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Impairments of the medial olivocochlear system increase the risk of noise-induced auditory neuropathy in laboratory mice

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Abstract

Hypothesis—Impairments of the medial olivocochlear system (MOCS) increase the risk of environmentally induced auditory neuropathy spectrum disorder (ANSD).

Background—ANSD is a problem in the neural transmission of auditory information that accounts for 10 – 15% of the cases of pediatric hearing loss. The underlying mechanisms of the disorder remain poorly understood, but noise exposure is an important risk factor. The goal of this study was to identify environmental conditions and genetic predispositions that lead to ANSD. Our approach was based on the assumption that noise induces ANSD by impeding the functional maturation of the brain's sound coding pathways. Because the MOCS adjusts the sensitivity of the inner ear to noise, impairments of this feedback are predicted to increase the disruptive effects of environmental exposures.

Methods—An animal model of ANSD was created by rearing mice in noise. MOCS protection was assessed by comparing the incidence of noise-induced ANSD among knock-out (KO) mice lacking feedback and wild-type (WT) controls. The mice were screened for ANSD with distortion product otoacoustic emissions (DPOAEs), auditory brainstem responses (ABRs), and behavioral measures of gap detection. Single-unit recording procedures were used to link these deficits to impaired synaptic transmission in the ventral cochlear nucleus.

Results—ANSD manifested in noise-reared mice as intact DPOAEs, abnormal ABRs, and impaired gap detection. The phenotype was not observed among quiet-reared WT mice, but was occasionally noted among noise-reared WT mice. The incidence of ANSD significantly increased among KO mice, especially when they were reared in noise.

Conclusion—Noise promotes ANSD by altering the functional maturation of the brain's temporal pathways. Noise-induced impairments are reduced by the sound-attenuating effects of the MOCS. Noise levels do not need to be unnaturally loud to constitute significant risk in MOCS-compromised individuals.

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Auditory neuropathy spectrum disorder (ANSD) describes a prevalent hearing disorder that is characterized by normal cochlear outer hair cell (OHC) function and abnormal auditory nerve function (1). Minimum diagnostic criteria include normal otoacoustic emissions (OAEs) and missing or aberrant auditory brainstem responses (ABRs). Changes in the ABR are assumed to arise from a dys-synchronization of sound-driven activity in the auditory nerve. The loss of temporal processing can impair speech, sound localization, and listening in noise.

The impact of ANSD on speech has encouraged neonatal screening programs for early diagnosis of the disorder. These programs have revealed a higher incidence of ANSD among infants who have been treated in neonatal intensive care units (NICUs)(2,3). Although the health concerns that necessitate early hospitalization are varied, a constant factor in NICU treatments is the noise exposure that is generated by equipment, procedures, and staff (4,5). Several surveys have reported NICU noise levels consistently above the recommendations of the American Academy of Pediatrics (AAP) for both sustained (Leq) and maximum transient exposures (Lmax) (6).

At present, significant ambiguities remain on what constitutes an acceptable level of noise exposure in human infants. While it is clear that a single loud exposure at a young age can accelerate hearing loss and promote spiral ganglion degeneration (7), the effects of persistent moderate exposures are difficult to estimate because environmental noise exerts pervasive effects on the developing auditory system. The deficits often manifest as perceptual disorders that are independent of overt hearing loss (8,9).

Physiological studies of the cochlea and auditory nerve have provided detailed functional analyses of the medial olivocochlear system (MOCS). In normal individuals, the release of acetylcholine by the MOCS hyperpolarizes the membrane potentials of OHCs (10). The resulting decrease in cochlear mechanical sensitivity protects the ear from damaging levels of noise (11) and enhances auditory processing in distracting levels of noise (12,13). Recent clinical evidence suggests that ANSD is more prevalent among MOCS-compromised children (14). One explanation for this increased risk is that synaptic development of the central auditory pathways requires regulated sound-driven activity in the ascending inputs of the auditory nerve (15–17). When young individuals are exposed to environmental noise, MOC feedback may preserve normal synaptic development by regulating potentially disruptive levels of noise-driven activity.

