Influence of sound-conditioning on noise-induced susceptibility of distortion-product otoacoustic emissions^{a)}

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Cochlear damage caused by loud sounds can be attenuated by “sound-conditioning” methods. The amount of adaptation for distortion product otoacoustic emissions (DPOAEs) measured in alert rabbits previously predicted an ear’s susceptibility to a subsequent noise exposure. The present study investigated if sound-conditioning influenced the robustness of such DPOAE adaptation, and if such conditioning elicited more protection by increasing the amount of DPOAE adaptation. Toward this end, rabbits were divided into two study groups: (1) experimental animals exposed to a sound-conditioning protocol, and (2) unconditioned control animals. After base-line measures, all rabbits were exposed to an overstimulation paradigm consisting of an octave band noise, and then re-assessed 3 weeks post-exposure to determine permanent changes in DPOAEs. A major result was that prior sound-conditioning protected reductions in DPOAE levels by an average of 10–15 dB. However, DPOAE adaptation decreased with sound-conditioning, so that such conditioning was no longer related to noise-induced reductions in DPOAEs. Together, these findings suggest that sound-conditioning affected neural pathways other than those that likely mediate DPOAE adaptation (e.g., medial olivocochlear efferent and/or middle-ear muscle reflexes).

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I. INTRODUCTION

It has long been known that the mammalian cochlea is capable of actively adapting to certain high-level sounds by undergoing “exposure-experience training” or sound-conditioning (Canlon *et al.*, 1988). Presumably, in sound-conditioning, cochlear elements become resistant over time to the adverse consequences of excessive sound by providing a pre-exposure training experience using a moderate-level acoustic stimulus that, at a more intense level, eventually becomes the subsequent overexposure stimulus. Hence, under certain conditions, the mammalian cochlea is capable of dynamically adapting to the aftereffects of excessive sounds. Although the early research on sound-conditioning was conducted in several common laboratory animals including, for example, guinea pigs (Canlon *et al.*, 1988), rabbits (Franklin *et al.*, 1991), and chinchillas (McFadden *et al.*, 1997), a follow-up study in teenagers using a temporary-threshold shift (TTS) paradigm showed that the development of resistance exists in human subjects as well, at least, under brief TTS-exposure conditions (Miyakita *et al.*, 1992).

We have previously shown that the amount of distortion product otoacoustic emission (DPOAE) adaptation or efferent-system feedback strength in an alert rabbit’s ear, due to either the medial olivocochlear reflex (MOC-R) and/or the middle-ear muscles’ acoustic reflex (MEM-R), predicted an ear’s susceptibility to a subsequent noise exposure (Luebke *et al.*, 2014). Previous studies in the sedated guinea pig established that sound-conditioning enhanced both ipsilaterally and contralaterally evoked MOC-R strengths as assessed with DPOAEs (Kujawa and Liberman, 1999). However, when compared with the frequency range and magnitude of the frequency-related protection, these authors concluded that protection from sound-conditioning could not be completely explained by strengthening the MOC-R. Yet, using synaptophysin immunoreactivity as a marker for the medial olivocochlear (MOC) system, Canlon *et al.* (1999) showed that sound-conditioning significantly reduced noise-induced injury to MOC nerve terminals, thus, suggesting that the conditioning procedure might protect efferent-nerve terminals from morphological damage due to overexposure. However, in contrast to the earlier Kujawa and Liberman (1999) findings, results from a more recent investigation suggested that long-term sound-conditioning decreased MOC-R strength in alert guinea pigs thereby increasing DPOAE magnitudes (Peng *et al.*, 2007).

The purpose of the present study was to investigate if sound-conditioning influenced protection from the efferent

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system's feedback strength in the alert rabbit, and if such conditioning elicited its protection by increasing the amount of DPOAE adaptation. A major finding was that although sound-conditioning protected noise-induced reductions in DPOAE levels by an average of 10–15 dB, DPOAE adaptation actually decreased with such conditioning. That is, after sound-conditioning, DPOAE adaptation was no longer related to noise-induced decrements in DPOAEs, thus, suggesting that sound-conditioning affected neural pathways other than those mediating DPOAE adaptation through bolstering the feedback strength provided by the MOC-R or combined MOC-R/MEM-R processes. The lack of a correlation in conditioned ears between DPOAE-adaptation measures and the magnitude of DPOAE losses post-exposure may possibly explain the difficulties of other investigators (e.g., [Veuillet et al., 2001](#); [Shupak et al., 2007](#)), who have been unsuccessful in using DPOAE-adaptation measures to predict susceptibility to acoustic overstimulation effects in humans that are regularly exposed to excessive noises. Presumably, those, who routinely work in noisy settings, eventually become sound-conditioned and, consequently, more resistant to the potentially harmful effects of intense sounds.

