Development of Cochlear Amplification, Frequency Tuning, and Two-Tone Suppression in the Mouse

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Song L, McGee J, Walsh EJ. Development of cochlear amplification, frequency tuning, and two-tone suppression in the mouse. J Neurophysiol 99: 344–355, 2008. First published November 7, 2007; doi:10.1152/jn.00983.2007. It is generally believed that the micromechanics of active cochlear transduction mature later than passive elements among altricial mammals. One consequence of this developmental order is the loss of transduction linearity, because an active, physiologically vulnerable process is superimposed on the passive elements of transduction. A triad of sensory advantage is gained as a consequence of acquiring active mechanics: sensitivity and frequency selectivity (frequency tuning) are enhanced and dynamic operating range increases. Evidence supporting this view is provided in this study by tracking the development of tuning curves in BALB/c mice. Active transduction, commonly known as cochlear amplification, enhances sensitivity in a narrow frequency band associated with the “tip” of the tuning curve. Passive aspects of transduction were assessed by considering the thresholds of responses elicited from the tuning curve “tail,” a frequency region that lies below the active transduction zone. The magnitude of cochlear amplification was considered by computing tuning curve tip-to-tail ratios, a commonly used index of active transduction gain. Tuning curve tip thresholds, frequency selectivity and tip-to-tail ratios, all indices of the functional status of active biomechanics, matured between 2 and 7 days after tail thresholds achieved adulthood values. Additionally, two-tone suppression, another product of active cochlear transduction, was first observed in association with the earliest appearance of tuning curve tips and matured along an equivalent time course. These findings support a traditional view of development in which the maturation of passive transduction precedes the maturation of active mechanics in the most sensitive region of the mouse cochlea.

INTRODUCTION

The final stages of cochlear development in mammals are associated with the anatomical refinement of the organ of Corti and the formation of a stable place-frequency map, the so-called tonotopic map. In altricial mammals, this anatomical transformation of the end organ is accompanied by the evolution of functional correlates in which individuals first acquire the capacity to respond to low-frequency, intense airborne sounds and subsequently acquire adultlike sensitivity across the adult audible frequency range, as well as mature frequency resolving power and dynamic operating range (see Walsh and Romand 1992 for an overview). This account of peripheral auditory development is based largely on findings from auditory nerve fiber studies in the cat (Dolan et al. 1985a; Pujol and Marty 1970; Romand 1979, 1983; Walsh and McGee 1986, 1987, 1990), spiral ganglion recordings from the gerbil (Echteler et al. 1989), and frequency-threshold curves derived from cochlear potentials (Carlier et al. 1979; Puel and Uziel 1987; Shnerson and Pujol 1981).

Because large scale auditory nerve fiber studies requiring the collection of data from a sizeable population of fibers in a single animal are not practical when studying developing mice, alternate approaches must be developed to confirm the widely held assumption that peripheral auditory function in mice mimics the developmental profile described in other mammals thus far studied. Furthermore, although histological representations of cochlear development in mice are generally consistent with those observed in other mammals, strain differences among mice stress the importance of characterizing developmental profiles in each group independently, particularly with regard to the timing of peripheral events that develop rapidly in mice.

In this study, a noninvasive, evoked potential approach is used to assess peripheral auditory development generally, and the development of active and passive aspects of the transduction process more specifically. Threshold versus frequency curves, otherwise known as tuning curves (TCs), in this case derived from auditory nerve responses [i.e., wave I of the auditory brain stem response (ABR)], were used to track the evolution of peripheral auditory function in BALB/c mice. Tuning curves derived from evoked potentials have been shown to accurately represent the tuning characteristics of single auditory nerve fibers (Bauer 1978; Brown and Abbas 1987; Dallas and Cheatham 1976; Dolan et al. 1985b; Gorga et al. 1983; Harris 1979; Harris and Dallas 1979), and in turn, auditory nerve fiber TCs closely reflect the tuning characteristics of basilar membrane mechanics (Narayan et al. 1998; Ruggero et al. 2000). The basilar membrane is very sharply tuned in adult mammals, and frequency selectivity is attributed to an active transduction mechanism powered by electromotile outer hair cells (OHCs).

