

LOCALIZATION OF TONES AND NOISE IN THE HORIZONTAL PLANE BY UNRESTRAINED HOUSE MICE (*MUS MUSCULUS*)

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SUMMARY

House mice (*Mus musculus*, outbred strain NMRI) were trained to locate loudspeakers at the margin of a wire-mesh covered circular platform. Sound signals were tone bursts of 1, 15, 50 and 80 kHz and noise bursts (bandwidth 15–80 kHz). Localization acuity as represented by orientation angles (α) toward the speaker was determined at 5 radial distances from the centre of the platform. If the animals could localize under closed-loop conditions (with repetitive stimulation), the distributions of (α) showed a significant peak at the speaker position (0°) and mean orientation angles ($\bar{\alpha}$) for the different stimuli all varied around 0° . Distributions of (α) from open-loop tests were not peaked, i.e. mice did not localize the sound source. We calculated the median angle (β) of the distributions of orientated runs and used (β) as a measure for the accuracy of localization. Smallest values of β were 12° for 1 kHz, 15° for 15 kHz, 9.5° for 50 kHz, 8.5° for 80 kHz tone bursts and 7° for the noise bursts. The results are discussed in relation to possible localization mechanisms in mice.

INTRODUCTION

The call repertoire of house mice consists of harmonic calls of varying bandwidths in the frequency range of 2–80 kHz and of pure ultrasounds in a frequency range of 40–90 kHz (Ehret, 1975; Haack, Markl & Ehret, 1983). With regard to the different call structure (frequency bandwidth, frequency range, pure tone *vs* broad spectrum) we might expect different accuracies of localization. Different acuities have been demonstrated for tonal and for wide-band stimuli, like noise, in barn owls (Knudsen & Konishi, 1979), cats (Casseday & Neff, 1973) and monkeys (Brown, Beecher, Moody & Stebbins, 1980). The study of mice is of special interest in relation to the localization abilities of larger mammals. Because mice are small and their ears are close together, interaural arrival time differences (ΔT) are small, interaural intensity differences (ΔI) are certainly small or non-existent at low frequencies, and interaural phase differences ($\Delta \Phi$) can be processed only up to about 5 kHz, where phase coding in the auditory nerve rapidly decreases (Rose, Brugge, Anderson & Hind, 1967), so that phase is not helpful for localizing pure tones in the major part of the frequency range of the mouse auditory system (1–100 kHz; Ehret, 1974).

Key words: Sound localization, behavioural tests, mice.

In the present study we used tone and noise bursts to measure localization accuracy under free-field sound presentation and in a test paradigm in which the mouse had to localize a sound source while moving towards it. These conditions restrained the animals minimally and allowed us to obtain data from freely moving animals. Thus we can expect results on localization accuracy which come close to what the mouse may achieve under natural conditions.

MATERIALS AND METHODS

Conditioning and testing procedures

Female laboratory mice (*Mus musculus*, outbred strain NMRI) aged 2 months at the beginning of the tests were used. An operant water-reward procedure was applied. Training and testing was done in a sound-proof and anechoic room. Dim white light (<1 lx) was present during training sessions. Tests were run under a minimum of dark red light ($<<1$ lx) necessary for the video camera (National MV-341 N/G). The mice moved freely on a wire-mesh covered circular platform (diameter 155 cm). At the margin of the platform, three speakers (separated by 120°) were mounted at the head level of the mice independently of the platform. A water spout was fixed in front of each speaker at the margin of the platform. In the centre of the platform there was another water spout, and under the surface were mounted three speakers (separated by 120°) which directed their sound field upwards towards the margin of the platform.

The mice, water-deprived for 24 h before a training or test session, were trained to run from the centre of the platform toward that speaker which emitted sound signals (tone bursts of 15 kHz, see below) in order to obtain a water reward there. Only runs ending within $\pm 45^\circ$ from the speaker were rewarded if the animals finally touched the water spout. Then, in response to sound signals from the speakers under the centre of the platform, animals had to move back to the centre where they were rewarded again. After that, a new run towards a speaker at the margin was initiated. Sound signals in successive runs were presented in random order from the speakers at the margin. The position of the active speaker was not correlated with the initial body or head orientation of the mice in the centre. Only runs from the centre to the margin were videotaped and analysed. During training and testing a maximum of 10 such runs could be recorded for each mouse. After 15 days of conditioning, with one training session of 15–20 min per mouse per day, 8 of 10 females at the beginning reached a criterion of more than 50 % runs ending within $\pm 45^\circ$ of the speakers in one session. These females were used in the tests.

