 2063 (1992)
7. Knudsen, E.I., Konishi, M.: ibid. *41*, 870 (1978)
8. Schuller, G., Radtke-Schuller, S.: Exp. Brain Res. *79*, 192 (1990)
9. Harris, D.M., Dallos, P.: J. Neurophysiol. *42*, 1380 (1979)
10. Yin, T.C.T.: Neuroscience *14*, 5170 (1994)
11. Wilson, W.W., O'Neill, W.E.: Assoc. Res. Otolaryngol. Abstr. *1995*, 257
12. Semple, M.N., Spitzer, M.W.: ibid. *1995*, 520