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distributed over a discrete and narrow portion of the cochlear spiral when set into motion by tonal stimulation (Brownell et al. 1985; Cody 1992; Nilsen and Russell 2000; Ruggero and Temchin 2005). The frequency band associated with active mechanics is coupled conceptually with the “tip” of the tuning curve (see Fig. 1) and the most sensitive frequency within the active band is referred to as the “characteristic” or “best” frequency (CF). The tuning curve includes an off-CF, relatively insensitive low frequency region frequently referred to as the “tail.” Transduction dynamics are passive in this frequency region; i.e., the process is linear. The physiological status of passive and active aspects of transduction can be assessed by considering tuning curve tail thresholds and tip-to-tail ratios, respectively. Tip-to-tail ratio refers to the difference between tip and tail threshold values, and those differences are useful estimates of the gain of the active transduction process, a process commonly referred to as “cochlear amplification” (Davis 1983). Although it is impossible to unambiguously differentiate the explicit contributions of passive and active systems operating within the cochlea during all stages of development using this approach, the relative expression of these systems can be assessed at any developmental time point.

In developing altricial mammals, it is generally held that the micromechanics of active transduction achieve maturity later than passive elements. If correct, one consequence of this developmental order is the loss of transduction linearity, because an active, physiologically vulnerable process is hypothetically superimposed on passive elements of transduction. However, this view of peripheral auditory development has been called into question on the basis of basilar membrane measurements from the basal end of the gerbil cochlea during development (Overstreet et al. 2002b). The purpose of this study was to determine whether developmental changes in the TCs representing the most sensitive region of the cochlea of mice support the traditional or alternative view.

METHODS

Animals

A total of 74 mice from 30 litters were used in this study. All experimental animals were bred in-house and were derived from offspring of breeding pairs originally obtained from the BALB/c colony (48 mice), as well as from the Tshr<sup>hryt</sup> colony (26 mice), at The Jackson Laboratory (Bar Harbor, ME). The Tshr<sup>hryt</sup> colony is maintained on a BALB/c background, and, although mice expressing FIG. 1. A forward masking paradigm was used to acquire frequency-threshold curves (tuning curves) from auditory nerve responses. A: tuning curves were derived from auditory brain stem response (ABR) wave I measurements in response to a 3-ms probe tone that followed a 50-ms masker stimulus, leaving a 10-ms interval between masker offset and probe onset. B: examples of ABRs elicited in response to a probe tone at 11.3 kHz in the absence of a masker (bottom waveform) and in the presence of a 13.5-kHz masker. C: the level of the masker that reduced wave I amplitude by 50% was designated “threshold” for the 13.5-kHz masker. D: masker frequency was varied and the paradigm was repeated, producing a tuning curve (TC) with a characteristic frequency corresponding to frequency of the probe tone. Data shown were obtained from an adult mouse at P25.
homozygosity for the mutant allele exhibit abnormalities associated with resistance to thyroid stimulating hormone, those carrying a single mutant allele (heterozygotes) exhibit normal thyroid and auditory function (Song et al. 2006a; Sprenkle et al. 2001) and are indistinguishable from wild-type mice.

Animals were studied between P12 and P90 (P represents postnatal day and P0 is designated the day of birth), and the study population consisted of randomly selected males and females, a subset of which were studied longitudinally. A group of animals from four litters were tested in half-day intervals between P13 and P15 to assess changes occurring during the rapid period of auditory system development. The care and use of all animals included in this study were approved by the BTNRH Institutional Animal Care and Use Committee.

Animal preparation

Animals were anesthetized with chloral hydrate (480 mg/kg, ip), and supplemental doses (120 mg/kg) were administered as needed, with younger animals receiving lower doses (240–360 mg/kg initial dose and 90–120 mg/kg supplemental doses). During recording sessions, body temperature was thermostatically regulated and maintained at ~38.5°C using a servo-controlled electrical heating blanket (Harvard Apparatus). Subdermal recording electrodes were positioned at the vertex (active, noninverting), infra-auricular mastoid region (reference, inverting), and in the neck region (ground). All recordings were conducted in a double-walled sound-attenuating chamber (Industrial Acoustics).

Acoustic system and calibration

Two independent channels of the sound generating system were used to deliver stimuli. Computer-generated digital waveforms were transformed by 16-bit digital to analog converters. Stimuli were amplified by a low distortion amplifier (Crown D75) and attenuated using custom-built attenuators with a range of 127 dB. Free field sound stimuli were delivered through two high-impedance piezoelectric tweeters (Radio Shack) positioned along the animal’s midline and placed 4 in. from the vertex. Tone bursts were symmetrical and ramped with a 1-ms cosine rise and fall times. Probe stimuli had a 1-ms plateau and were 3 ms in duration, whereas masker and suppressor stimuli were 50 ms in duration. Stimulus levels were calibrated using a 0.5-in. Brüel and Kjar microphone (Model 4134) positioned at the approximate location of the subject’s head during recording sessions and are reported in decibels sound pressure level (dB SPL: referenced to 20 μPa).