II. METHODS

A. Animal subjects

Young ($n = 11$, 2–3 kg, 2–3 months old, males = 5, females = 6, ears = 20) New Zealand pigmented rabbits (*Oryctolagus cuniculus*) obtained from a local commercial supplier were used as experimental subjects. All rabbits initially underwent aseptic surgery under general anesthesia (40 mg/kg ketamine hydrochloride, 10 mg/kg xylazine) to permanently affix a head-brace device consisting of an inverted stainless-steel screw to the dorsal surface of the skull. This brace supported the animal's head and also provided the stability necessary for obtaining repeated DPOAE measures over sequential test sessions because it permitted the accurate replication of head position over different test times. Animals were allowed to recover from the head-post surgery for 3 weeks before experimentation was initiated. Average DPOAE adaptation or MOC-R and MEM-R indices (see below) were determined in these 11 alert rabbits (20 ears) under binaural acoustic-stimulation conditions.

B. Study groups

The two study groups included a set of sound-conditioned rabbits ($n = 5$, 9 ears), along with a non-sound-conditioned ($n = 6$, 11 ears) series of animals. This latter group of control subjects consisted of the rabbits in the study by [Luebke et al. \(2014\)](#) that underwent adaptation testing, and noise exposure using the identical intense octave band noise (OBN) overstimulation that was utilized in the present study, but in the absence of the sound-conditioning treatment.

C. Measures of DPOAEs and DP-grams

DPOAEs at the $2f_1$ – f_2 frequency were recorded using an emissions-measurement system consisting of commercially available components. These elements included separate f_1 and f_2 ear-speakers (ER-2, Etymotic Research, Elk Grove Village IL), and an acoustic probe/microphone assembly (ER-10, Etymotic Research, Elk Grove Village IL). As detailed in a prior report ([Martin et al., 1998](#)), stimulus generation and response acquisition were controlled by a personal microcomputer through an on-board digital signal processor operated by customized software. In combination, this system was capable of obtaining DPOAEs in the form of routine distortion-product grams (i.e., DP-grams or DPOAE levels as a function of f_2 frequency). Such standard DP-grams were obtained over a range of f_2 frequencies ($f_2 = 1.6$ – 18 kHz), at 0.1-octave (oct) steps (i.e., for $n = 31$ test frequencies at $n = 8$ synchronous samples per frequency), with the f_2/f_1 ratio set at 1.25. Equilevel ($L_1 = L_2$) primary tones ranged from 45 to 75 dB sound pressure level (SPL), in 10-dB steps thus yielding four standard DP-grams per ear. These data were used to confirm that study subjects exhibited emissions that were within the normal range based upon the laboratory's extensive database for rabbit DPOAEs.

D. Determination of the average DPOAE adaptation index

The single-frequency fast adaptive measure developed by [Lieberman et al. \(1996\)](#) was modified to include two distinct stimulus-presentation protocols (for schematic details, see Fig. 1 in [Luebke et al., 2014](#)) incorporating a selected, condensed DP-gram frequency range of ~ 3 – 13 kHz, in 0.2-oct steps (i.e., $f_2 = 3.162$ – 12.647 kHz, $n = 11$ frequencies). This truncated select DP-frequency range was chosen because it spanned the 2-oct frequency extent that was centered about $\frac{1}{2}$ oct above the subsequent OBN exposure and, thus, was expected to exhibit the largest noise overexposure effects. The initial or baseline 31-frequency standard DP-gram measures were elicited monaurally at a time interval that was ~ 5.7 ms after the onset of the ramped 46-ms primary tones. The second set of 11 condensed-frequency DP-grams were elicited using the adaptive paradigm that consisted of a delayed measure in which the f_1 and f_2 primaries were presented binaurally for 1046 ms, resulting in a repetition rate of about once every 3.5 s, with the DPOAE being measured from the test ear only during the final 46 ms of the lengthy stimulation period. In both the monaural standard and binaural condensed protocols, each DPOAE measure was separated by a 2.5-s interstimulus interval to allow for sufficient time, particularly, during the presentation of the adaptive paradigm, for the efferent-feedback induced effects to dissipate before the next set of primary tones was presented. To compute a DPOAE-adaptation index, the differences between the DPOAE levels obtained during the baseline monaural and the longer-lasting delayed binaural condition were then computed for each of the 11 truncated DP-gram frequencies. These differences, which were treated as absolute values to accommodate either efferent system-

related decreases or increases in DPOAE levels, were then summed and divided by the number of test frequencies ($n = 11$) in each condensed DP-gram to obtain the average DPOAE-adaptation index.

