Postnatal development in the acoustic system of the house mouse in the light of developing masked thresholds

Günter Ehret

Fachbereich Biologie, Universität Konstanz, D-775 Konstanz, Federal Republic of Germany
(Received 21 June 1976; revised 23 February 1977)

Postnatal development of masked auditory thresholds in the house mouse *Mus musculus* were behaviorally measured from the 10th to the 24th day. An unconditioned start reflex and an unconditioned pinna reflex to tones were used for threshold determinations. Masked threshold levels first decrease from day 10 to day 12, then increase or remain constant until day 14, and finally decrease until day 16–18, where the adult levels are reached. The results are in agreement with anatomical and electrophysiological data in the literature. It was concluded that in the developing acoustical system of the mouse two effects superimpose: peripheral completion and central maturation. The data can be interpreted such that the peripheral development which lasts until day 14 dominates between the 12th and 14th postnatal day, whereas development in the central nervous system influences thresholds between day 10 and day 16–18. The widths of critical masking bands are assumed to be constant during development and determined by peripheral mechanisms.

PACS numbers: 43.66.Gf, 43.66.Dc, 43.63.Bq, 43.66.Cb, 43.80.Lb

INTRODUCTION

Auditory thresholds of pure tones which are masked by white broadband noise are the basis for the calculation of critical ratios (Fletcher, 1940), which can be seen as frequency bands of summated sound evaluation or as bandpass filters acting somewhere in the acoustic system. In this paper data from measurements of developing masked thresholds are presented for the house mouse. The mouse was selected because anatomical (Weibel, 1957; Kikuchi and Hilding, 1965; Mlonyeni, 1967) and electrophysiological (Schmidt and Fernandez, 1963; Alford and Ruben, 1963; Mikaelian and Ruben, 1965) results on the development of the acoustic system are available. In addition the development of absolute thresholds (Ehret, 1976a) and masked thresholds of the adults (Ehret, 1975, 1976b) have also been measured. Thus the present investigation has a basis on which it is attempted to decide whether the width of critical masking bands is influenced by peripheral and (or) central nervous system mechanisms. If peripheral and central factors differentially influence behavioral thresholds during development, the temporal sequence of dominance of each factor may be inferred from the present results.

I. MATERIALS AND METHOD

A. Animals

Juvenile house mice (*Mus musculus*, outbred strain NMRI) from altogether seven litters (aging 10–24 days) were used in the tests. 94 subjects (Ss) were included in the measurements. To guarantee uniform stages of development only mice from litters with 12–15 pups were tested. No sex differentiation was made.

B. Apparatus

Measurements were conducted in a sound-shielded room (430×210×320 cm³). The inner walls were covered with sound absorbing rock wool. Total background noise measured between 1 and 20 kHz was maximally 28 dB *re* 0.0002 dyn/cm² (Rockland 852, bandpass from

1 to 20 kHz; Brüel & Kjær (B & K) sound-level meter 2203 with 4131 microphone), and was most probably below that at higher frequencies.

The speaker and the test area were fixed to a frame made out of metal bars (diameter 1 cm) hanging in the center of the room. The distance between speaker and test area was always 25 cm.

Pure tones of definite frequencies (Kontron counter-timer 400B) were generated in a function generator (Exact 129) and passed through an attenuator (Hewlett-Packard 350D) to channel I of an electronic switch. In the switch the tones were shaped into pulses of 100-msec duration (flat top) with additional linear rise and fall times of 10 msec. Three single pulses (300-msec intervals) formed the test signal. Broadband white noise was produced in a Wavetek 132 generator and passed through a filter (Rockland 852, bandpass from 1 to 101 kHz) and attenuator (Hewlett-Packard 350D) to channel II of the electronic switch. Passing the switch the broadband noise was mixed to the pure-tone signal and the complete stimulus finally went through a power amplifier (Exact 170) to an electrostatic speaker after Kuhl *et al.* (1954) in the modified version of Machmerth *et al.* (1975). Sound pressure levels (SPL's) of pure tones and noise were measured independently by a calibrated $\frac{1}{4}$ in. microphone (B & K 4135). The microphone output was amplified (Hewlett-Packard 466A), filtered (Krohn-Hite 3500), read on a storage oscilloscope (Tektronix 5103N), and the rms values were converted into SPL. All SPL's are expressed in decibels *re* 0.0002 dyn/cm². The noise intensity is given as the spectrum level (L_{WN} ; see Zwicker and Feldtkeller, 1967).