Recording procedure

ABRs were recorded differentially using standard procedures (Walsh et al. 1986a). Voltages recorded from the scalp were amplified 100,000 times using a Grass P511 preamplifier, band-pass filtered between 0.03 and 10 kHz, and digitized at a 20-kHz sampling rate over a 15-ms epoch using a Cambridge Electronics (CED 1401plus) A/D converter. Trials containing peak-to-peak voltages exceeding 70 μV were automatically rejected and the trial was repeated. A total of 200 trials were averaged for each stimulus condition using customized software to acquire evoked potentials.

ABR thresholds were obtained in half-octave steps, and level series were obtained in 10-dB steps from 2 to 32 kHz, except near threshold where level was changed in 5-dB steps, in approximately one half (52%) of the animals studied as part of this investigation. The results of those analyses are included in a previous report (Song et al. 2006b). Threshold was determined, and a level series was obtained in response to probe alone after stimulus delivery.

A

B

C

D

FIG. 2. The paradigm used to acquire 2-tone suppression areas from auditory nerve responses was implemented after acquisition of tuning curves. A: a suppressor tone was presented simultaneously with the masker tone, and the amount of suppression was estimated by measuring the degree to which the probe-elicited response was released from the influence of the masker. B: masker tones were matched in frequency to the probe and adjusted to the lowest level producing 100% masking (2nd waveform from bottom) of the probe-elicited response (bottom waveform). Suppressor level was varied as indicated for the waveforms shown in B, and suppression magnitude was estimated at each suppressor level (C) and was used to generate suppression areas shown as the cross-hatched areas in D. The horizontal lines in D represent the levels of the suppressor tones, and the amount of suppression produced by suppressor tones is reported as the percentage that the probe-elicited response was released from masking. The TC with a characteristic frequency (CF) corresponding to the frequency of the probe tone is also shown in D (●) in relation to suppression areas. Data shown were obtained from an adult mouse at P25.
to the probe tone alone before the acquisition of tuning curves and suppression areas in the case of remaining animals.

**Tuning curve acquisition**

A forward-masking (FM) procedure was used to derive TCs from scalp-recorded evoked potentials. As shown in Fig. 1A, probe stimuli (3-ms duration) at either 8 or 11.3 kHz were presented ~5–15 dB above threshold to produce a control response of ~1.3 μV, and a masker tone burst (50-ms duration) was presented before the probe, leaving a 10-ms interval (Δt) between masker offset and probe onset. Masker level was varied in 5-dB steps, or smaller, from threshold to a level that reduced the potential (wave I amplitude) elicited by the probe by >50%. Examples of ABRs obtained in response to the probe alone and in the presence of a masker at the indicated masker levels are shown in Fig. 1B. The amplitude of wave I of the ABR was quantified using a triangulation procedure (Walsh et al. 1986b) and plotted as a function of masker level as shown in Fig. 1C. The level of the masker that produced a 50% reduction of the probe-elicited response was computed and defined as masked threshold. Approximately three to five masker frequencies above the probe tone were presented, separated by 1/8th octave intervals, and approximately four frequencies were presented below the probe frequency in 1/8th octave intervals, followed by an additional four to five maskers at more distant frequencies in 1/4th octave intervals. For each masker frequency, a level series was obtained, and masked threshold was computed by linear interpolation. Probe alone conditions were regularly interleaved with masker conditions to assure that the amplitude of the control response did not vary significantly during the recording session. The level of each masker producing a 50% reduction in the probe-elicited response was used to construct a TC by plotting those levels (i.e., thresholds) as a function of masker frequency (Fig. 1D). Tip thresholds were determined by recording the value observed at the probe frequency, and tail thresholds were determined at 1.0 octave below the probe frequency in the case of the 8-kHz probe and at 1.5 octaves below the probe frequency for the 11.3-kHz condition. Sharpness of tuning was computed by dividing the probe frequency by the bandwidth measured 10 dB above threshold at the probe frequency (Q10). Finally, tip-to-tail ratio was determined by computing the difference in decibels between tip and tail thresholds.