To investigate this novel developmental role for the MOCS, the feedback system was silenced in laboratory mice by a genetic modification of cholinergic signaling (18). The mice were reared in background noise, and then screened for ANSD using established diagnostic criteria. The audiological implications of the observed processing deficits were further delineated with a behavioral assay of temporal acuity. The refinement of this animal model allowed us to relate common clinical observations to direct electrophysiological recordings from the post-synaptic targets of the auditory nerve in the ventral cochlear nucleus. These recordings revealed significant functional impairments of the synaptic specializations that dictate the quality of temporal coding in the auditory brainstem.

METHODS

Interactions between the potential environmental and genetic precursors of ANSD were evaluated with a 2×2 factorial design. Environmental factors were investigated by rearing mice in quiet or noisy environments. Genetic factors were assessed by comparing noise effects in WT and KO mice.

Breeding pairs of KO mice were obtained from Jackson Labs (CBACaJ;129S-*Chrna9*^{tm1Bedv}/J, stock #005696). This strain was generated with a mixed CBA/CaJ and 129S6/SvEvTac background to control for the early onset of age-related hearing loss. The mice were born and housed in the test environments for the duration of the study. They were periodically removed for 1-h behavioral sessions and concurrent DPOAE and ABR recordings. All testing was completed by eight weeks of age. DPOAE input-output functions, conventional ABR thresholds, and cochlear histology showed no indications of hearing loss among KO or WT mice.

It has been previously established that a null mutation of the $\alpha 9$ nicotinic acetylcholine receptor subunit in the KO strain eliminates MOC-mediated control of cochlear sensitivity (18,19). MOC projections remain intact but their release of acetylcholine has no effect on OHCs in the absence of functional $\alpha 9$ receptors. Homozygous wild-type (WT) mice from the same background strain maintain a functional MOCS. A total of 34 KO mice and 31 WT mice were tested. Both male and female mice were used.

Environmental noise levels were manipulated by rearing mice in institutional vivaria with different patterns of investigator access (20). Daily noise levels in the two facilities are shown in Fig. 1. The heavy-traffic room provided high-density housing for a large number of investigators. Sustained increases in ambient noise levels were produced by high-volume air exchange systems. High-energy transients were associated with bursts of investigator activity. The low-traffic room was quieter and tended to have fewer high level transients. Investigator activity decreased during non-working hours in both facilities (20), but air-exchange systems remained louder in the heavy-traffic room. For comparison, the time scale of the noise records is expanded in Fig. 1C to match published noise levels in a neonatal intensive care unit (NICU)(4). Although the acoustic environment of the heavy-traffic vivarium was complex, it provided a conservative estimate of the NICU exposure.

Assessments of outer hair cell survival patterns

Functional assessments of the integrity of outer hair cells (OHCs) were based on published measures for relating sensorineural hearing loss to DPOAEs in patient populations (21). General methods for recording DPOAEs in mice are described by Parham et al. (22). Because ANSD is attributed to the abnormal transmission properties of the auditory nerve (23), DPOAEs and OHC survival patterns were expected to remain normal in all treatment groups (24).

DPOAEs were measured using an ear insert consisting of independent conduction tubes for two remote speakers and a microphone (Etymotic ER-10C). F2 primary tones with a frequency of 16 kHz were presented from one speaker. F1 primary tones with an F2/F1 ratio of 1.2 were presented simultaneously from the second speaker. The level of F1 was varied from 5 – 65 dB SPL to produce input-output functions relating DPOAE magnitude to stimulus level. The level of F2 was always 10 dB lower than the level of F1. Responses were averaged over 50 repetitions.