The response of the electrostatic speaker was flat within ± 2 dB from 15 to 100 kHz and decreased linearly below 15 kHz with a slope of about 8 dB/octave (Machmerth *et al.*, 1975). This decrease was considered in calculating thresholds.

SPL's were measured with ± 1 -dB accuracy every 1 cm in the area in which the animal could move its head.

Thus in the test, the animal's position was considered in determining actual SPL's.

C. Tests

It is difficult to get stable behavioral responses to acoustical stimulation in young developing animals. In a previous paper (Ehret, 1976a) it has been shown that two unconditioned reactions to tones, a stop reflex and a pinna reflex, can be used for threshold determinations in mouse pups. The pinna reflex should not be confused with the "Preyer reflex," which appears as a startle response to intense sound, whereas the present pinna reflex can be elicited at low intensities and can be regarded as an orientation response. In house mice the Preyer reflex appears first nine days after birth (Alford and Ruben, 1963; Mikaelian and Ruben, 1965). The Preyer reflex, however, is not a reliable measure to indicate hearing sensitivity at least in quantitative studies (Knecht, 1958; Markl and Ehret, 1973). Therefore the more sensitive pinna reflex in an orientation situation is used in the present study. Unfortunately this pinna reflex becomes visible first at day 12 after birth when the pinna detaches from the scalp. This is why one has to look for another response in tests before day 12. Here a stop reflex to tones which also can be interpreted as an orientation response is quite useful. The stop reflex, however, becomes more and more unstable after day 11; crawling mouse pups no longer stop regularly to a clearly suprathreshold tone. The problem arises, how to compare results from both methods? It is possible, however, to get data from both methods at day 12 after birth, thus method-related differences can be recognized and in such a case be considered in the discussion.

Masked thresholds were measured at 0 and 20 dB spectrum level and at the following frequencies (in the sequence of testing): 10, 5, 2, 15, 20, 30, 40, 50, 60, 80 kHz. For every age 15 subjects (always 5 Ss from one litter) from three of the seven litters were tested. The Ss of each litter were individually marked by a color code and then selected randomly for the tests. Methods of the test procedure are detailed in Ehret (1976a), and only a short description is given here.

The unconditioned stop reflex was used in 10, 11, and 12 day old mice. After the broadband noise was switched on, a S was placed on a running board (15×4 cm² with a border at the long sides) so that it faced the speaker. When the S slowly crawled towards the speaker it received the test signal of known frequency and intensity. The positive reaction was a stop on the signal often accompanied by lifting the head and sniffing around. Each S was tested in one daily session at all frequencies and both spectrum levels of noise. Masked thresholds at 0-dB l_{WN} were measured first. Before the measurement at 20-dB l_{WN} started the S had a rest of about one hour in which it was put back into the home cage. In the test the following modified method of limits was employed: first, test all frequencies at a sub-threshold level, then gradually increase the SPL in 5-dB steps, testing only those frequencies for which the threshold was not reached before. The threshold for

one frequency was defined as the lowest SPL to which a S responded positively at least twice out of three sequential signal presentations; if the S positively responded to a level three times and to the 5-dB smaller one only once, then the higher SPL was taken as threshold (criterion).

In 12–24 day old mice the described pinna reflex was taken as indicator for hearing. The reflex to the test signal could be obtained best, if the animals, crawling very slowly, were exposed to the sound from behind or from the side. The test and the criterion were as described above.

Masked auditory thresholds for adult mice were previously measured by a third technique, a conditioned eyelid reflex. This method works very well in adult mice but it is not applicable for measurements of development. Conditioning can be started at day 13 when the eyes are open, and the animals need at least three days until the response is stable. Thus, thresholds are first measurable at day 16 after birth, too late to indicate developmental changes.