**FIG. 3.** A and B: TCs, generated using the forward masking procedure at probe tones of 8 (A) and 11.3 kHz (B) are shown for BALB/c mice between the ages of P24 and P90. Each curve represents the recording from an individual animal. TCs in B are shown in comparison to ABR thresholds (a) obtained across frequency to simple tone burst stimuli (ABR audiogram). Note that the difference in threshold between the tips of the TCs and the audiogram threshold at the corresponding frequency is ~15 dB and corresponds to the level that the probe tone was placed above the ABR threshold. C and D: average adult TCs ± SD derived from the forward masking procedure (FM TC) at probe tones of 8 (C) and 11.3 kHz (D) are plotted overlying the upper boundary (solid line) of auditory nerve fiber TCs (ANF TC) having a similar CF. Frequency plotted along the abscissa was normalized relative to the probe tone or CF. Inset in C represents tuning sharpness (Q10) for FM TCs in adults (○) compared with those from individual ANFs (●) and error bars represent ±SD. Data from single ANFs were obtained in CBA/CaJ mice by Taberner and Liberman (2005) and audiogram data in B are from Song et al. (2006b).
Measurement of two-tone suppression

Two-tone suppression (2TS) was estimated by measuring the capacity of a suppressor tone to affect the magnitude of a response to a probe stimulus by releasing the probe response from the influence of a masker tone presented at a matching frequency. Suppressor effects derived from evoked potential measurements have been shown to be similar to the suppressive effects produced by a second tone on probe-evoked discharge rates of auditory nerve fibers (Dolan et al. 1985c; Harris 1979) and reflect the suppressive effects of a second tone on basilar membrane mechanical responses (Cooper 1996; Nuttall and Dolan 1993; Rhode 1977; Ruggero et al. 1992).

The stimulus conditions used to elicit 2TS are depicted in Fig. 2A. The duration and timing of the masker and probe tones were identical to those used to acquire tuning curves, and a third tone, the suppressor, was gated on and off at the same time as the masker tone and was delivered through a second transducer. The level of the probe tone was maintained at the same level used to acquire tuning curves, and the masker was equal in frequency to that of the probe and adjusted to the lowest level producing 100% masking. Suppressor level was varied as shown in a series of responses in Fig. 2B. An example of a suppression magnitude versus suppressor level curve used to generate suppression contours reported in this study is shown in Fig. 2C. This procedure was repeated for suppressor tones placed at frequencies both above and below the probe frequency using the same frequencies and step sizes used for acquiring TCs. The amount of suppression produced by suppressor tones is reported as the percentage of the probe-elicited response released from masking and is shown as hatched areas in Fig. 2D, overlying the tuning curve (○). Suppression magnitude is indicated by the length of a line segment extending down from the associated suppressor frequency-level coordinate.

Data analysis

TC tip and tail thresholds, tuning sharpness (Q10), and tip-to-tail ratios were the primary parameters of interest in this study, and changes associated with each parameter were assessed during the early period of rapid development (P12–P15) and throughout the developmental period in general (P12–P90) for each probe frequency studied. Developmental rates observed during the rapid developmental period were determined on the basis of the outcome of a least-squares linear regression analyses. Lines were fit to values extending throughout an arbitrarily determined linear phase extending between P12 and P15 and P12 and P14.5 for tip and tail thresholds, respectively; in the case of tail thresholds, the average value on P15 was slightly higher than the average on P14.5 and was consequently excluded from the regression. Likewise, in the case of tip-to-tail ratios, regressed values were restricted to measurements made between P13 and P15 because values remained constant until P13. Similar restrictions were applied in the case of tuning curve sharpness (measurements were made between P13.5 and P15) for the same reason. Estimates of adult values were based on the average of all measurements ≥P24. The significance of each regression was tested using an ANOVA approach, and developmental rates were compared in the case of each probe frequency. Results were considered statistically significant when P < 0.05, unless otherwise specified. Developmental endpoints (i.e., the age that any given parameter achieved maturati) were determined by computing the age that threshold estimates achieved values within 1 SD of average adult values.