Data were obtained in two different stimulus presentation paradigms. (A) Under the closed-loop paradigm, sound stimuli were presented repetitively while the animals moved from the centre of the platform to the margin. Thus the females could correct their orientation during their runs to the speaker. (B) Under the open-loop condition, sound stimuli were presented only when the mice were within a 15-cm radius around the centre of the platform. Since open-loop orientation was very much less accurate, the females often reached the margin of the platform outside the $\pm 45^\circ$ reward zone. In order to avoid the experiment terminating as a result of the females not being rewarded at the end of a run, about every third run was conducted under closed-loop conditions. However, only the open-loop runs were evaluated.

Stimulus generation

Closed-loop tests were done with tone bursts of 1, 15, 50 and 80 kHz and with noise bursts (white noise, bandwidth 15–80 kHz). Open-loop orientation was tested only with 15-kHz tone bursts. These frequencies were selected because they represent characteristic points in the mouse audiogram. The most sensitive frequency range is around 15 kHz while another relative sensitivity maximum is at 50 kHz; 1 kHz and 80 kHz are, respectively, close to the low and high frequency cut-offs of the audiogram (Ehret, 1974). All stimuli had a duration of 100 ms with additional rise and fall times of 10 ms and interburst intervals of 100 ms. The three speakers in the centre of the platform always emitted 10 kHz tone bursts independent of the test stimulus emitted by the speakers at the margin.

Pure tones of known frequency (Kontron counter-timer 400B) were generated by an oscillator (Wavetek 130) and passed through an electronic switch for burst shaping. Then the tone bursts ran through an amplifier (Hewlett-Packard 465A), attenuator (Hewlett-Packard 350D), and another amplifier (Exact 170) to the speakers. Continuous white analogue noise was produced in a function generator (Wavetek 132) and passed through a bandpass filter (Krohn-Hite 3323R; 48 dB/octave initial slope) to the electronic switch and from there, as described above, to the speakers. The 1- and 10-kHz tone bursts were emitted by dynamic speakers (Dynaudio 28) while those of 15, 50 and 80 kHz and the noise bursts were emitted by electrostatic speakers (see Machmerth, Theiss & Schnitzler, 1975), which had a linear ± 1 dB frequency response between 15 and 100 kHz. Sound pressure levels were measured with a calibrated 6.35 mm condenser microphone (Bruel & Kjaer 4135) and sound level meter (Bruel & Kjaer 2606). The tests were run at the following SPL values (re. $20 \mu\text{N m}^{-2}$) in the centre of the platform: 79 dB at 1 kHz; 57 dB at 15 kHz; 75 dB at 50 kHz; 87 dB at 80 kHz; 80 dB total SPL for the noise band; 75 dB at 10 kHz (speakers in the centre). The SPL values were chosen to represent intensities of 60 dB above the lowest measured thresholds of the mouse at 10, 15 and 50 kHz and for the noise band (Ehret, 1974 and unpublished). At 1 and 80 kHz, intensities of only 55 and 32 dB above threshold could be reached because the equipment could not produce higher SPL values.

Evaluation of runs

All videotaped runs were drawn from a television screen onto transparent paper. Then the length of each run and the orientation was determined from the drawing. We marked five concentric circles on the platform at distances of 12.5, 25, 37.5, 50 and 62.5 cm from the centre. Measuring points (m_n) for the orientation during the approach of a speaker were the intersections of the running tracks with these circles (Fig. 1). Orientation angles (α), the angle of deviation of a running track from the straight line to the speaker, were measured (with 0.5° accuracy) between the line m_n and m_{n+1} and the line m_n and the centre of the speaker. These angles were plotted in frequency distributions with a class width of 5° (see Results). In addition, the total distribution (sum of distributions from the five circles) was plotted for each sound signal.

