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THE NEONATAL CHINCHILLA COCHLEA: MORPHOLOGICAL AND FUNCTIONAL STUDY

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Abstract

The developmental time scale of the cochlea varies from species to species. We investigate here the condition of the neonatal cochlea in the chinchilla, a species increasingly used in auditory research. We have examined the morphology of cochlear hair cells using scanning microscopy, and the development of auditory function during the first postnatal month by monitoring auditory brainstem evoked responses (ABR). We find that although there were some outer hair cell kinocilia present in middle and apical areas, the hair cells otherwise were mature at 24 hours after birth. Furthermore, cochlear auditory thresholds are adult-like at birth. However, whilst there was little change in ABR thresholds over one month, there is evidence of continued maturation of the central auditory system as shown by the shortening of the PI-P5 latency from 9.1 ms to 7.7 ms.

Key Words: Chinchilla, organ of Corti, development, scanning electron microscopy, auditory brainstem evoked response audiometry.

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Introduction

The development of the cochlea, morphologically and functionally, varies from species to species. In precocious animals, such as man and guinea pig, which are born in a relatively mature state, the onset of cochlear function is prior to birth (Romand, 1983). In altricious animals, such as the rat, the onset of cochlear function is not noted until 8-12 days after birth. This function usually corresponds to the time of onset of the cochlear microphonic, the action potential typically being detectable 1-2 days later. The chinchilla (*Chinchilla laniger*) is a species commonly used in auditory research, including studies on developmental plasticity. For example, in previous studies, we have examined the effects of ototoxic lesions upon plasticity in the neonatal chinchilla (Harrison *et al.*, 1993, 1996). However, whilst we know that the chinchilla is a precocious animal, no studies (to our knowledge) have shown the functional and anatomical stage of cochlear development at birth. The purpose of this study was twofold: first, to examine the morphology of the sensory epithelium of the neonatal chinchilla cochlea; second, to ascertain the level of cochlear function in the newborn animal and to follow the maturation process. For the anatomical study, we used scanning electron microscopy to assess, in particular, the maturity of inner and outer hair cells in subjects at postnatal day two. For the functional assessment, we measured auditory brainstem evoked responses (ABR) to tonal stimuli, and examined thresholds and response latencies over time from postnatal day 1 to 28. We have found some immaturity in apical cochlear hair cells but almost adult-like ABR thresholds.

Materials and Methods

Morphological studies

Normal and healthy newborn chinchilla pups were used for a morphological evaluation of the 48-hour-old cochlea. Under general anesthesia (see section below), cardiac perfusion was carried out with fixative (1.25%