Because TC features other than tail thresholds exhibited an extended period of gradual maturation, developmental changes in tuning curve parameters other than tail thresholds were alternatively described by an exponential in the form of $y = a + be^{-cx}$. The independent variable, $x$, represents age in postnatal days, and the dependent variable, $y$, represents either tip threshold in dB SPL, $Q_{10}$, or tip-to-tail ratio in dB, and $e^{-cx}$ is the exponential function, $exp(-cx)$. The value of $a$ denotes asymptote, the sum of $a$ and $b$ is the $y$-intercept, and the reciprocal of $c$ is the time constant of the function. Maturational endpoints were determined by computing the age that values fell within 5% of asymptotic values. The proportion (or percentage) of the total variation associated with each relevant parameter that was accounted for by the fitted curves was determined for both exponential fits ($R^2$) and linear regressions ($r^2$).

Comparison to data drawn from the literature was accomplished by enlarging published figures and digitizing x,y-coordinates of interest using a 12 × 12-in. computer graphics tablet (Summasketch). Selected data were plotted as described below.

RESULTS

Development of tuning

TCs derived from ABR wave I using a FM protocol and representing animals between P24 and P90 exhibited adultlike properties, as shown in Fig. 3, A and B. The most sensitive region of the TC (i.e., tip frequency) always matched the probe frequency, and tip thresholds varied over a relatively narrow
range; i.e., thresholds representing TCs centered on 8 kHz ranged from 45 to 56 dB SPL, and 39 to 56 dB SPL in the 11.3-kHz case. Measurements of tuning sharpness, estimated by computing Q10 values, were similar to those observed by Taberner and Liberman (2005) in TCs derived from single auditory nerve fiber (ANF) recordings in mice, ranging from ~4 to ~8 as shown in the inset of Fig. 3C. Furthermore, the properties of TCs generated using the FM procedure closely resembled TCs derived from the responses of individual ANFs of adult mice in virtually all aspects, as shown in Fig. 3, C and D, in which TCs derived from wave I of the ABR are shown to match the contour of TCs derived from single ANF responses (note that frequency was normalized relative to probe or CF and the frequency axis is represented in octaves relative to CF). Although tip thresholds were elevated relative to those of ANFs, much of this difference can be accounted for by noting that probe tone levels were generally 15 dB greater than threshold to the probe tone in the absence of the masker, as indicated by the audiogram (▲) in Fig. 3B.

The shapes of TCs recorded from immature mice were notably different from those of adult controls, as shown for 8- and 11.3-kHz probe tones in Fig. 4. On the first day that animals were sufficiently sensitive to acquire the masking functions necessary to generate TCs (i.e., P12–P13), TC tips were either absent or grossly elevated, and masked thresholds were uniformly high (i.e., >100 dB SPL). Neonatal animals (P13 and P13.5) were also less sensitive to high-frequency tone bursts than they were to lower-frequency stimuli; i.e., thresholds were inversely related to stimulus frequency except in the case of the 11.3-kHz probe on P13, at which time thresholds were universally in the vicinity of 120 dB SPL (Fig. 4, □ and ▒).

![Figure 5](https://www.jn.org/)

**FIG. 5.** The development of TC tip thresholds (A), tail thresholds (B), tip-to-tail differences (C), and frequency selectivity or tuning sharpness (Q10) (D). Probe frequencies (CF) were 8 and 11.3 kHz as indicated in the symbol key in A. Data values falling in the rapid development period were fitted using a least squares linear regression. Values obtained at ages greater than or equal to P24 were averaged and appear as horizontal lines. Data points represent means and error bars represent SD; for clarity, error bars are shown in 1 direction for each probe frequency (upward for 8 kHz and downward for 11.3 kHz). Note the scale change at P20 along the abscissa. Scatter plots and associated exponential fits are shown as insets.
Although thresholds improved somewhat by the middle of the 13th day, overall changes were unremarkable. On P14, thresholds continued to improve, and in the case of 11.3 kHz, relatively clear tips (CFs) were observed, although tip-to-tail ratios were small, on the order of 5–10 dB. Threshold improvement continued at a steady pace during the second half of day 14, as tip regions acquired greater definition. By P15, TCs exhibited adultlike form, although tip thresholds remained slightly elevated relative to adult values, ending a period of rapid maturation during which individual TCs nearly achieved maturity.

To quantitatively assess the development of key TC features, tip thresholds, tail thresholds, tip-to-tail ratios (i.e., cochlear amplifier gain), and tuning sharpness were plotted as a function of age as shown in Fig. 5. This exercise is particularly useful in the effort to track the development of sensitivity to low-frequency stimuli lying in the TC tail region given the relatively small range over which tail thresholds improve during development. While maturation could be reasonably represented as an exponential process when considering tip thresholds, sharpness of tuning, and tip-to-tail ratios, indicating that a period of gradual development occurs following the rapid, linear developmental phase (Fig. 5, insets), tail thresholds developed along a more linear time line (Fig. 5B) and efforts to represent developmental trends as exponential were unsuccessful.