Eight cochleae were processed (4 quiet-reared KO, 4 noise-reared KO) for direct visualization of OHC survival patterns in the apical, middle, and basal turns of the organ of Corti. The cochleae were perfused with 4% buffered paraformaldehyde, post-fixed at 4°C for three hours, and rinsed in phosphate buffer. Individual turns of the organ of Corti were separated from the tectorial membrane and removed for processing as whole-mounts. Hair cells were stained with antibodies directed against myosin 7a (25). Olivocochlear terminations were labeled with antibodies targeting synaptic vesicle protein 2 (SV2)(26).

The auditory brainstem response

ANSD patients show missing or abnormal ABRs, presumably because the conduction of sound-evoked activity through the auditory nerve is abnormally dys-synchronized (27). This observation was replicated by comparing the effects of rearing environment and MOCS status on the magnitude of ABR waveforms.

ABRs were recorded in a sound isolation chamber under light ketamine anesthesia. Sound-driven activity was evoked by free-field presentations of 8-kHz tone bursts with a duration of 20 ms and rise/fall times of 0.5 ms. The inter-stimulus interval (ISI) ranged from 1 to 15 ms. This protocol was designed to assess the quality of the ABR over the same timing conditions that produced the most robust indications of ANSD during behavioral testing.

Sound-evoked potentials were recorded with a triad of platinum needle electrodes that were attached to the vertex (non-inverting electrode), to the skin covering the bulla (inverting electrode), and to a hind limb (ground electrode). Electrical activity was amplified (300,000 $\times$), band-pass filtered (0.3 – 3 kHz), and digitally sampled (30-msec recording at a 10-kHz sampling rate). The averaged waveform was based on 300 stimulus repetitions.

Single-unit recording in the ventral cochlear nucleus

The analysis of ABR waveforms was supplemented with direct electrophysiological recordings of single-unit activity in the ventral cochlear nucleus. Equipment for single-unit recording in the ventral cochlear nucleus has been described in previous publications (28,29). Physiological protocols were implemented on programmable real-time processors (Tucker-Davis Technologies, RX6) using custom MATLAB software. Auditory stimuli were generated at a 200-kHz sampling rate, attenuated to control presentation levels (Tucker-Davis Technologies, PA5), and amplified (Crown D-75) before being passed to a calibrated free-field speaker (Tannoy, Arena). Single-unit activity was amplified (10,000 $\times$), low-pass filtered (5 kHz), and digitized with a voltage level discriminator.

Electrophysiological methods were based on a surgical exposure of the dorsal surface of the ventral cochlear nucleus in anesthetized mice (xylazine and ketamine). A tungsten microelectrode was placed on the surface of the nucleus and slowly advanced with a hydraulic micromanipulator. When the repeated presentations of search tones revealed a well-isolated auditory unit, its best frequency (BF, the most sensitive frequency) and threshold were determined by manually sweeping the frequency and level of the tone bursts.

The early components of the ABR have been previously linked to the synchronized activity of globular bushy cells (GBCs), which receive endbulb synapses from the auditory nerve (30). These excitatory synapses are among the largest in the central nervous system (31), and presumably are specialized to ensure the secure transmission of temporal information. The objective of single-unit recording was to identify GBCs, and then measure how their ability to encode temporal information was affected by early noise exposure and the elimination of MOCS feedback.

GBCs were identified during single-unit recording by their responses to short BF tone bursts (50-msec on, 200-msec off)(32–34). These responses were used to construct a peri-stimulus time histogram (PSTH) plotting the timing of action potentials relative to stimulus onset (32,34,35). GBCs produce a primary-notch PSTH that has a precisely timed onset response followed by a brief period of inactivity. Spherical bushy cells (SBCs) integrate a smaller number of endbulb synapses and produce a primarylike PSTH with less onset precision and no notch. Multipolar cells receive auditory nerve inputs through conventional bouton synapses and produce a PSTH with a regularly timed “chopped” appearance.