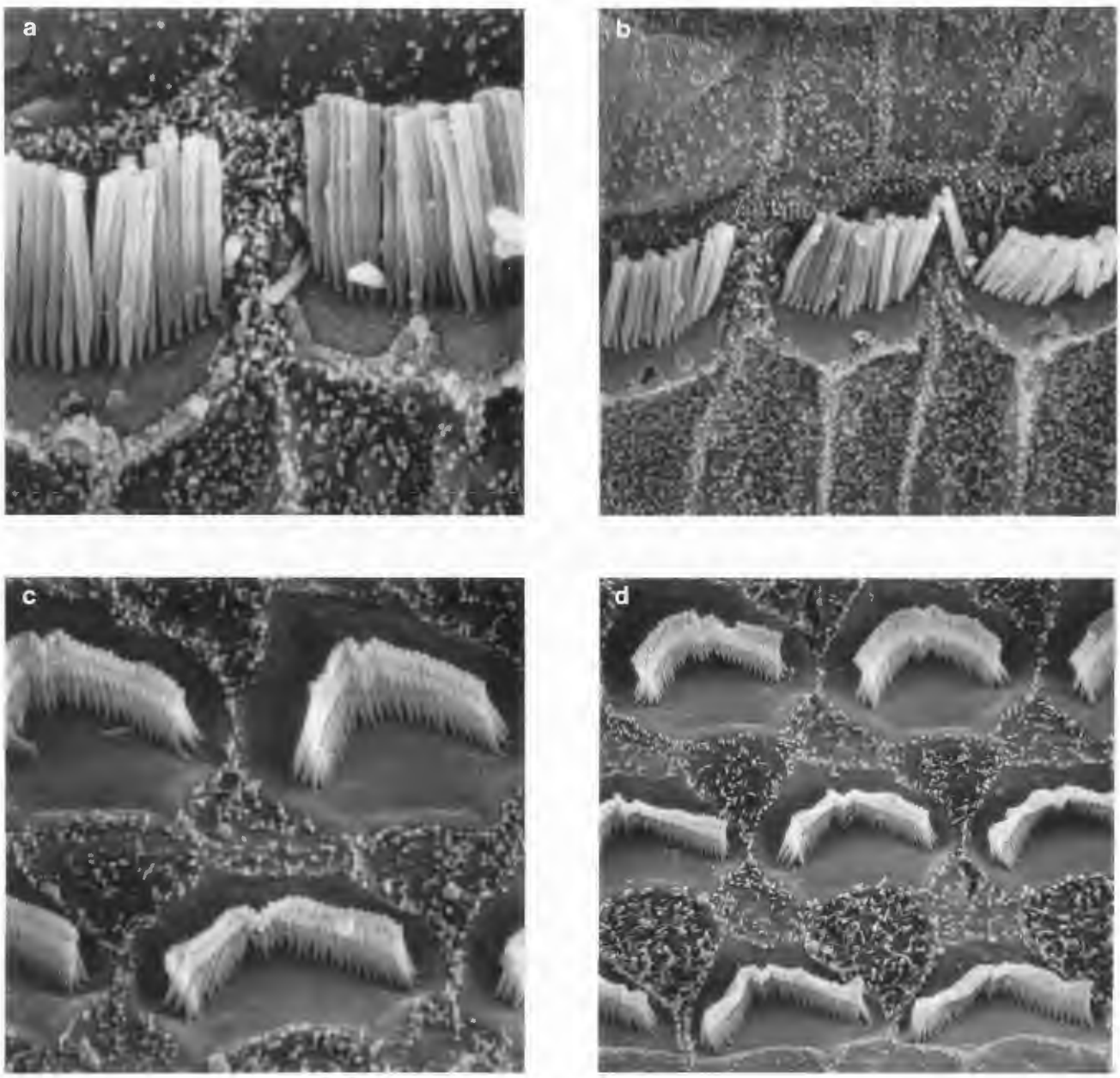

Figure 1. Typical basal turn hair cells of a 48-hour-old chinchilla pup. **(a, b)** Inner hair cell stereocilia are normal in appearance. **(c, d)** Outer hair cells appear adult-like. Photo width (P.W.) = 11 μm **(a)** 16 μm **(b)**, 13 μm **(c)** and 20 μm **(d)**.

glutaraldehyde, 1% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4), and the cochleas were removed. After opening the round and oval windows, the cochleas were slowly perfused with 2.5% glutaraldehyde in 0.1 M phosphate buffer and were prepared for scanning electron microscopy (SEM). Specimens were post-fixed 1 hour with 1% osmium tetroxide, stained with tannic acid (1%; 30 minutes), re-osmified (0.5%; 15 minutes) then dehydrated in a graded ethanol series. Following cochlear dissection, specimens were critical point dried, sputter coated with gold and examined to assess cochlear morphology.

Cochlear function

Four newborn chinchillas were used to examine cochlear function. The animals were anesthetized with a combination of ketamine (15 mg/kg), xylazine (2.5 mg/kg) and atropine (0.004 mg/kg). ABRs were then

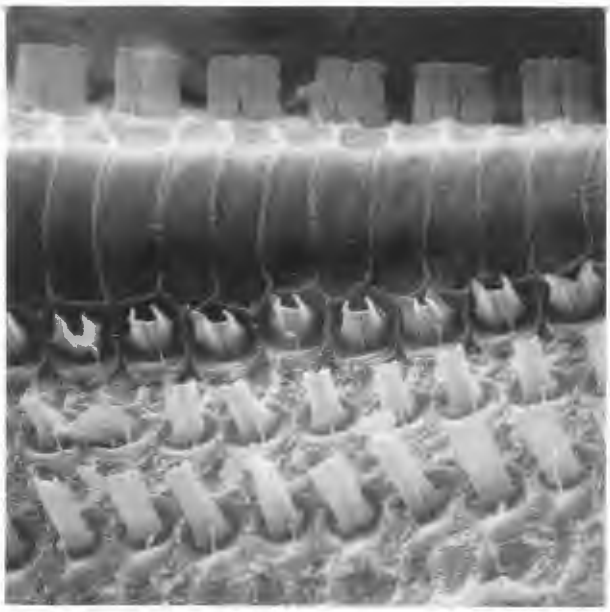

Figure 2. Middle turn of a typical 48-hour-old chinchilla pup. Inner hair cells are adult-like in appearance; single vestigial kinocilium are present at the lateral edge of each outer hair cell. P.W. = 48 μm.