Therefore to facilitate the comparison of tip and tail thresholds during development, maturational rates were determined by computing the slope of a best-fitting line associated with the early rapid phase of development, as shown in Fig. 5. A comparison of maturational rates recovered from regression analyses is shown in Fig. 6. Tip thresholds and cochlear amplifier gain, as well as ABR thresholds in general, matured at a significantly greater rate than tail thresholds at both 8 and 11.3 kHz. However, in the context of this study, it is most notable that tail thresholds achieved adultlike status between the 13th and 14th postnatal day, ~2 days earlier than tip thresholds when the rapid period of development was considered exclusively (Fig. 6B). In addition, significant differences in the rate of tail threshold improvement were not observed between 8 and 11.3 kHz; however, tip thresholds and amplifier gain at 11.3 kHz matured at a slightly faster rate than at 8 kHz ($P < 0.05$). As shown in Fig. 6C, TC sharpness matured at approximately the same rate for both the 8- and 11.3-kHz probe tone conditions.

These findings were consistent with developmental rate and maturational endpoint estimates based on exponential regression analysis. Maturational rates for tip threshold, amplifier gain, and tuning sharpness were higher at 11.3 than 8 kHz, and based on estimates of the age that tip thresholds achieved 95% of adult (asymptotic) values, maturity was achieved by approximately P20 and P17 for 8 and 11.3 kHz, respectively. Although efforts to fit tail threshold-age data with exponential functions failed, the delayed development of tip thresholds relative to tail thresholds is emphasized when exponential estimates of tip threshold endpoints are compared with endpoint estimates of tail thresholds based on linear projections; i.e., whereas tail thresholds matured between P13 and P14 at both probe frequencies, tip thresholds did not achieve maturity for an additional 7 days for the 8-kHz probe and 4 days for the 11.3-kHz probe conditions, respectively.

**Fig. 6.** A: comparisons between growth rates computed as slope estimates derived from least-squares linear regression fits to ABR threshold, TC tip threshold, amplifier gain (computed as the difference between the TC tip threshold and tail threshold), and TC tail vs. postnatal age plots. B: maturational endpoints (i.e., age at maturity) computed as the age corresponding to 1 SD of mean adult values. In the case of threshold estimates, ages correspond to adult values plus 1 SD, whereas in the case of amplifier gain and tuning sharpness, ages correspond to adult values minus 1 SD. C: growth rates representing sharpness of tuning ($Q_{10}$). The proportion of variance ($r^2$) accounted for by age was converted to a percentage and is indicated above each bar in A and C. Values for ABR threshold development are from Song et al. (2006b).
FIG. 7. The development of 2-tone suppression contours (color-coded) is shown in relation to the development of tuning curves (dark line) at 8 (left) and 11.3 kHz (right). Postnatal ages are indicated on the left. Suppression magnitude is plotted as the fraction of the probe-elicited response that was released from masking by the suppressor tone. The data shown are averages.
Development of two-tone suppression

Like other features of peripheral auditory development, little evidence of 2TS was observed on either P13 or P14, as shown in a sequence of suppression contour plots in Fig. 7. However, 2TS was observed on P15 and developed rapidly over the course of the following day, such that nearly adultlike suppression contours were observed by P18 for both the 8- and 11.3-kHz probe tone conditions. As shown in Fig. 7, although a distinctive TC tip was observed in the 11.3-kHz case on P14, 2TS was barely detectable, if detectable at all, at this developmental stage. This observation notwithstanding, when considered in a qualitative context, suppression generally appeared in concert with sharp tuning and appeared above and below the probe frequency at approximately the same developmental stage and grew in strength in proportion to tip sensitivity and frequency selectivity.

To quantify developmental trends associated with two-tone suppression, the maximum amount of suppression produced by suppressor tones, expressed as a percentage of the probe-elicited response, was analyzed for the 11.3-kHz condition, and the results are shown in Fig. 8. On P13, when TCs were essentially flat and individuals were grossly insensitive to stimulation, suppressor tones were completely ineffective for “low-side” suppressor tones (i.e., suppressor tones below the probe frequency), although very low levels of suppression (<10%) were detected in the case of “high-side” suppressor tones.