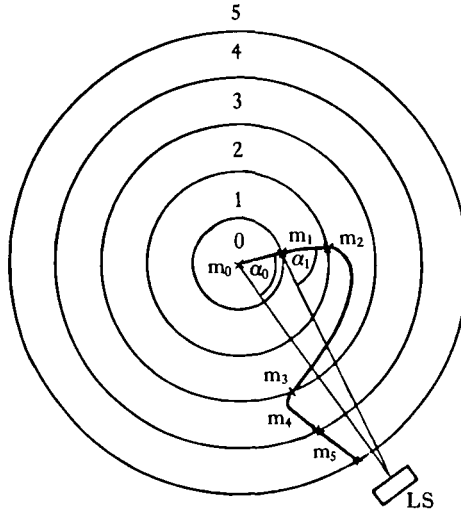


Fig. 1. Diagram which shows how orientation angles (α) were obtained from running tracks. m_0 , centre of the platform (diameter 155 cm); m_1 – m_5 , measuring points for (α) on the five circles. LS, loudspeaker.

Statistics

Circular statistics (Batschelet, 1981) were applied to determine the directedness (length of vector r) of the distributions and the mean orientation angle ($\bar{\alpha}$), which is the angle between the vector (r) and the direction to the centre of the speaker (0°):

$$r = \sqrt{\bar{x}^2 + \bar{y}^2},$$

$$\bar{\alpha} = \arctan \bar{y}/\bar{x} \text{ for } \bar{x} > 0,$$

$$\bar{\alpha} = 180^\circ + \arctan \bar{y}/\bar{x} \text{ for } \bar{x} < 0,$$

with $\bar{x} = 1/n \sum \cos \alpha$, $\bar{y} = 1/n \sum \sin \alpha$, α = orientation angle, n = number of angles measured. The Rayleigh test was used to decide whether the orientation angles (α) were randomly distributed or directed. The Watson-Williams test was applied to compare mean orientation angles ($\bar{\alpha}$).

RESULTS

We recorded and evaluated 231 runs at 1 kHz, 280 at 15 kHz (closed-loop), 260 at 15 kHz (open-loop), 265 at 50 kHz, 239 at 80 kHz and 206 with the noise band. Fig. 2 shows a sample of the original running tracks of a single mouse at 80 kHz test frequency. It is evident that most of the runs have curves and bends and that some are undirected with regard to the speaker position. Sound stimulus onset often had a first effect on the mice, initiating quick turns of head and/or body in the area between the centre of the platform and the first circle, and after that a run in a seemingly arbitrary direction. Such a run may have been modified by spontaneous directional changes which occur when mice examine their environment. If the sound signal could not exert additional directing effects because the mouse did not 'pay attention', the runs could remain undirected.

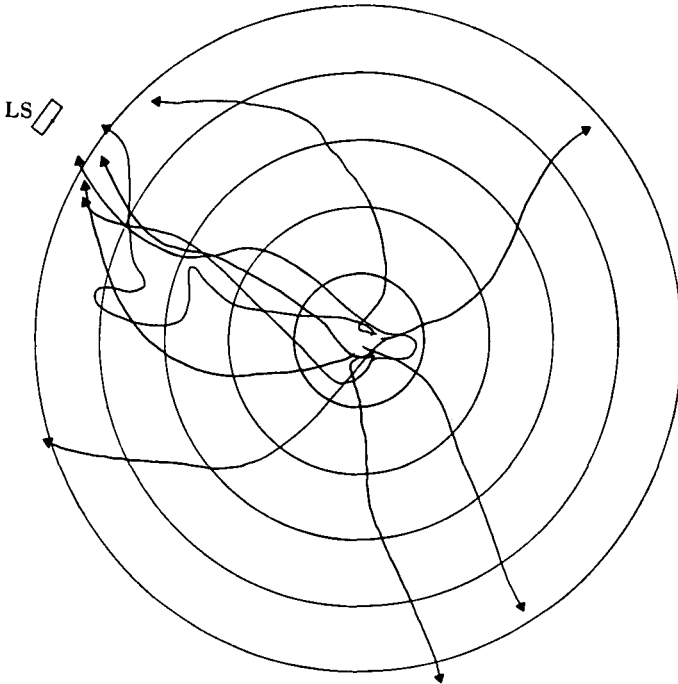


Fig. 2. An example of original running tracks of a single mouse in one test session at 80 kHz tone stimulation. The running tracks to all speakers are superimposed. LS, loudspeaker. Diameter of the platform, 155 cm.