For comparative purposes masked thresholds for 16–18 day old Ss were determined in the present study by means of the conditioned eyelid reflex as done with adults by Ehret (1975). The Ss worked in a cage ($13 \times 3 \times 4$ cm³) made out of metal bars (diameter 3 mm). Through the bottom an electroshock of 40 V ac and 0.5-sec duration could be administered. On every shock the Ss reacted with closing the eyes. The Ss were trained with tone-shock pairs (10-kHz, 70-dB test tone, 0-dB l_{WN}) until they invariably produced the reflex to tone stimulation alone. The threshold for each frequency was defined as the lowest SPL to which a S responded positively (eyelid reflex) on at least two out of three sequential signal presentations (test and criterion, see above). Conditioning started with 13 day old mice whose eyes had just opened. The Ss were trained twice a day during three to four days (depending on the subject) until the response became stable. The first measurements were made at day 16 after birth.

D. Statistics

A parameter-free rank test after Wilcoxon was used to test threshold differences in mice of different ages, and a Wilcoxon matched-pairs signed rank test (Sachs, 1974) was employed in testing threshold differences between frequencies in the curve for one age.

II. RESULTS

Figure 1 presents mean masked thresholds for each frequency, noise level, and age. It must be noted that at days 10–13 the animals did not respond to frequencies below 5 and above 40 kHz at the maximum available SPL's. In addition, at day 10 only 6 mice out of 15 responded; thus in this case the threshold values in Fig. 1 present only the mean of six animals. Values at 2, 5, and 10 kHz are corrected for the decrease in the emitted spectrum level at these frequencies.¹

Figure 2 shows mean thresholds and standard deviations from both stop and pinna reflex used at day 12

FIG. 1. Masked threshold curves in the postnatal development of the house mouse. Each single diagram shows masked thresholds (—●—) for 20-dB spectrum level (upper curves) and 0-dB spectrum level (lower curves) at a specific postnatal day starting at day 10. Standard deviations are exemplarily shown in masked thresholds at day 10, 11, and 12. In the other curves they are of the same magnitude but are omitted for clearness. For comparative purposes the development of absolute thresholds (curves are taken from Ehret, 1976a, ---□---) is also presented.

after birth. It can be seen that stop reflex thresholds are mostly higher than those of the pinna reflex, but significant differences appear only at 15 and 20 kHz in the case of 0-dB spectrum level ($p < 0.01$), and at 5 kHz in the case of 20 dB L_{WN} ($p < 0.05$). In all other cases no significant differences can be measured ($p > 0.1$) that is, both methods generally lead to the same thresholds which are plotted as the averaged values in Fig. 1.

Starting at the 11th postnatal day the shapes of the curves for absolute and masked thresholds are practically identical until day 13. In 11, 12, and 13 day old mice masked thresholds for 0-dB spectrum level are not significantly different ($p > 0.1$) from the absolute thresholds. Up to day 13 masked thresholds for 0 and 20 dB L_{WN} do not differ by 20 dB as would have been expected (Zwicker and Feldtkeller, 1967; Ehret, 1975). The 20-dB difference appears, however, at day 14 and stays throughout the following days. This unexpected result led to the measurement of developing absolute thresholds for white noise (10–100-kHz bandwidth) in ten additional animals. The following thresholds (expressed in spectrum levels) were found: 16.5 dB at day 11, 3 dB at day 13, -3.5 dB at day 14, -9.5 dB at day 18. These results indicate that 11–13 day old Ss were unable to hear a 0-dB spectrum level, but were already able to hear a 20-dB L_{WN} . Thus in these mice masked thresholds for 0 dB L_{WN} and absolute thresholds must be identical, which is confirmed by the experimental results (Fig. 1). Real masked thresholds for both noise levels appear from day 14.

If one now looks at the masked thresholds for 20-dB L_{WN} (Fig. 1) they show a significant decrease at all frequencies ($p < 0.01$) between day 10 and day 11, and a further significant decrease at 10 kHz ($p < 0.05$), at 15 and 30 kHz ($p < 0.01$) and at 20 kHz ($p < 0.02$) between the 11th and 12th postnatal day. In 12 and 13 day old

mice masked thresholds are not significantly different ($p > 0.05$) at all frequencies except 30 kHz, where a further decrease is noticeable ($p < 0.05$). Thresholds of 13 and 14 days old Ss differ significantly only at 40 kHz ($p < 0.01$). Thresholds of mice aged 12 days are significantly lower at 10 kHz ($p < 0.02$) and 15 kHz ($p < 0.01$) than those of 14 day old Ss. Finally, masked thresholds decrease together with absolute thresholds from day 14 to day 16 rather steadily. Values for masked thresholds at day 16 are significantly lower at all frequencies ($p < 0.02$) compared with those at day 14.