Although the TC shown in Fig. 7 was essentially flat on P13, evidence of an emerging tip was observed as an inflection, or an irregularity, in the frequency region of the probe tone. Additionally, barely detectable evidence of two-tone suppression was observed in what will become the frequency range of high-side suppressor tones in mature individuals. The first sign of low-side suppression was observed on P14. It is notable that a clearly defined TC tip was also observed at this age. On P14.5, the suppressive power of both low-side and high-side suppressor tones increased simultaneously, achieving a maximum suppression magnitude of 30–40%, approximately one half of the suppression power of suppressor tones observed in adults. By P15, low-side suppressor tones were in the adult range, as indicated as the cross-hatched area in Fig. 8, whereas high-side suppressors remained effective, but generally immature. Adult conditions were observed by the 18th postnatal day, although average suppression power of high-side suppressors was on the low end of normal.

The range of stimulus levels over which effective suppression was observed also increased during development and varied relative to the degree of suppression produced. For example, the range of suppressor levels necessary to release the probe-evoked response from the influence of the masker by 10% (i.e., the vertical length of the suppression region for a criterion magnitude of suppression as shown in Fig. 7) grew in an orderly manner for both high-side and low-side suppressors and achieved adultlike values by P18 (Fig. 9A). Similar results were observed when considering stimulus conditions producing greater amounts of suppression (Fig. 9, B–D).

**Discussion**

Although basic aspects of cochlear development have been studied and characterized with relative thoroughness in altricial mammals (Carlier et al. 1975; Dolan et al. 1985a; Pujol and Marty 1970; Romand 1979; Shnerson and Pujol 1981; Walsh and McGee 1986, 1987, 1990), attempts to precisely determine the timing of developmental events associated with passive and active aspects of cochlear micromechanics have been limited. It is the case, nonetheless, that findings from studies designed to track auditory nerve fiber responses to two-tone stimuli in developing cats suggest that transduction events are initially passive and are followed by the maturation of active, nonlinear processes (Fitzakerley et al. 1994a,b,c; Tubach et al. 1996).

Support for a model of cochlear development in which the micromechanics associated with active transduction are expressed later than those associated with passive transduction can be found in the outcome of studies designed to track the development of peripheral auditory tuning by recording from single auditory nerve fibers (Dolan et al. 1985a; Romand 1979, 1983; Walsh and McGee 1986, 1987), spiral ganglion cells located in the cochlear base (Echteler et al. 1989), and compound action potentials (CAPs) of the auditory nerve (Carlier et al. 1979; Puel and Uziel 1987; Shnerson and Pujol 1981). During the earliest stages of functional development, both passive and active aspects of transduction are distinctly immature based on the extreme degree of insensitivity to acoustic stimulation observed during this period (thresholds in excess of 100 dB SPL). Additionally, the generally linear character of transduction dynamics observed among altricial mammals during early stages of functional development suggest that transduction mechanics are entirely passive in character; i.e., that the cochlear amplifier operates at unity gain, or near-unity gain, during this developmental stage. The relative contribution made by active and passive mechanisms cannot be determined during this period of extreme immaturity because both transduction processes are in a state of developmental flux. However, in the final stage of cochlear differentiation, TC tail thresholds, and passive transduction by association, acquire mature features while active processes continue to develop;
i.e., tip thresholds and frequency selectivity continue to improve. Findings reported here strongly suggest that the same basic developmental profile observed in cats and other mammals applies in the case of mice.