The relative lengths of the running tracks are a first indicator of the directedness of the runs under different stimulus conditions. Fig. 6A shows that runs at 15 kHz under open-loop conditions were, on average, about one-quarter longer than closed-loop runs, which is a significant difference ($P < 0.01$, U-test). There are also significant differences between the lengths of the runs at 15 kHz compared to 80 kHz ($P < 0.05$) and to the noise band ($P < 0.05$). Thus it appears that high tone and broad band stimuli have a higher directing effect than comparatively low frequency tones.

Examples of the distributions of the orientation angles (α) are shown in Figs 3–5. Significant differences between the mean orientation angles for the different speakers did not occur, therefore values from all runs to all three speakers have been combined. All distributions from closed-loop tests are significantly directed ($P < 0.01$ except d.5 at 15 kHz: $P < 0.05$). They all have a peak near 0° (speaker position) and the values of the mean orientation angles ($\bar{\alpha}$) vary unsystematically around 0° (Table 1). This shows that mice did not prefer a certain direction in space independent of the sound presented.

The distribution d.1 from the 15 kHz open-loop test has to be considered as closed-loop, since the first circle was within the 15 cm radius within which mice could still hear the tone bursts. This distribution is significantly directed ($P < 0.05$), the mean orientation angle ($\bar{\alpha}$), however, deviates significantly ($P < 0.05$) from that of the d.1, 15 kHz closed-loop (compare Table 1), although the situation of orientation for the mice was identical in both tests. A possible reason for this difference is the less determined test structure under open-loop conditions (see Methods), which seemed to make the animals more nervous during the tests.

