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ELECTROPHYSIOLOGICAL STUDY OF THE MATURATION OF AUDITORY RESPONSES FROM THE INNER EAR OF THE CHICK

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SUMMARY

Three electrophysiological functions of the chick basilar papilla were studied during development by recording the compound action potential (AP) at the round window. The autidory thresholds showed a continuous maturation between the fifteenth day of incubation (E15) and the first post-hatching day (P1), when they attained adult values. Responses matured first to low frequencies and later to high frequency stimuli. The input–output (intensity–amplitude) functions matured regularly and never demonstrated the classical two slopes seen in mammals. The tuning properties, studied by tone-on-tone masking of the AP, achieved mature values before the thresholds: the Q10s reached adult values at E17 for a 500-Hz probe tone and at E19 for a 1000-Hz probe tone. The fact that a low-to-high frequency developmental trend was found with the embryonic middle ear cleared of fluids further suggests that this property of auditory ontogeny may be a function of changes in the transduction properties of the cochlea.

INTRODUCTION

The avian embryo is proving to be an excellent model for studying the relationship of structural and functional maturation in the auditory system. Like the human but unlike rodents, it is precocial with respect to auditory function. However, unlike mammals, the avian embryo is accessible for investigation and/or manipulation at any age. In addition, we are beginning to amass considerable information on the ontogeny

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of the avian auditory system, particularly in the duckling and chicken: the ontogeny of the basilar papilla has been studied at the light microscope and EM level[4,14,15,20]; the morphological development of the brain stem auditory pathways has been investigated in normal and deafferented conditions[18,22,25,32,36]; the physiological ontogeny of the cochlear nuclei has been described both in response to tonal stimuli and direct stimulation of the 8th nerve[16,21,34]; and the behavior of embryonic and hatchling chicks and ducklings in response to simple and complex acoustic stimuli has been extensively studied[11,12,13,17,19,31]. One obvious gap in this list is ontogenetic electrophysiological analyses of the output of the cochlea, that is of compound action potentials or single unit tuning properties of the 8th nerve.

In most respects the findings on avian auditory system development coincide well with what has been found in mammals[26,27,28,35] in spite of differences in the structure of the inner ear and the central auditory pathways, again suggesting that the chick may provide an appropriate model for understanding the ontogeny of the vertebrate auditory system in general. However, to fulfill this function it is crucial to further document the normal development of inner ear function and the relation between structural and functional development of the avian cochlea.

A particular problem that has been noted by many investigators is the paradoxical relationship between the ontogeny of cochlear structure and the development of responsiveness to sound; the cochlea of most animals shows a generally basal-to-apical developmental gradient, while animals respond first to low or middle frequencies and only later to high frequencies[28]. Two explanations of this apparent paradox have been forthcoming. One suggests that the embryonic middle ear, particularly when fluid-filled, results in better transmission of low rather than of high frequencies[34]. The other explanation suggest that there are ontogenetic changes in the transduction properties of the cochlea itself, such that the early maturing basal portion responds first to only low or middle frequencies, and only later to high frequencies[28,29].

The purpose of the present study is to begin describing the maturation of cochlear function in the chick. The 8th nerve compound action potential (AP) response recorded from the round window was used. The ontogeny of AP thresholds across the chick's frequency range are described and observations on the development of input–output functions and AP tone-on-tone tuning curves are presented. It is particularly important to note that all of these results were obtained folowing drainage of all fluids and mesenchymal tissues from the middle ear.

MATERIALS AND METHODS

Three groups of Hubbard X Hubbard embryonic chicks (15, 17 and 19 days of incubation; E15, E17 and E19) and two groups of hatchlings (1 day and 10 days post-hatch; P1 and P10) served as subjects. Fertilized eggs were obtained from a commercial breeder, incubated in a forced draft incubator at 37.6 °C and turned 4 times daily. All groups contained 5 chicks except P10 which had 10 subjects. Animals were anesthetized with 80 ml/kg of Ketalar (i.m.) and 1.5 ml/kg of Chloropent (i.p.). For embryos, the shell and the membranes of the egg were opened and the head of the

embryo was gently pulled out of the egg and paralyzed with 0.1 ml of Flaxedil injected in the neck muscles.