Dys-synchronization of the auditory nerve synapse was characterized by measuring how well action potentials followed dynamic changes in the amplitude envelope of a BF tone (36,37). The envelope was sinusoidally amplitude modulated (SAM) in discrete frequency steps from 50 – 1000 Hz. The envelope following response was quantified by the synchronization coefficient (SC) (38). An SC of 1 indicates that all spike times coincided with the same phase component of the periodic envelope. An SC of 0 indicates a random distribution of spike times.

Behavioral performance

ANSD typically manifests in adults as a difficulty in speech comprehension (39). Because language-based testing is problematic in animal subjects, our behavioral assay used gap detection to estimate the acuity of temporal processing. Where a normal listener might hear two sounds separated by a brief gap, a temporally impaired listener hears one sound. Previous studies confirm that ANSD patients show elevated gap detection thresholds (40,41).

Gap inhibition of the acoustic startle reflex (GIASR) is a variant of pre-pulse inhibition (42), in which the offset of background noise before the onset of a startle-eliciting stimulus decreases startle magnitude. The magnitude of the inhibitory effect indicates the detectability of the silent gap between the two stimuli.

GIASR tests were implemented with real-time digital processors (Tucker-Davis Technologies, RP2) that monitored the subject's activity, presented acoustic stimuli, and recorded the magnitude of startle responses. The testing procedure was automated in a MATLAB programming environment running custom software (17).

Behavioral testing was conducted in 70 dBA background noise inside a sound-isolation box. The mouse was held at a calibrated sound location by a tube-shaped cage that was coupled to a movement sensor. Trials were presented at random time intervals, when automated monitoring of the movement sensor indicated that the mouse was sitting quietly.

During gap trials, the noise was turned off just before the presentation of the startle-eliciting stimulus. During reference trials, the noise was not turned off. Gap inhibition was quantified as the relative magnitude of the startle response on gap versus reference trials. A relative magnitude of less than 1 indicated the mouse startled less on gap trials, which was interpreted as gap detection. Subjects showing statistically less gap detection than quiet-reared WT mice were considered to test positively for ANSD. Tests were conducted with gap durations of 2 and 10 ms to determine the consistency of ANSD screening with different degrees of task difficulty.

RESULTS

The hypothesized protective role of the MOCS was tested by rearing KO and WT mice in different levels of background noise. The heavy-traffic vivarium represented a high-risk noise exposure. The low-traffic vivarium was considered a low-risk quiet exposure.

As predicted, noise-reared KO mice replicated the existing diagnostic criteria for ANSD. The presence of normal OHC function, as verified by intact DPOAEs, was then extended to a direct histological assessment of hair cell survival patterns. The dys-synchronization of auditory nerve transmission was demonstrated with conventional ABR procedures and then expanded to single-unit recordings in the ventral cochlear nucleus. Temporal coding deficits were specific to neurons that displayed the physiological properties of GBCs. Behavioral screening procedures revealed significantly impaired temporal acuity among noise-reared

KO mice. The expression of the perceptual deficit was highly individualized within each treatment group.

Outer hair cell function and structure

All treatment groups met the diagnostic criteria for ANSD by producing normal DPOAEs. Representative measures are shown for a noise-reared KO mouse in Fig. 2A. A cubic distortion product is clearly evident in this example at 10.6 kHz ($2F_1 - F_2$).

Input-output functions relating the magnitude of the cubic distortion product to the level of the primary tones are shown in Fig. 2B. Results are presented for WT mice reared in quiet and KO mice reared in quiet or noise. A statistical analysis of the effects of F2 level and treatment group was performed using a two-way ANOVA (implemented with the *anova2* function in MATLAB). The effect of F2 level was significant ($P < 0.05$) but the effect of treatment group was not.

A typical example of cochlear histology is shown Fig. 2C. The tissue was collected from the middle turn of the cochlea in a noise-reared KO mouse. Immunolabeling indicated a full complement of both inner and outer hair cells (myosin 7a) and MOCS terminals (SV2).