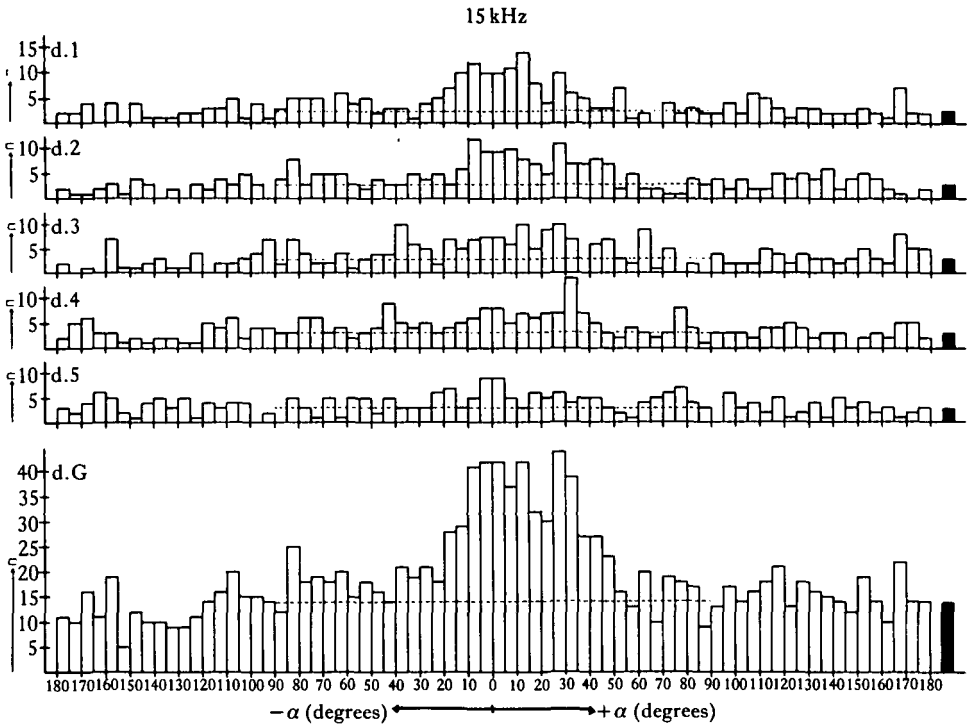


Fig. 3

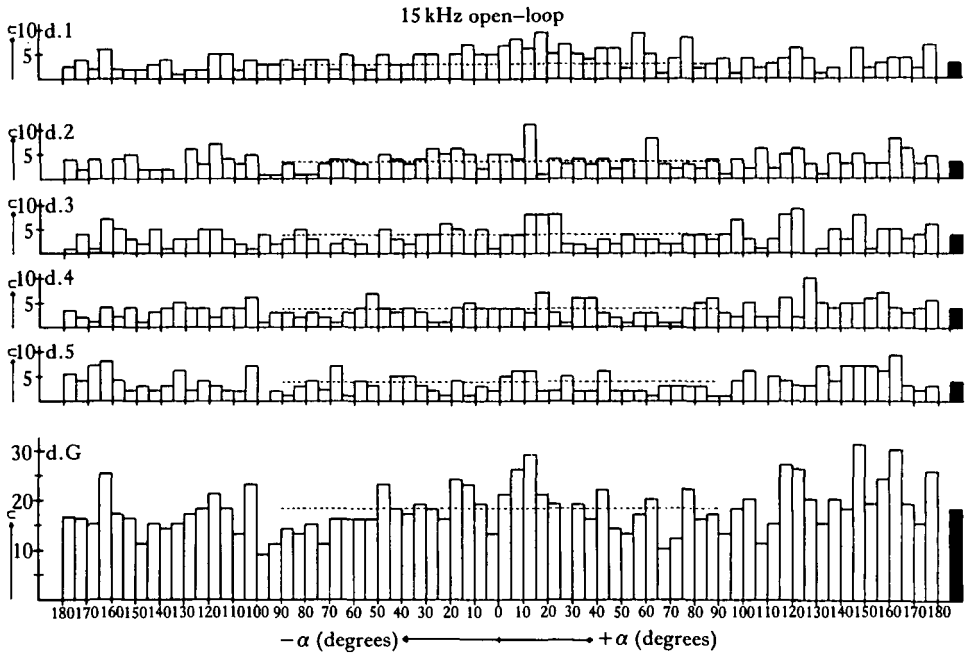


Fig. 4

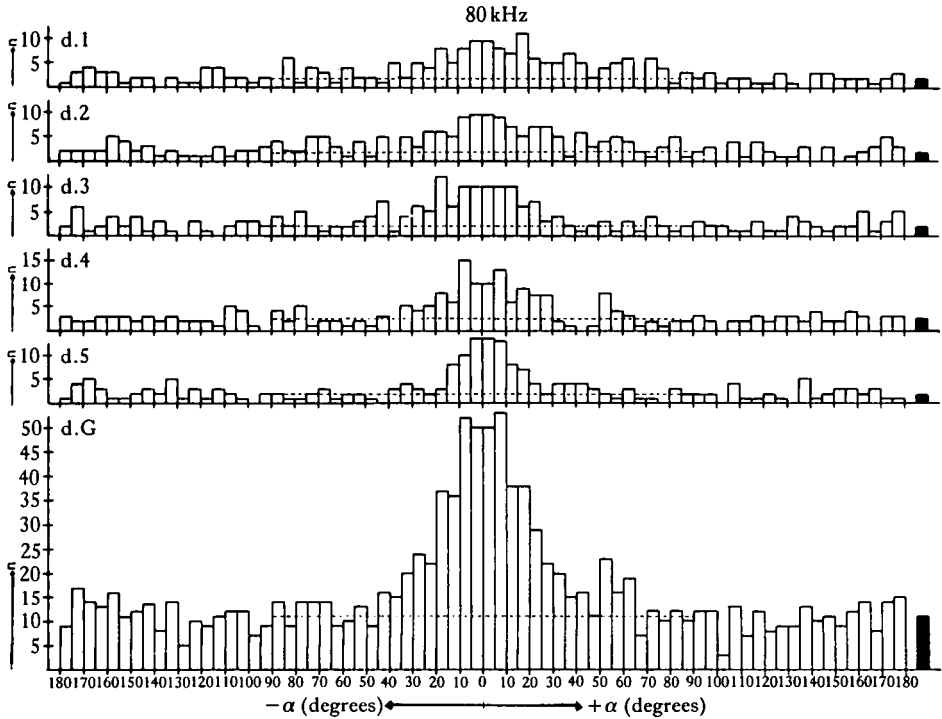


Fig. 5. An example of the distributions of the orientation angles (α) for each circle (d.1–d.5) on the platform and for the total distributions (d.G., sum of d.1–d.5). The ordinate presents the number of values of α in the respective classes of 5°-width. Speaker position, 0°. Black bars and dashed lines show the average frequency of angles in the classes outside $\pm 90^\circ$.

The peaks of all distributions of (α) decreased to the average background level within $\pm 90^\circ$ (compare Figs 3–5). Outside this range the frequencies of angles within the classes are randomly distributed around an average value which is indicated by the black bars in the figures. If we subtract this average frequency of unorientated runs from the values in all classes and then concentrate on the remaining angles within $\pm 90^\circ$, we obtain a separate distribution for the truly orientated runs (above the dashed lines in Figs 3, 5) on the background of randomly distributed unorientated runs. In Fig. 6B the vector lengths calculated from only the orientated runs are plotted. The values indicate that the runs are highly directed. The directedness (vector length) generally increases (except for 15 kHz tone bursts) with increasing distance from the centre of the platform. Mean orientation angles ($\bar{\alpha}$) of the orientated runs are given in Table 1. There are no systematic differences among $\bar{\alpha}$ of the distributions of all runs and of the orientated runs only.