Figure 3 shows the just-described development of masked thresholds (at 20 dB L_{WN}) in another manner. Here threshold values for five characteristic frequencies are plotted against the postnatal days. The dashed curve, which follows the mean values from these fre-

FIG. 2. Masked thresholds at the 12th postnatal day measured in stop-reflex and pinna-reflex tests. The vertical bars indicate standard deviations.

FIG. 3. This figure is a partial replot of Fig. 1, but here with the postnatal days as variables. It shows the development of masked thresholds at 20 dB L_{WN} and at five characteristic frequencies. The dashed curve follows the mean threshold values of the five frequencies and demonstrates clearly the decrease, small increase, and decrease of threshold levels.

quencies, again shows very clearly the decrease of threshold values from day 10 to day 12, then the small increase to day 14, and the following decrease.

No significant differences between masked thresholds of day 16, 18, and 24 (Fig. 1) can be measured, which indicates that the level of the adults is reached. This can also be recognized from Fig. 4, where threshold values of 16–18 day old Ss, gained by the conditioned eyelid reflex, are plotted together with thresholds of adults measured by the same method (Ehret, 1975).

Summarizing all results, it can be stated that masked thresholds (valid only for 20-dB L_{WN}) first decrease from day 10 to day 12, then increase or remain constant until day 14, and finally decrease until day 16–18, where the levels of the adults are reached (Figs. 1, 3 and 4).

III. DISCUSSION

A. Course of the threshold curves

Figures 1 and 3 demonstrate that masked thresholds (20 dB L_{WN}) decrease from day 10 to day 12, remain constant or show a small increase (depending on the frequency) up to day 14, and then decrease again until day 16. At day 12 the testing method was changed from the stop reflex to the pinna reflex. Figure 2 shows to what extent the change could have influenced the general course of the thresholds. Although no significant differences (except at 5 kHz where $p < 0.05$) were measurable between results from both responses at 20-dB spectrum level, the stop reflex thresholds tend to be higher than those of the pinna reflex. That is to say, taking only the stop reflex the decrease in thresholds from day 10 to day 12 would be smaller, and considering only the pinna reflex the increase in thresholds from

day 12 to day 14 would be larger than indicated in Fig. 3. It follows, however, that in any case the original statement of decrease until day 12 and small increase up to day 14 of masked thresholds does not change.

Finally the comparison of Figs. 1 and 4 shows that in fact at day 16 to day 18 masked thresholds of the young mice reach the level of the adults. Only at 80 kHz do significant differences appear ($p < 0.02$) between young and adults (Fig. 4). The different absolute levels (at equal shapes) of the masked thresholds between pinna reflex and conditioned eyelid reflex tests reflect different sensitivities of both methods to measure auditory thresholds (Markl and Ehret, 1973).

FIG. 4. Masked thresholds measured by a conditioned eyelid reflex in 16–18-day-old mice together with values for adults (from Ehret, 1975), which were determined by the same method. Thresholds were measured at 0-dB L_{WN} (lower curves) and at 20-dB L_{WN} (upper curves).

B. Comparative analysis and consequences

Mikaelian and Ruben (1965) reported that the development of middle and inner ear structures in the mouse is largely completed at day 10 after birth. Weibel (1957) in a more detailed light microscopic study of the inner ear of the mouse found the termination of development at day 13, and Kikuchi and Hilding (1965), who investigated the cochlea of the mouse by light and electron microscopy, stated "that the organ of Corti appears well developed by the 14th day." Ruben (1967) found, however, that mitoses in the region of the organ of Corti, especially near the base, can be recognized up to the 18th postnatal day in the mouse. This late development at the base of the cochlea may be responsible for the threshold differences at 80 kHz between the adults and 16–18 day old mice (Fig. 4).