ABR screening for ANSD

ABR-based hearing thresholds were measured by presenting 5-ms tone bursts at a relatively slow 10 burst/sec repetition rate ($ISI = 95$ ms). The average ABR thresholds ($\pm SE$) of the four treatment groups are presented in Fig. 3. Results are shown for frequencies of 8, 16, and 32 kHz, where noise-reared KO mice showed deficits in gap detection. A statistical analysis (two-way ANOVA) indicated a significant effect of frequency ($P < 0.05$) but no threshold difference between treatment groups.

ANSD-like processing deficits were demonstrated by increasing the demands of stimulus recovery during ABR recordings. Tone duration was increased to 20 ms and ISIs were decreased from 1 – 15 ms. Representative ABR waveforms from each treatment group are presented in Figs. 4A and B. The shorter delay between stimuli had no effect on WT mice. The quiet-reared KO mouse produced a relatively normal ABR at longer ISIs but the magnitude of the response declined at shorter ISIs. In contrast to WT controls, the noise-reared KO mouse produced a featureless ABR at all ISIs.

The effects of ISI on the magnitudes of ABR waves 1 and 2 are compared across treatment groups in Figs. 4C and D. A separate statistical analysis (two-way ANOVA) of each wave found no effect of ISI, but a significant effect of treatment group ($P < 0.05$). Post-hoc contrasts with bonferroni corrections (implemented with the *multcompare* function in MATLAB) linked the effect to significantly smaller waves 1 and 2 in KO mice relative to the baseline values of quiet-reared WT mice. Noise-reared WT mice produced wave 1 amplitudes that were smaller but not significantly different from WT controls.

The abnormal ABRs of KO mice met the diagnostic criteria for ANSD. Decreases in the magnitude of waves 1 and 2 imply that temporal coding deficits were present in both the auditory nerve and ventral cochlea nucleus. Both groups of KO mice expressed similar auditory nerve abnormalities (wave 1), but noise-reared mice displayed consistently more aberrant cochlear nucleus responses (wave 2).

Temporal processing deficits in the ventral cochlear nucleus

The neural correlates of aberrant ABR waveforms were characterized with single-unit recording procedures. Wave 2 of the ABR has been attributed to the synchronized activity of

GBCs in the ventral cochlear nucleus (30). Other unit types show onset latencies that are too long to account for the evoked potential. Consequently, explication of the underlying mechanisms of wave 2 abnormalities required the identification of GBCs using established physiological criteria and then a quantitative measure of the precision of temporal coding.

Figure 5A provides examples of the major PSTH response types that were encountered in the mouse. Primary-notch neurons showed a fast first spike latency, a precisely timed onset, and a brief refractory period (notch). Primarylike neurons produced a similar PSTH but did not display a notch because their onset responses were less precisely timed. Chopper neurons fired a train of action potentials with uniform intervals between spikes, producing a PSTH with a “chopped” appearance. Intracellular labeling of physiologically characterized neurons has assigned the primary-notch PSTH to GBCs, the primarylike PSTH to spherical bushy cells, and the chopper PSTH to multipolar cells (33).

Figure 5B compares the average SC scores of KO mice to the normal baselines of WT controls. Data from 70 neurons have been separated according to the physiological response types in Fig. 5A. Each panel indicates how well the unit type remained synchronized to a broad range of envelope frequencies. The three functions within each panel describe how the quality of temporal coding was influenced by noise exposure and the functional status of the MOCS.

As in previous studies of temporal coding in the ventral cochlear nucleus, primary-notch and primarylike neurons produced low-pass temporal modulation functions that extended to high envelope frequencies, while chopper neurons produced band-pass functions that peaked at intermediate frequencies.