Fig. 3. An example of the distributions of the orientation angles (α) for each circle (d.1–d.5) on the platform and for the total distributions (d.G., sum of d.1–d.5). The ordinate presents the number of values of α in the respective classes of 5°-width. Speaker position, 0°. Black bars and dashed lines show the average frequency of angles in the classes outside $\pm 90^\circ$.

Fig. 4. An example of the distributions of the orientation angles (α) for each circle (d.1–d.5) on the platform and for the total distributions (d.G., sum of d.1–d.5). The ordinate presents the number of values of α in the respective classes of 5°-width. Speaker position, 0°. Black bars and dashed lines show the average frequency of angles in the classes outside $\pm 90^\circ$.

Table 1. Mean orientation angles ($\bar{\alpha}$) in degrees of the distribution of α for all stimuli used (compare Figs 3-5)

Distribution	1 kHz		15 kHz (c.l.)		50 kHz		80 kHz		Noise band		15 kHz (o.l.)	
	all	orientated	all	orientated	all	orientated	all	orientated	all	orientated	all	orientated
d.1	7	4.5	1	-2.5	-2	-2	12	9	6	-1.5	23	17
d.2	4	-6	13	4	5.5	2.5	2.5	3	1	2	-	-
d.3	-5	-7.5	8	5	-1.5	-2.5	-6	-6	6	4.5	-	-
d.4	-13	-7	9	6	10	4	-3	0.5	8	-0.5	-	-
d.5	-12	-12	20	9	1.5	-4.5	4	4.5	1	-3.5	-	-
d.G	-3	-6.5	10	6	2	-0.5	3	3	2	-0.5	-	-

The angles ($\bar{\alpha}$) are presented separately for all runs and for the orientated runs (those within $\pm 90^\circ$ above the dashed lines in Figs 3,5). Since the distributions d.2-5 and d.G open-loop are not directed, it is not meaningful to calculate (α). c.l. = closed loop, o.l. = open loop.