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recorded using skin needle electrodes in a standard vertex to post-aural configuration. Acoustic stimuli were short tone pips (1 ms rise/fall, 2 ms plateau) at frequencies between 0.5 and 16 kHz, and presented at a range of intensity levels down to threshold. Care was taken not to acoustically traumatize the ears. Evoked potentials were band pass filtered (150 Hz - 3 kHz) and amplified conventionally. After A-D (analog-to-digital) conversion and artifact rejection, signals were averaged {Cambridge Electronic Design (Cambridge, U.K.) 1401 intelligent interface with an 80286 host). Typically, three hundred averages were used; the time window was 25 ms. Stimulus intensity was adjusted in 5 dB steps; ABR threshold was defined as the level of stimulation at which a reliable ABR signal could be just detected. Recordings were made at 24 hours, 2 days, 4 days, 1 week, 2 weeks and 1 month.

Results

Cochlear morphology

Figures 1, 2, and 3 show typical scanning electron micrographs of the hair cell bearing reticular laminas in the basal, middle and apical turns of the cochlea respectively. The inner hair cells are normal in all turns of the cochlea, having erect and well ordered stereocilia. There are no signs of immaturity, in particular, no evidence of a kinocilium. The small blebs on hair cell

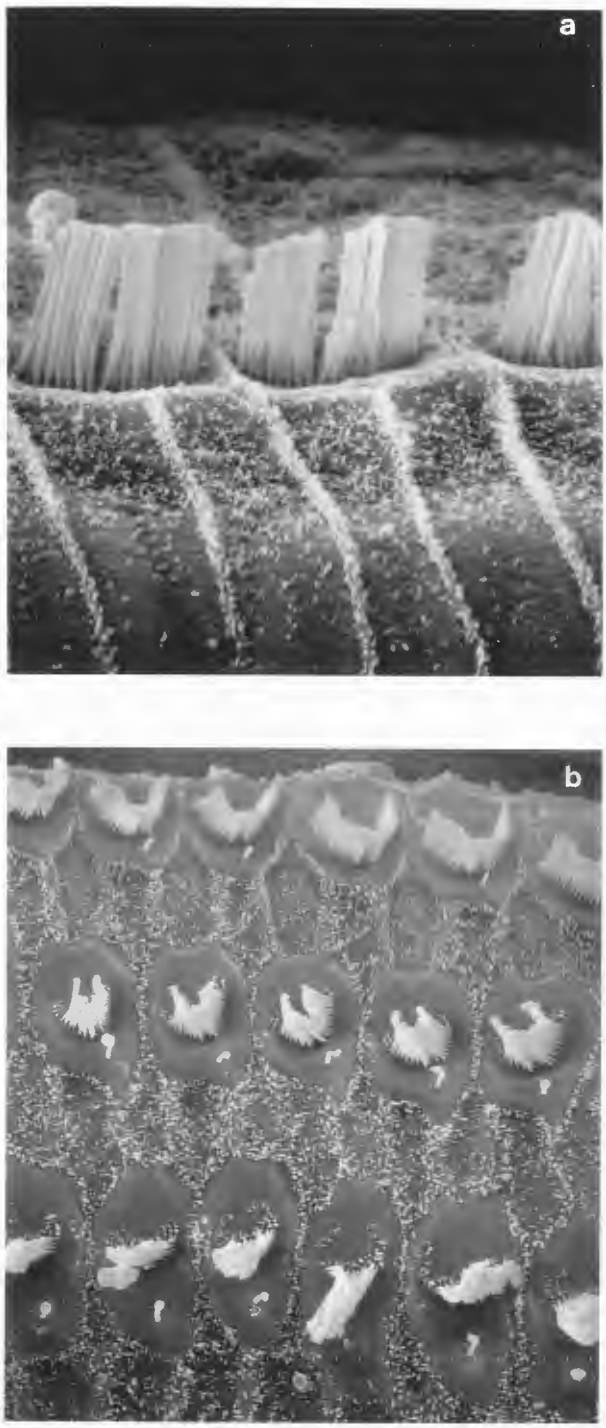

Figure 3. Hair cells of the apical turn in a typical 48-hour chinchilla pup. (a) Inner hair cells are adult-like. (b) Outer hair cells have a vestigial kinocilium, but are otherwise adult-like in appearance. P.W. = 20 μm (a) and 35 μm (b).

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surface, as evident in Figures 1a and 1b, reflect the vestigial basal body as are often found in mature cells.

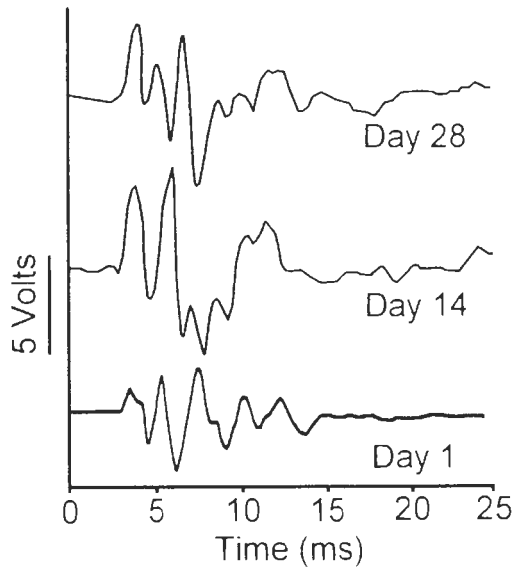