A statistical analysis (two-way ANOVA) of the primary-notch data set indicated a significant effect of treatment group ($P < 0.05$), but not modulation frequency. Post-hoc contrasts noted a significant decrease in the SC scores of noise-reared KO mice. These results establish a direct link between noise-induced ANSD and the defective endbulb transmission of GBCs. Primarylike neurons in quiet-reared KO mice did not show the deficit. Spherical bushy cells (primarylike PSTHs) and multipolar cells (chopper PSTHs) showed normal coding regardless of treatment group.

Behavioral consequences of ANSD

Previous clinical studies of ANSD have reported a decline in gap detection (41). This finding was replicated with behavioral measures of GIASR in noise-reared KO mice. The distribution of relative inhibition is described by the box plots in Figs. 6A and B. Each box shows the interquartile range (middle 50%) of inhibition for the four treatment groups. The line bisecting each box indicates the median score. Whiskers extend to the most extreme data points falling within ± 1.5 interquartile ranges. Data falling outside these limits are plotted as individual outliers.

Quiet-reared WT mice showed the lowest relative startle magnitudes (best gap detection) at both gap durations. The scores of these controls were also less variable. At the opposite extreme, noise-reared KO mice showed the highest startle magnitudes (worst gap detection) and largest inter-subject variability. Because the distributions were highly skewed by outliers, the startle magnitudes were ranked and analyzed with a non-parametric Kruskal-Wallis test (implemented with the *kruskalwallis* function in MATLAB). A significant effect of treatment group was noted at both durations ($P < 0.05$). Post-hoc contrasts revealed a statistically significant increase in the startle magnitudes of noise-reared KO mice relative to baseline measures in quiet-reared WT mice. No other contrasts attained significance.

The skewed distributions of startle responses in noise-reared KO mice suggest a heterogeneous sample with some subjects showing normal behavior while others manifest highly impaired gap detection. An important caveat from these comparisons is the potential to under-estimate the perceptual or physiological consequences of ANSD by mixing the results of normal and impaired subjects in the same treatment group.

To refine the behavioral criteria for distinguishing normal and impaired mice, a new screening procedure was derived from the limits of gap detection in quiet-reared WT controls. This approach is illustrated by the scatter plot in Fig. 6C, which compares each subject's relative startle magnitude for the 2 and 10-ms gaps. The largest startle score of the quiet-reared WT mice defined the worse case scenario for normal performance along each dimension. Scores that exceeded these limits along both dimensions indicated a positive test for ANSD. Scores that failed one criterion and passed the other were considered an intermediate test result.

When these diagnostic criteria were applied to the results of the GIASR tests, 53% (9/17) of noise-reared KO mice tested positively for ANSD. An additional 29% (5/17) of the mice displayed intermediate deficits. In addition to the highest incidence of ANSD, this treatment group produced the largest deviations from normal baselines. Quiet-reared KO mice yielded 35% (6/17) positive test results and 35% (6/17) intermediate results. Deficits in this group were consistently smaller than those observed in noise-reared KO mice. None of the noise-reared WT mice demonstrated a convincing departure from normal baselines.

CONCLUSIONS

An animal model of ANSD was created by exposing young mice to environmental noise. Individual susceptibility to the noise exposure varied within treatment groups, but was significantly increased among mice that lacked a functional MOCS. The successful induction of ANSD was confirmed with the diagnostic criteria of normal DPOAEs and aberrant ABR waveforms that are typically used to screen human infants (2,27). Mice that tested positively for ANSD demonstrated a significant impairment of gap detection, which is a perceptual deficit that has been reported in clinical studies of adult ANSD patients (41).

Single-unit recordings revealed selective defects in the transmission of temporal information from the auditory nerve to the specialized endbulb synapses of GBCs in the ventral cochlear nucleus. Although these transmission errors are implied by abnormalities of the ABR waveform (43), they have not been previously described at the neuronal level. Because GBCs provide critical inputs to the temporal pathways of the binaural brainstem, their disordered transmission properties are likely to have a pervasive impact on speech recognition, masking release, pitch perception, and sound localization (44). These dimensions of auditory processing are essential for the perceptual segregation of competing sound sources, which may explain why ANSD patients are uniquely challenged by complex listening environments (45,46).