In all chicks the middle ear was exposed, a silver electrode was secured at the round window and a reference was set in the neck muscles. In embryos, to respect the aerial transmission of the sounds, both the external and the middle ear were emptied of all embryonic fluids using a cotton wick and aspiration under a Zeiss operating microscope. The preparation was then positioned under a loud-speaker in a sound-attenuated room (IAC 1200). Body temperature was constantly regulated at 38 °C during the experiment. The sound level was measured by a General Radio Electret microphone connected to a G.R. Model 1900A Wave Analyzer.

Recording of the threshold curves

Compound action potential responses were recorded at 10 frequencies ranging from 250 Hz to 4000 Hz. The stimulations consisted of 30-msec tone-bursts with a 5-msec rise and fall time. Presentation rates were 0.5/sec for embryos and 2/sec for hatchlings. After amplification the potentials were averaged on line by a PDP 11/04 computer. For each frequency the tones were attenuated until the response disappeared. The lowest intensity able to evoke a clearly observable averaged response from 100 presentations was taken as threshold.

Input–output function

The input–output functions were determined at the most sensitive frequency for each subject. The 8th nerve response was evoked by a 10-msec tone with a rectangular envelope (i.e. rise-time and fall-time = 0). Thus amplitude of the averaged N1 wave was plotted relative to the lowest intensity at which a response could be obtained under these conditions (SL).

Tuning function

The simultaneous masking procedure for establishing tone-on-tone action potential tuning curves (APTCs) described by Dallos and Cheatham[6] was used with 500-Hz and 1000-Hz probe tones. A reduction of the amplitude of the probe tone response by one-half was arbitrarily set as the masker threshold. The Q10 value was determined as a criterion of tuning sharpness.

RESULTS

N1 thresholds

The ontogeny of the N1 thresholds as a function of frequency and age is shown in Fig. 1. At E15 the thresholds were obtained for very high sound pressure and only to relatively low frequencies. They then decreased quickly as a function of age to reach mature values at P1. These levels are quite comparable to the results obtained from cochlear nucleus evoked potentials at similar ages[34].

Over this same developmental period the minimum threshold, which was obtained by 250-Hz stimulation at E15, shifted towards higher frequencies (500 Hz

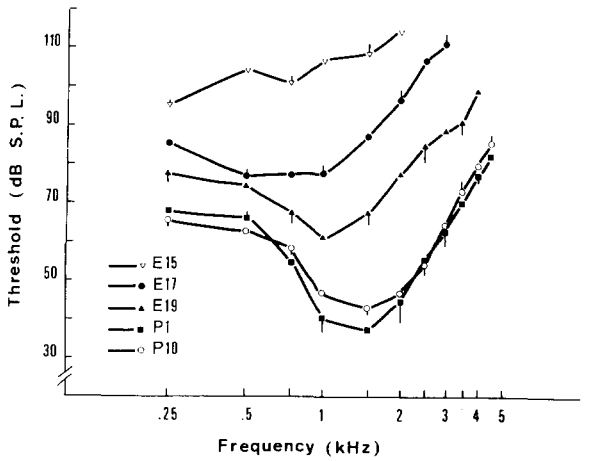

Fig. 1. Maturation of compound action potential (AP) thresholds. The thresholds are plotted as a function of frequency at embryonic day (E) 15, 17 and 19, and post-hatchling days (P) 1 and 10. Note the reduction of the threshold value and the shift of the lowest threshold toward higher frequencies as a function of age. Each curve represents the mean of 5 experiments except at P10 (10 experiments). Bars show S.E.M.

TABLE I

Evolution of the Q_{10} as a function of age for two probe tones

The adult values are obtained, respectively, at E17 and E19 for 500-Hz and 1000-Hz probe tones.