Figure 4. Typical chinchilla ABR waveforms measured 1, 14, and 28 days postnatally. Stimulus was a 4 kHz tone pip presented at approximately 30 dB above threshold.

The outer hair cells (OHC) are entirely normal in the basal turn, as shown in Figures 1c and 1d. However, in both the middle (Fig. 2) and apical turns (Fig. 3), we note the presence of a vestigial kinocilia. Otherwise, the OHCs in these regions appear to be morphologically normal and adult-like.

Auditory brainstem evoked responses

Figure 4 shows a typical ABR waveform as recorded within 24 hours of birth (lower trace), together with ABRs recorded at 14 and 28 days in the same subject with approximately the same stimulus parameters (4 kHz; 30 dB suprathreshold). The general pattern of this response did not change with subject age in that positive peaks 1 through 5 could be identified; however, the relative amplitudes of these peaks does change. Some of this variation relates to different electrode placement sites for the separate recording sessions, however, it is noticeable that in recordings at days 14 and 28, the earlier ABR peaks (particularly, P1) are more well defined. The relative latencies of the peaks also change. A standard latency measure, used clinically to assess "central conduction time," is that which is between the first and last (5th) positive peak. This P1-P5 latency was, on average, 9.2 ms, 24 hours after birth compared with 7.6 ms at age 28 days. These values are based on pooling ABR data from three subjects using, in each, tone pip stimuli at 0.5, 1, 2, 4, 8, and 16 kHz; the change in P1-P5 latency over the 28 day period is statistically significant, $p = < 0.0001$; data are presented in Table 1.

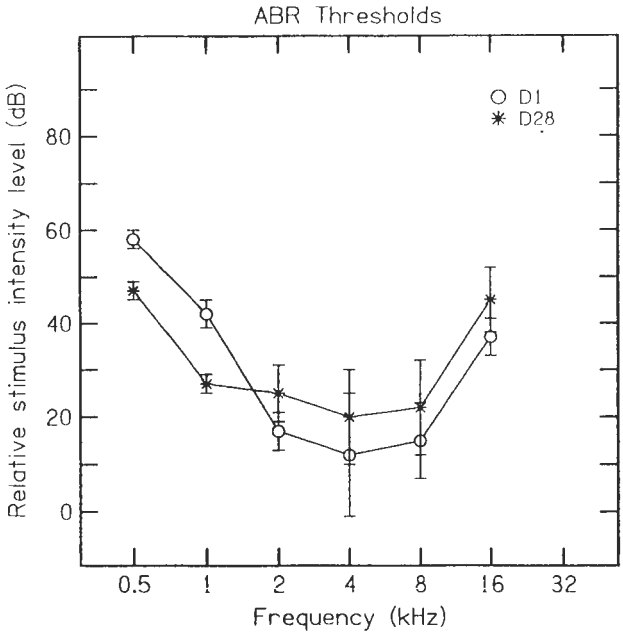

Figure 5. Average ABR thresholds for 3 pups (± 1 standard deviation) at day 1 and day 28. Frequencies above 2 kHz show no significant changes; low frequencies show some improvement with developmental age.