While the principal finding of this study suggests that the development of passive aspects of transduction micromechanics precede and do not limit the development of active transduction at the 8- to 12-kHz place in the cochlea of BALB/c mice, this conclusion is open to question in the absence of direct measurements of basilar membrane motion. However, reports of basilar membrane recordings from developing mammals are limited to a single study from a single location deep in the base of the gerbil cochlea (Overstreet et al. 2002b), making it difficult to comment on the perspective of biomechanics. Despite this, circumstantial evidence supporting the primary conclusion of this study is considerable, and findings from Muller (1996) are especially informative. Muller reported that CAP responses to acoustic stimuli centered on 32 kHz acquired mature sensitivity in gerbils by roughly the 18th postnatal day in a subset of animals included in the study. This finding is consistent with the view that the auditory periphery is either mature or, more likely, rapidly approaching maturity by this age in the general cochlear region studied by Overstreet et al. (2002b). Although no evidence of an active contribution to basilar membrane movement was observed in 20-day-old gerbils generally, a subset of animals studied by Overstreet et al. (2002b) exhibited minimally compressive input-output functions, as in Muller (1996), suggesting that this extreme cochlear base had entered the final stage of development, that passive aspects of transduction were mature and that the process of acquiring active transduction mechanics was occurring dynamically. Findings from Harris and Dallos (1984), in which changes in the upper cut-off frequency of cochlear microphonic (CM) isoresponse versus frequency curves, the CM equivalent of tuning curves, were tracked between the 12th and 19th postnatal days, also support this view; i.e., although nominally adultlike conditions were achieved by P19, sharp tips did not appear on the leading edge of CM isoresponse functions until later, again supporting the position that gain associated with mature cochlear amplification is acquired relatively late in the development of cochlear function in the gerbil. This suggestion is also supported by the work of Finck et al. (1972), Woolf and Ryan (1984, 1988), Echteler et al. (1989), McFadden et al. (1996) and Mills and Rubel (1996). Ignoring the possibility that findings reported in Overstreet et al. reflect cochlear trauma, a common pitfall of...
the procedure (Overstreet et al. 2002a), results of that study seem entirely consistent with the view that the maturation of passive transduction precedes the appearance and subsequent maturation of active processes.

In the most clearly relevant reports from mammals other than the gerbil, no evidence of two-tone suppression or cubic distortion product generation has been observed in the discharge patterns of auditory nerve fibers recorded from neonatal animals (Fitizakerley et al. 1994a-c; Tubach et al. 1996), a finding that is consistent with the outcome of distortion product otoacoustic emission (DPOAE) studies (Henley et al. 1990; Lenoir and Puel 1987; Mills and Rubel 1996; Norton et al. 1991), and a finding that is consistent with the view that cochlear transduction is an essentially linear process during the earliest stages of functional development. In addition, single auditory nerve fiber TCs from neonatal cats are either tipless or, if tips are detectable they are broadly tuned, and acquire mature, nonlinear properties later in perinatal life, after tail thresholds are adultlike (Dolan et al. 1985a; Romand 1979; Walsh and McGee 1987).

Although the clearest interpretation of findings reported here, as well as findings from other laboratories, is that outer hair cell (OHC) function, and the cochlear amplifier by inference, is among the last of cochlear elements to mature, the finding that OHCs are motile in vitro (Belyantseva et al. 2000; He et al. 1994; Oliver and Fakler 1999; Pujol et al. 1991) before clear evidence of active transduction is observed in vivo is inconsistent with the view that active processes mature later in development than do passive contributions. A potential explanation of this otherwise paradoxical condition may be found in hypothyroid animals. Although electrophysiological findings suggest that the active component of transduction fails to develop in congenitally and profoundly hypothyroid mice (Walsh and McGee 2001), OHC somatic motility, the source of active mechanics, appears normal (Walsh et al. 2003), as does cochlear anatomy in general (Walsh et al. 2007). Although the precise explanation of this relationship is not immediately clear, the finding serves to emphasize the need to study cochlear amplification in vivo. Additional support for the notion that active mechanics mature in series with passive contributions was provided in findings from Walsh et al. (1998), who showed that the cochlear amplifier fails to develop properly following cochlear deafferentation in neonatal cats. Because the efferent innervation of the cochlea, and OHCs explicitly, is the last major developmental inner ear event to occur in mammals, one might also expect the activation of the amplifier to occur in concert with this event if the efferent system does indeed trigger the final step in OHC differentiation or otherwise influence the functional disposition of OHCs; e.g., alter the operating point of OHCs.

Novel and potentially important as the suggestion of Overstreet et al. (2002b) may prove to be, data collected from a variety of laboratories support the alternative position. Specifically, aside from the findings of McGuirt et al. (1995), whose endocochlear potential and compound action potential findings are in line with the suggestion of Overstreet et al. (2002b), others have shown that the sensitivity of gerbils to tone bursts in the 35-kHz range are mature by 20 postnatal days of age, as is the endocochlear potential and all other aspects of passive transduction (McFadden et al. 1996; Mills and Rubel 1998; Norton et al. 1991; Woolf and Ryan 1984, 1988). Nonetheless, if the suggestion of Overstreet et al. (2002b) proves to be an accurate characterization of basilar membrane development in the extreme base of the cochlear spiral, it will be important to determine whether the developmental dynamic that governs transduction at that location generalizes to the rest of the cochlea. Until that time, the view that passive mechanics mature first, followed by the maturation of active processes remains the most secure model of cochlear development generally.

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