Age	*E17*	*E19*	*P1*	*P10*
Q_{10} (500 Hz)	4.97	4.27	4.15	4.95
Q_{10} (1000 Hz)	3.24	4.96	4.93	5.01

E17, 1000 Hz at E19). Minimum thresholds finally reached mature values (1500 Hz) at P1.

Tuning function

The APTC were not obtainable at E15 because thresholds were just below the maximum intensities available with our stimulating system without producing distortions.

As shown in Table I, the Q10s obtained with a 500-Hz probe tone did not vary significantly between E17 and P10. With a 1000-Hz probe tone, the Q10 increased very quickly and remained stable after E19. Representative AP tuning curves are shown in Fig. 2.

Input–output function

For the same reason as mentioned above it was impossible to record meaningful intensity functions at E15. Input–output functions for older subjects are shown in Fig. 3. At no time was it possible to differentiate clearly the two slope functions usually

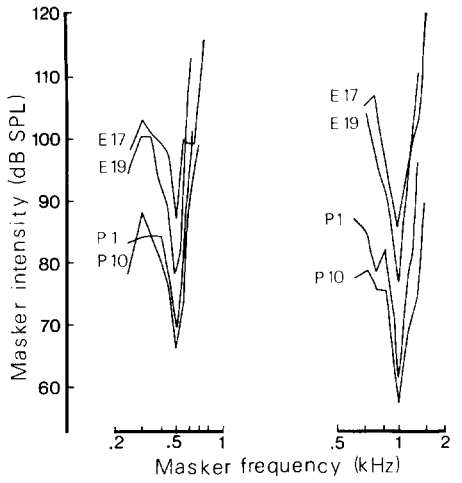

Fig. 2. Development of compound AP tuning curves in chicks. Examples of tuning curves recorded at E17, E19, P1 and P10 for two probe-tone frequencies (500 Hz and 1000 Hz). Note the increase slope with age, especially at the higher frequency (see also Table I). Age of a subject is shown on the left of each curve.

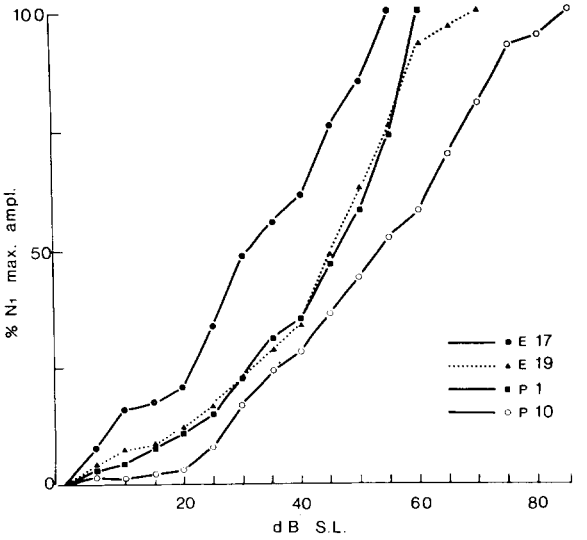

Fig. 3. Maturation of the N1 input–output curves recorded on the round window in chickens at E17, E19, P1 and P10. Each curve represents the mean of 5 experimental curves. The two slope function usually described in mammals cannot be clearly recognized at any age.

described in the literature on mammalian auditory system. At E17 the curve increased in an essentially linear fashion. At E19 and P1 a discrete two slopes appearance could be imagined but at P10 the curve presented a short segment of shallow slope followed by a long and linear intermediate slope. Interestingly at no age was a negative slope found even though greater than an 80 dB dynamic range was tested in the oldest animals.