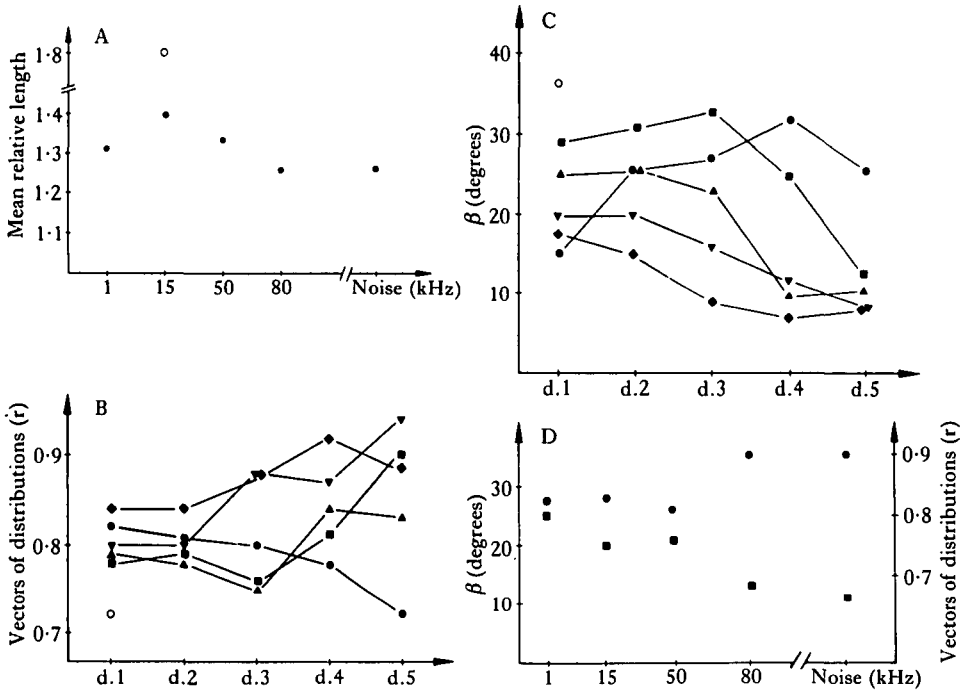


Fig. 6. (A) Mean relative lengths of the running tracks for the different stimulus conditions. The actual lengths are divided by the radius of the platform (72.5 cm). Open circle, open-loop. (B) Lengths of vectors (r) of the distributions (d.1–d.5) of the orientated runs, i.e. those contributing angles above the dashed lines within $\pm 90^\circ$ in Figs 3, 5. ■, 1 kHz; ●, 15 kHz; ▲, 50 kHz; ▼, 80 kHz; ◆, noise band. (C) Median angles (β , for definition, see text) calculated from the orientated runs (same symbols as in B). (D) Angles (β) (■) and vectors (●) from orientated runs for each stimulus calculated from the summed distributions (d.G) (compare Figs 3, 5).

The vector lengths and the mean orientation angles ($\bar{\alpha}$) are measures of the average directedness and the average deviation angle from the speaker position. These measures can give information on whether the runs as a whole are directed toward the sound source and whether the localization is unbiased by possible other influences. Neither measure is, however, very useful for estimating and predicting the actual accuracy with which mice can localize a sound source. We therefore calculated the median angle (β) of the absolute values of the angles (α) of the orientated runs, which is a measure of the variability of the directed runs and thus a measure of the localization accuracy. The angle (β) is taken from the summed distribution of respective positive and negative angles (α). The values obtained for (β) are plotted in Fig. 6C. Except for 15 kHz tone burst stimulation, β generally decreases with the distance from the centre of the platform. The average localization accuracy obviously improves (except for 15 kHz) the longer the mice could hear the sounds and the closer they came to the speakers. This is comparable to the increase of the vector lengths in Fig. 6B. Localization accuracy is best (β is smallest) for the noise band followed by the 80 kHz tone bursts, it is worst for 1 and 15 kHz tone bursts. Fig. 6D shows β and r , calculated from the summed distributions of the orientated runs, in relation to the sound stimulus. The reciprocal courses of r and β are obvious. Both measures indicated

highest directedness and localization accuracy for the noise band and the 80 kHz tone. The smallest values of β measured were 12° (1 kHz), 15° (15 kHz), 9.5° (50 kHz), 8.5° (80 kHz) and 7° (noise band).