Environmental risk factors

It is well established that the functional status of the auditory nerve must be preserved to maintain synaptic development in the central auditory system. Others have shown that deafness impedes the maturation of endbulb synapses in the ventral cochlear nucleus (15,47), and their targets in the medial nucleus of the trapezoid body (16). Our results indicate that these same developmental processes are altered when the auditory nerve is driven to unnaturally high levels of activity by background noise. In defining noise levels for the prevention of ANSD, it is important to distinguish the immediate damaging effects of

very loud exposures from the insidious long-term developmental consequences of moderate exposures.

The complex acoustic environment of the heavy-traffic vivarium represents a conservative estimate of risk factors that are posed by NICU exposures (4,5). Although the suitability of this experimental manipulation was ultimately confirmed by its ability to induce ANSD in a high percentage of young mice, this approach did not isolate which environmental factors represented the most significant risk. For example, sustained noise levels in the vivarium exceeded the Leq exposures that are recommended by the American Academy of Pediatrics (6). The ambient environment also contained high levels of transient energy that exceeded Lmax recommendations. At present, it is not possible to determine whether either type of noise was necessary, or sufficient for inducing the developmental impairments.

The mice in the present study were reared from birth in noise and screened for ANSD as soon as they reached an adult age. Activity-dependent development of the central auditory system is known to begin with the onset of hearing. Consequently, our findings can only restrict the induction of ANSD to a period that begins as early as birth and persists as late as early adulthood. It is not known whether starting the environmental exposure at a later age would interrupt ongoing developmental processes, or whether stopping the exposure prior to maturation would restore normal development. These unanswered questions are highly relevant for the prevention and treatment of ANSD. The present mouse model of ANSD is an ideal experimental context for future investigations of critical periods for induction and recovery.

Individual susceptibility

Relative to quiet-reared WT mice, the incidence of ANSD was increased in both groups of KO mice. Some mice with compromised feedback maintained normal behavior when reared in noise, while others developed deficits when reared under quiet conditions. While these patterns of susceptibility suggest distinct influences of MOCS feedback and noise exposure, they also point out how the risk factors are amplified by a powerful interaction between rearing environment and genetic predisposition.

Although the MOCS is found in all general mammalian species and many non-mammalian vertebrates, there is a high degree of anatomical variation within a species. As a result, individuals may show substantial differences in the strength of MOCS feedback. Presumably, the noise-reared WT mice that tested positively for ANSD developed the disorder because their naturally weak MOCS failed to provide sufficient protection. These findings encourage the early screening of MOCS function in human infants to identify individuals with an increased risk for noise-induced ANSD. Contralateral suppression of DPOAEs is a non-invasive method for the rapid assessment of efferent function that can be efficiently coordinated with existing infant screening protocols (48).

Underlying mechanisms and their perceptual consequences

ANSD has been attributed to the dys-synchronized transmission of temporal information by the auditory nerve (49). This interpretation is largely inferred from the unusual pattern of DPOAE and ABR measurements in patient populations. The introduction of adequate animal models makes it possible to explore the underlying mechanisms of the disorder with direct anatomical and physiological methods. Recent studies in mice have used this approach to confirm structural abnormalities of the spiral ganglion (24). The present study extends those observations to deficiencies in central coding and their perceptual consequences.

Abnormalities of ABR waves 1 and 2 were demonstrated in KO mice regardless of rearing environment. Wave 2, however, showed larger deficits and less recovery at longer ISIs when it was recorded in noise-reared KO mice. Because this treatment group showed the highest incidence of impaired temporal processing during behavioral testing, wave 2 appears to provide a better prediction of the perceptual consequences of ANSD. One interpretation is a progressive loss of coding that first affects the auditory nerve (wave 1) and then progresses to the cochlear nucleus (wave 2). From this perspective, noise-reared KO mice exhibited larger deficits because ANSD had reached a more advanced state.