In Figure 5, average ABR thresholds to tone pip stimuli, at octave intervals between 0.5 and 16 kHz are shown for three animals on day 1 compared to day 28. For frequencies above 2 kHz, there is no significant change over this period. At lower frequencies (0.5 kHz and 1 kHz), there appears to be some improvement in threshold.

Discussion

The chinchilla is a precocious animal with audiometric characteristics similar to that of man (Miller, 1970; Heffner and Heffner, 1991). This species is increasingly used as an animal model in exploration of peripheral and central auditory function, and in developmental studies (this is particularly the case in North America, where chinchilla farms provide a very convenient source of experimental animals). We have shown here some of the characteristics of the cochlear condition in the neonate. Although there were some outer hair cell kinocilia present in middle and apical turns, the hair cells otherwise were mature in appearance at 24 hours after birth. As for cochlear auditory function, we suppose that there is quite a degree of maturity at birth, as judged indirectly from ABR thresholds across frequency. Thus, the ABR audiograms of Figure 5 indicate normal thresholds for high frequencies (above 2 kHz). It is possible that the improvements in low frequency ABR

Table 1. Mean difference in latency (wave V-I); n = 3; $p \leq 0.0001$.

Frequency	Day 1	Day 28
0.5	9.4	7.7
1	9.9	8.4
2	9.3	7.8
4	9.0	7.6
8	8.5	6.9
16	9.2	7.2
Mean	9.22	7.60

thresholds (at 0.5 and 1 kHz) could relate to the apparent immaturity of more apical cochlear hair cells at birth. However, we cannot determine if threshold improvements at low frequencies relate to some apical cochlear maturation or to a conductive change relating to middle ear function. The other aspect of ABR change after birth relates to the shortening of the P1-P5 latency, which perhaps is evidence of continued post natal maturation of the central auditory pathways (at least to the midbrain level).

It has been a general finding that final stages of cochlear development extend both apically and basally from the mid-cochlear region (Lorente de No, 1933; Romand and Romand, 1982). It has also been previously noted that the inner hair cells mature before the outer hair cells. Thus, any immature feature are most likely to be seen in the outer hair cells; this was certainly the case in our observations of vestigial kinocilia.

Three stages of final maturation, common to all species, have been defined by Romand (1983) as follows. The first stage is before the onset of cochlear function when the cochlea is morphologically immature. Stage II is the stage where cochlear function begins and potentials can be recognized. The hair cells lose their kinocilia at this stage, and inner hair cell innervation is more mature than that of the outer hair cells (Pujol *et al.*, 1978). Myelination of the spiral ganglion neurons also begins. This stage is reached 10-15 days after birth in altricious animals. In man, however, it is reached at 20-35 weeks gestation, and in other precocious animals, prior to birth. It is interesting to note that the kinocilia are lost from the hair cells during this phase. Remnants of these were noted in outer hair cells of the apical-most two turns of the neonatal chinchilla cochlea. Thus, whilst the degree of maturation of the hearing threshold would indicate that stage II had been reached well before birth, outer hair cells still show this marker of immaturity. Stage III development is a slow phase where final myelination of the spiral ganglion neurons occurs. This occurs post-natally in most species including man. Functional development of the cochlea can be defined as the time when evoked potentials can be elicited with acoustic stimulation. As stated above, this usually coincides with late stage II of the morphological development. The time of onset of the cochlear microphonic (CM) varies from species to species. In altricious animals such as the rat or mouse, it is noted 8-12 days after birth, and for the dog or rabbit, 5-12 days postnatal. It can be recorded prenatally in more precocious animals such as the cat or guinea pig. The compound action potential usually develops a day or two after the CM. The evolution of the normal short latency of click evoked responses is complete in a few days in the rat (Uziel *et al.*, 1981), but requires many weeks in the cat (Moore, 1981). In part, this is governed by the spatial development of the cochlea, especially at the basal end, where the most synchronized and shortest latency responses arise. In our assessment of ABR waveform latency, we used frequency specific stimuli so as to avoid spatial spread of cochlear activity. Furthermore, the use of a relative latency measure between P1 and P5 allows us to be more confident that we are indeed monitoring a maturational change central to the peripheral organ.