DISCUSSION

The results have shown that house mice can localize a sound source and can do so with considerable accuracy in the high ultrasonic range. This, and the fact that the animals did not localize in the open-loop paradigm, is important when we consider, for example, pup searching behaviour of adult females (Ehret & Haack, 1982). The females have to localize ultrasound-emitting pups that are displaced outside the nest. Since the pups repeatedly emit series of calls, the females are able to localize very accurately under closed-loop conditions. The lack of orientation under open-loop conditions clearly shows that the animals depended on acoustic cues in our localization tests, when other cues, for example olfactory and visual ones, were not available for them. They needed repetitive sound stimulation during their approach, and this obviously allowed them to correct their course and to keep their movement goal-directed. We have not yet investigated why mice are unable to localize a sound source under open-loop conditions. It may be because mice have a poor short-term spatial memory or because the high motivation to run and the spontaneous changes in running direction interfered with the spatial memory to such an extent that localization of the speakers became impossible.

Suggested localization mechanisms

With a distance between the entrances of both ears of nearly 2 cm in our strain of mice, phase cues for localization are not available at 15 kHz and the higher frequency stimuli used. Only at 1 kHz can phase differences between the two ears play a significant role because the wavelength at this frequency is more than twice as long as the ear distance (Konishi, 1977). We ruled out intensity differences (ΔI) at 1 kHz as a localization mechanism by measuring sound pressure levels at the ear drum of three mice as a function of the incident angle of the sound. Intensity differences between the two ears were always smaller than 0.5 dB for angles between $\pm 90^\circ$ (G. Ehret & A. Dreyer, unpublished results). Saunders & Garfinkle (1983) also failed to measure an intensity difference larger than 0.5 dB between the two ears of the mouse at any angle of sound incidence. They found, however, interaural intensity differences of more than 6 dB at azimuths of 45, 90 and 135° of the sound source for frequencies between 10 and 20 kHz. The sound shadow effect of head and body can be expected to be even larger at higher frequencies, causing a substantial ΔI even at azimuths close to 0° . Therefore, intensity differences between the two ears are the most likely localization cues at our 15, 50 and 80 kHz test frequencies and for the noise bursts.

We found that the distributions from noise band localization were generally more peaked than those from localization of tone bursts, the frequencies of which were all included in the noise bandwidth. This is evidence for the use of spectral differences between the two ears as localization cues. Spectral differences, i.e. differences in the intensity of the frequency components of a broad-band spectrum at the two ears, arise in the same frequency range as the intensity differences for pure tones and are th

result of the frequency dependence of the sound diffraction by the head and of the sound shadow effect. In addition, the movable pinnae may contribute to intensity and spectral differences between the ears if the mouse locates high frequencies the wavelengths of which are similar to or smaller than the dimensions of the pinnae.

If arrival time differences were the only cue for sound localization in the present tests, we would have been unable to measure differences in directedness and peakedness among the distributions of the different test signals, since the temporal structure of the signals was always the same. If arrival time differences do play a significant role, the mouse must have a very precise comparison mechanism because a deviation angle of 10° , for example, means a ΔT of only about $10 \mu\text{s}$, which is the minimum interaural time difference humans can resolve under optimum conditions (e.g. Mills, 1972). It has been shown, however, that interaural time differences of the order of only $8 \mu\text{s}$ may be perceived by barn owls and used as localization cues (Knudsen, 1980), so that ΔT cannot be completely ruled out as a possible mechanism in mice.

The disadvantage of having a comparatively small interaural distance for sound localization is compensated in the mouse by hearing high up in the ultrasonic range where intensity and spectral differences are available as localization cues. Thus the mouse can achieve localization accuracies comparable with other vertebrates. If we consider β as a good approximation to the true acuity we find that noise burst localization is only slightly worse in mice (7°) than in barn owls (5° ; Knudsen & Konishi, 1979), opossum (4.5° ; Ravizza & Masterton, 1972), rat (4.5° ; Kelly, 1980), cat (5° ; Casseday & Neff, 1973) and monkey ($4-6^\circ$; Brown *et al.* 1980).

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