Schmidt and Fernandez (1963) measured the development of the endocochlear potential in the mouse. This potential increases from birth up to the 14th postnatal day with the largest increment between day 11 and 14. Investigations of developing cochlear potentials in the mouse (Mikaelian and Ruben, 1965) show an increase in the frequency response range from day 8 to day 11 and an increase in sensitivity from day 8 to day 14 with the largest increment from the 11th to the 14th day. Mikaelian and Ruben (1965) also measured the development of the VIII nerve action potential which can be first recorded at day 9 from the round window and at day 10 from the VIII nerve itself. The amplitude of the potential increases up to day 14 with the largest increment between day 12 and 14. In conclusion, it was demonstrated that at the 14th postnatal day structures and electrical activity in the cochlea of the mouse (measured between 0.5 and 40 kHz) have reached the values of the adults. Between day 12 and 14 the developmental speed with regard to the electrical potentials appears to be largest.

These results, especially the temporal pattern of the development of the cochlea, correspond excellently with the behavioral measurements of developing masked (present study) and absolute (Ehret, 1976a) thresholds in the mouse, and are the basis for the interpretation of the present data. It is obvious now that the decrease in masked and absolute thresholds from day 14 to day 16–18 (Figs. 1 and 3) is not due to a development in the middle and inner ear and in the auditory nerve, but must be the result of a further differentiation and maturation in higher nervous centers of the brain. In fact, Mlonyeni (1967) showed for the mouse that the number of differentiated neurons in the ventral part of the cochlear nuclei reached adult values at about the 11th postnatal day but in the dorsal part at about day 16. This result indicates that development in higher centers continues well after day 14. Pujol (1972) demonstrated electrophysiologically that in the acoustic centers of the cat's brain development proceeds centripetally. This most probably holds for the mouse too and means that development in midbrain and cortical centers continues after day 16. A functionally incomplete nervous system can be assumed to have a higher "internal noise level" than a system which is mature. If this internal

noise adds to the external masking noise, then a reduction of the internal noise level leads to an improvement of signal-to-noise ratio and by that to a decrease of masked thresholds as demonstrated during the development (Figs. 1 and 3). There are some results (Evans, 1975; Ehret, 1977) from which one can conclude that the masking bandwidths derived from critical ratios are determined within the cochlea. If this is true, the widths of these critical masking bands in the mouse remain constant after maturation of the inner ear that is from day 14 at least. What then can change is, in fact, the signal-to-noise ratio within the bandwidths.

Yet unexplained is the course of masked thresholds from day 10 to day 14. During this time the inner ear develops. The development may influence the widths of the masking bands so that they decrease according to the decrease of masked thresholds between day 10 and 12 or increase when the threshold levels increase between day 12 and 14 (Figs. 1 and 3). Following Evans (1975) and Ehret (1977) a variation of the width of masking bands should either become visible in a variation of tuning curve characteristics of auditory nerve fibers or should be based on changes in innervation characteristics within the cochlea. There are no investigations, however, dealing with developing tuning curves or innervation patterns in the mouse. Therefore it cannot be decided yet, whether the decrease and increase of masked thresholds up to day 14 are related to a decrease and increase of masking bandwidths. The alternative is that the widths of masking bands do not change after day 10; then a decrease of masked threshold values is attributed to an improvement of signal-to-noise ratio in the nervous system and an increase of masked thresholds is due to an improvement of sound transfer in the inner ear.² The advantage of this last possibility against a variation of masking bandwidths is that the steady decrease of absolute thresholds and the decrease and small increase of masked thresholds up to day 14 (Fig. 1) can be explained without further implications.

Summarizing the discussion, the following conclusions are, without further evidence, most reasonable: a development in the acoustic centers of the brain (improvement of signal-to-noise ratio) contributes to a decrease of masked and absolute thresholds from day 10 to day 16–18. This central influence is superimposed by a development within the cochlea up to day 14. The rate of cochlear development is largest between day 12 and day 14 and by that it has the largest effects on masked and absolute thresholds during this time—masked thresholds show a small increase and the sensitivity optimum at 15 kHz in the absolute threshold curve appears (Fig. 1). These conclusions are valid only if the widths of critical masking bands are determined within the cochlea and are not changing during the time of development for which behavioral thresholds were measured.

ACKNOWLEDGMENT

I wish to thank Dr. H. Markl for fruitful discussions.

*This work was supported by the Deutsche Forschungsgemeinschaft, Eh 53/1.