DISCUSSION

Onset of the basilar papilla function

Our first responses were obtained at E15. Had higher sound pressures been used, we presumably could have seen responses as much as 2–3 days earlier[34]. Hirokawa[15] indicates that the afferent fibers begin to have some synaptic contacts with the hair cells in the basal region at E14, but that the synapses are not formed throughout the length of the papilla at this time. Some efferent fibers are seen but they do not connect with the sensory cells. Cohen and Fermin[4] on the other hand were able to recognize afferent synaptic complexes in the basal portion of the chick cochlea on the 10th or 11th day of incubation. The correlation of these anatomical and physiological findings is rather unimpressive, presumably due to the inexactness of various measures that have been used and variations between breeds of chickens. In any case, from these data, those of Saunders[34] and behavioral data[17], it seems that the onset of cochlea function and hearing quickly follow the establishement of the first afferent connections.

Saunders et al.[34] report that cochlear nucleus evoked potentials to tone stimulation are first seen as early as E11–E12. Two explanations of this apparent discrepancy with our data are available. First and probably most important, Saunders et al. used levels of stimulation ranging up to 120 dB (SPL), while we were not able to produce levels much above 100 dB without introducing significant distortion components. In the present context this did not cause serious concern since it is highly unlikely that embryonic chicks are ever normally exposed to sound pressure levels above 100 dB. A second possible reason for earlier responses in this previous study is that potentials were recorded through an electrode placed directly in the cochlear nuclear complex, while our round window placement is more remote from the neural generators. Given this later difference, the thresholds reported here are quite comparable (within 5 dB) of those recorded from the cochlear nuclei.

Maturation of the thresholds

Of particular interest is the fact that in this study as well as in previous reports on the cochlear nuclei[34] the first responses were evoked by relatively low frequency stimuli, and that low frequency thresholds approached mature levels more quickly than high frequency responses. In addition, in both studies the most sensitive frequency gradually increased with age until the time of hatching. For example, as seen in Fig. 1, the most sensitive frequency shifted from 250 Hz at E15, to 500 Hz at E17, to 1 kHz at E19, and to 1.5 kHz at P1 and P10. (The greatest sensitivity to 1.5 kHz at older ages was also found in our brain stem evoked potential analyses[19].) Aside from the placement of the recording electrode, the only difference between the recording conditions in the present study and that of Saunders et al. is that we were very careful to evacuate all fluids from the middle ear cavity. Thus the observed predominance of low frequencies in the younger animals is unlikely to be due to the presence of unresorbed fluids in the middle ear. This fact, together with the relative immaturity of apical portions of the cochlea which has been observed in young embryos lends some support

to the notion of ontogenetic changes in transduction at the level of the basilar membrane[28,29].

It is impossible at this time to directly correlate changes in AP thresholds with the morphological development of the basilar membrane. Descriptive studies[4,10,14,15,20] do suggest that important (and presumably functional) changes are occurring between embryonic days 11 and 19 and that there is a generally basal (proximal) to apical (distal) gradient of such changes. However, no quantitative studies have been forthcoming. In view of the present observations it would obviously be of great interest to have available quantitative information on developmental changes along the length of the basilar papilla.

Development of the tuning properties

The tuning properties seem to achieve their maturation slightly before thresholds: between E17 and E19 depending on the frequency. If we consider the data of Dallos and Cheatham[6] who show that in adult guinea pigs the Q10 decreases when the probe tone becomes louder, one could imagine that the development of the tuning properties and the maturation of the threshold are related. Our data show that these two phenomena are to some extent independent.

In the mammal it is usually suggested that the outer hair cells (OHC) play some role in the sharpening of the tuning curves. Evans[9] and Dallos and Harris[7] showed that a selective degeneration of the OHC induces a degradation of the tuning properties. They propose that the OHC could be part of the hypothetical 'second filter' while Russel and Sellick[33] propose a mechanical action of OHC on IHC to explain the tuning of intracellular potentials from inner hair cells. Other authors[2,3,23] consider a possible role of the efferent innervation to outer hair cells. In the chick, Cohen and Fermin[4] indicated that the efferent synapses reach mature structure at the base of the short hair cells at E19 which seems to nicely correspond to the maturation of the tuning properties of the basilar papilla.

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