In summary, the chinchilla, a precocious animal, is commonly used in North America for audiological research. We have shown that the cochlear hair cells have mature appearance at birth, apart from some small kinocilia in the upper turns. We have shown neonatal chinchilla to have normal auditory (ABR) thresholds to acoustic stimuli above 2 kHz, but with some delay in developing normal thresholds to lower frequencies. Postnatally, there appears to be some further central development, as shown by a reduction of P1-P5 latency of the ABR waveform.

Acknowledgments

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Discussion with Reviewer

R. Romand: It would be interesting to hear your views or suggestions related to the first disappearance of kinocilia on OHCs in the chinchilla compared with the rat?
Authors: It is rather difficult for us to make cross-species comparisons, particularly between an animal which is relatively precocious at birth (the chinchilla) with a species like the rat, which is immature at birth. In addition, given that hair cells appear to mature differently along the length of the cochlea, such a comparison would have to be between comparable frequency regions. In any case, our observations are just a "snapshot" during early development. All we can say is that in the chinchilla, the *vestiges* of OHC kinocilia are still present 48 hours after birth in middle and apical cochlear turns, but we have no notion as to when the kinocilia degeneration was initiated. It is clear that, in the chinchilla, such rudimentary kinocilia persist to a stage when there is good auditory function. This presumably corresponds to developmental stage II as defined by Romand (1983).

R. Romand: Do you know if a 28 DAB (days after birth) chinchilla corresponds to a full mature animal from an auditory point of view?
Authors: The chinchilla is a very precocious animal; for example, it has very good motor activity at birth and appears to develop rapidly. Our data showed that the auditory thresholds as indicated by ABR to frequency specific stimuli are very low at 24 to 48 hours after birth and are similar to animals at one month of age. At this point, we do regard the peripheral system to be fully mature; cochlear thresholds and ABR waveforms are the same as in two-year-old animals. However, we cannot say that we regard the whole system as mature. It may well be that the very highest levels are still not mature at 28 days (and may never be fully mature).

R. Romand: In the discussion, you said that the cochlear function is mature at birth, but what evidence is there? You did not specifically study cochlear potentials compared with the adult.
Authors: You are correct in noting that we did not directly record cochlear microphonics or cochlear action potentials from our neonatal animals. We are judging the maturity of the peripheral auditory system on the basis of ABR thresholds to tone pip stimuli, which appear to have normal values (other than some small elevations for the low frequencies). It is on this basis that we contend that cochlear function is mature at birth. We do not think that it is a large leap of faith. Thresholds sensitivity of cochlear mechanisms depends on good IHC (inner hair cell) function as well as a mature OHC amplifier. Low ABR thresholds reflect low cochlear thresholds and are, therefore, a sensitive measure of the integrity of the peripheral transduction mechanism.

K. Hutson: The authors state that in general cochlear development extends towards apex and base from mid-regions of the cochlea. However, their results for chinchilla suggest that, at least for outer hair cells, the basal cochlea reaches a mature state prior to complete maturation of the middle and apical turns. Please comment.
Authors: While we are describing the cochlear morphology of the new born animals, we cannot say too much about the dynamics of the changes because we have not done repeated morphology assessments.

K. Hutson: Given that the results suggest that inner heir cell development is virtually complete by 24 hours, while outer hair cell development is skewed towards the apex (apex maturing later than base), would the authors go so far as to suggest that the reduction in ABR threshold at low frequencies (Fig. 5) correlates with final development of outer hair cells in the apical turn of the cochlea?
Authors: We might conclude this if we were confident that the only influence on the low frequency thresholds related to cochlear mechanisms. However, we cannot rule out the possible influence of middle ear transmission which could change over the first few days postnatally. Such effects would be expected to particularly change the low frequency transmission.