¹Noise level measurements for narrow-band noise (bandwidth 1-6, 1-10, and 5-15 kHz related to 2-, 5-, and 10-kHz test frequency) were made and the differences in spectrum level (17, 11, and 4 dB) to the spectrum level for broadband noise (1-101 kHz) was added to the respective masked thresholds. This correction leads to a good approximation for the true masked thresholds at these frequencies. Figure 4, shows that corrected thresholds of 16-18 day old Ss can be compared with those of adults, which were measured in a flat noise spectrum.

²An improvement of sound transfer over a frequency band has the effect of masked threshold increase (assuming that the widths of masking bands do not change), because the increase of total noise energy within the bandwidth is larger than the increase of energy of the masked tone.

Alford, B. R., and Ruben, R. J. (1963). "Physiological, behavioral and anatomical correlates of the development of hearing in the mouse," *Ann. Otol. Rhinol. Laryngol.* 72, 237-247.

Ehret, G. (1975). "Masked auditory thresholds, critical ratios, and scales of the basilar membrane of the house-mouse (*Mus musculus*)," *J. Comp. Physiol.* 103, 329-341.

Ehret, G. (1976a). "Development of absolute auditory thresholds in the house mouse (*Mus musculus*)," *J. Am. Audiol. Soc.* 1, 179-184.

Ehret, G. (1976b). "Critical bands and filter characteristics in the ear of the housemouse (*Mus musculus*)," *Biol. Cybernetics* 24, 35-42.

Ehret, G. (1977). "Comparative psychoacoustics: Perspectives of peripheral sound analysis in mammals," *Naturwissenschaften* (to be published).

Evans, E. F. (1975). "Cochlear nerve and cochlear nucleus," in *Handbook of Sensory Physiology*, Vol. V/2, *Auditory System, Physiology (CNS) Behavioral Studies Psychoacoustics*, edited by W. D. Keidel and W. D. Neff (Springer, Berlin).

Fletcher, H. (1940). "Auditory patterns," *Rev. Mod. Phys.* 12, 47-65.

Kikuchi, K., and Hilding, D. (1965). "The development of the organ of Corti in the mouse," *Acta Oto-Laryngol.* 60, 207-222.

Knecht, S. (1958). "Ist der Preyersche Ohrmuschelreflex als Hörtest verwendbar?" *Z. Vgl. Physiol.* 41, 331-341.

Kuhl, W., Schodder, G. R., and Schröder, F. K. (1954). "Condenser transmitters and microphones with solid dielectrics for airborne ultrasonics," *Acustica* 4, 19-52.

Machmerth, H., Theiss, D., and Schnitzler, H.-U. (1975). "Konstruktion eines Luftschallgebers mit konstantem Frequenzgang in Bereich von 15 kHz bis 130 kHz," *Acustica* 34, 81-85.

Markl, H., and Ehret, G. (1973). "Die Hörschwelle der Maus (*Mus musculus*). Eine kritische Wertung der Methoden zur Bestimmung der Hörschwelle eines Säugetieres," *Z. Tierpsychol.* 33, 274-286.

Mikaelian, D., and Ruben, R. J. (1965). "Development of hearing in the normal CBA-J mouse," *Acta Oto-Laryngol.* 59, 451-461.

Mlonyeni, M. (1967). "The late stages of the development of the primary cochlear nuclei in mice," *Brain Res.* 4, 334-344.

Pujol, R. (1972). "Development of tone-burst responses along the auditory pathway in the cat," *Acta Oto-Laryngol.* 74, 383-391.

Ruben, R. J. (1967). "Development of the inner ear of the mouse: a radioautographic study of terminal mitoses," *Acta Oto-Laryngol. Suppl.*, 220.

Sachs, L. (1974). *Angewandte Statistik*, 4th Ed. (Springer, Berlin).

Schmidt, R. S., and Fernandez, C. (1963). "Development of mammalian endocochlear potential," *J. Exp. Zool.* 153, 227-235.

Weibel, E. R. (1957). "Zur Kenntnis der Differenzierungsvorgänge im Epithel des Ductus cochlearis," *Acta Anat.* 29, 53-90.

Zwicker, E., and Feldtkeller, R. (1967). *Das Ohr als Nachrichtenempfänger* (Hirzel, Stuttgart).