Electrophysiological recordings indicated that early noise exposure distorted the neural coding of rapid envelope modulations. Noise-induced deficits were limited to the GBCs of MOCS-compromised mice (i.e., neurons with a primary-notch PSTH). GBCs are unique because they integrate a large number of auditory nerve inputs via powerful endbulb synapses (50). Multipolar cells receive more inputs, but they are communicated by weaker bouton synapses. Spherical bushy cells receive larger endbulbs, but they are fewer in number. Consequently, GBCs display exceptional fidelity for the coding of stimulus onsets and envelope modulations (51,52). The selectivity of the deficit among physiological response types implies that ANSD has less effect on non-temporal aspects of auditory coding such as threshold, loudness, and frequency selectivity.

Artificial rearing environments are known to alter sound processing throughout the central auditory system. For example, an immature state persists in the lateral superior olive when one ear is deafened (16). The abnormalities can be traced back to defective inputs from the medial nucleus of the trapezoid body (MNTB), and presumably the impaired endbulbs of GBCs. These findings suggest that ANSD reflects a cascade of synaptic changes that begins with the auditory nerve and eventually manifests throughout the auditory pathways. The resulting deficits may be most apparent for the coding of temporal information because small errors in the timing of action potentials are magnified by large errors in the perceptual identity of auditory signals.

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FIG. 1.

Daily noise levels in the two rearing environments. **A.** Bursts of investigator-generated noise are louder and more frequent in the heavy-traffic vivarium than in the low-traffic vivarium.

B. Long-term spectra also indicate higher sustained noise levels in the heavy-traffic vivarium. **C.** The time scale has been expanded to facilitate comparisons with published noise levels in a typical NICU (adapted from Busch-Vishniac et al., 2005). Gray lines representing the 80-dB noise floor in the NICU are superimposed on vivarium recordings.

FIG. 2.

Effects of early noise exposure on outer hair cell function and structure. **A.** Frequency spectrum of a DPOAE in a noise-reared KO mouse. The emission shows a prominent cubic distortion product (DP). The signal level and noise level at the DP frequency was 28.4 and -24.4 dB SPL, respectively. **B.** Input-output functions relating the magnitude of DP to F2 sound levels. The average DP magnitudes ($\pm$ SE) of KO mice are compared with the normal baselines of quiet-reared WT mice (bold gray line). Numerical labels indicate the number of subjects in each treatment. **C.** Immunolabeling showing intact hair cells and olivocochlear terminals in a noise-reared KO mouse.

FIG. 3. Average ABR thresholds ($\pm$ SE) of each treatment group at 8, 16, and 32 kHz. The thresholds varied by frequency but not by group.

FIG. 4. Effects of early noise exposure on rapidly evoked auditory brain responses (ABRs). **A,B.** Representative ABRs at inter-stimulus intervals of 1, 4, and 15 ms. **C,D.** The average wave 1 and 2 magnitudes ($\pm$ SE) for all subjects in the four treatment groups.

FIG. 5. Effects of early noise exposure on single-unit activity in the ventral cochlear nucleus. **A.** Representative peri-stimulus time histograms (PSTHs) for three major physiological response types. **B.** Temporal modulation functions showing the synchronization coefficient (SC) in relation to envelope frequency. Each panel indicates the average SC ($\pm$ SE) for all neurons with the PSTH shown on the left. Results from KO mice are compared with the normal baselines of quiet-reared WT mice (bold gray line).

FIG. 6.

Effects of early noise exposure on gap detection. **A,B.** Box plots summarizing gap inhibition of the acoustic startle reflex (GIASR) at gap durations of 2 and 10 ms. Lower startle magnitudes indicate better gap detection (arrows). See text for a description of plotting conventions. **C.** Scatter plot of the same data. Behavioral criteria for ANSD are defined as a decrease in gap detection relative to the normal range of quiet-reared WT mice.