E. Determination of the MEM-R index

The potential contribution that a primary-tone induced activation of the MEM-R made to the mean DPOAE-adaptation index was also estimated. To this end, a measure of the average MEM-R-related activity was computed in a manner that was similar to that described above for the adaptation metric. That is, the amount of change to the middle-ear conduction apparatus introduced by the acoustic reflex was estimated as an alteration in middle-ear immittance, which was measured by monitoring changes in the level of the f_1 -primary tone in the outer ear canal of the test ear. Similar to the computations performed to establish the average DPOAE-adaptation index, the differences between the levels of the f_1 tones measured during the monaural baseline stimulation period using the brief 46-ms primary tones compared to f_1 -primary tone levels during the longer 1046-ms binaural stimulation interval were computed for each of the 11 frequencies that comprised the condensed DP-gram function. These difference values were then summed and averaged by dividing this amount by the number ($n = 11$) of tested frequencies in the condensed DP-gram, thus, yielding the average MEM-R index. It is interesting to note that for the awake rabbit acoustic-reflex thresholds elicited by ipsilateral pure-tone stimuli vary, on average, from about 65–70 dB SPL, over the truncated frequency range (~ 3 –13 kHz) measured here (Whitehead *et al.*, 1991).

F. Sound-conditioning and noise over-exposure

For both sound-conditioning training and noise-exposure delivery, rabbits were placed inside a sound-reverberant, noise-exposure chamber, where they were allowed free

access to food and water. The details related to the conditioning and exposure protocols are presented schematically in Fig. 1(A) for rabbits in the conditioned experimental group ($n = 5$ animals, 9 ears), while Fig. 1(B) illustrates the paradigm for non-conditioned control animals ($n = 6$ animals, 11 ears). For sound-conditioning, each animal in the experimental group was exposed to an OBN centered at 2 kHz, and presented at 85 dB SPL, over a 6-h on/18-h off schedule, for ten consecutive days, with sound-conditioning delivered from ~ 9 a.m. to 3 p.m. each day. The last conditioning episode was 6 days before the acoustic overexposure paradigm was initiated. Over the 5-day time period between the end of conditioning and the beginning of the overstimulation, standard 31-frequency DP-grams were measured at 1 and 5 days post-conditioning to determine the effects of the conditioning protocol on emission levels. The acoustic over-exposure consisted of a standardized 105-dB SPL, 2-kHz OBN that was presented for 6 h/day (9 a.m.–3 p.m.) over a 2-day period (Martin *et al.*, 1998). Following a 3-week recovery period, post-exposure DPOAEs were measured as standard 31-frequency DP-grams, so that pre- versus post-exposure levels could be compared. In this manner, the amount of permanent noise-induced changes in DPOAE levels were determined.

G. Determination of the average DPOAE loss index

To quantify the loss in DPOAE levels due to the OBN over-exposure, an additional measure, termed the average DPOAE-loss index, was computed as described in Luebke *et al.* (2014). Briefly, this measure represented the mean difference between the pre- versus post-standard DP-grams (i.e., DP-grams collected at routine 0.1-oct intervals) elicited by 65-dB SPL primaries at the 21 test frequencies making up the condensed frequency range ($f_2 = 3.16$ –12.65 kHz). These difference values were then summed and divided by number of frequencies tested ($n = 21$) to calculate the average DPOAE loss index (Luebke *et al.*, 2014). Note that in the

FIG. 1. Schematic illustrating the timing of the various study treatments across alert rabbits for the conditioned and non-conditioned groups. (A) Basically, two pre-conditioning standard DP-grams (test and retest) were obtained from the sound-conditioned group before a 10-day conditioning period in which animals were exposed to a moderate OBN. The conditioning interval was followed by a 5-day post-conditioning period in which DPOAEs were collected on post-conditioning days 1 and 5. “Conditioned” rabbits were then exposed to a more intense version of the conditioning OBN over a 2-day period. Post-exposure measures to determine permanent noise-induced changes in DPOAE levels were obtained at 3 weeks after cessation of the overstimulation episode. (B) Except for the conditioning interval and post-conditioning tests, non-conditioned control rabbits received identical exposure and post-exposure treatments. See Methods section for the specific details of each protocol.

standard DP-gram, which was obtained in 0.1-oct steps, the f_2 -frequency range from 3.16 to 12.65 kHz comprised 21 frequencies in contrast to the select truncated DP-gram collected in 0.2-oct intervals, which consisted of 11 frequencies over the same f_2 -frequency span.

H. Form of data and statistical analyses

The DPOAE responses illustrated below are typically displayed in the form of DP-grams that describe the absolute levels of the individual DPOAEs as a function of the f_2 frequency. However, when comparing MOC-R and MEM-R activity within the same animal, difference plots were used [see example in Fig. 2(C)]. Difference plots simply describe the amount of change in dB that occurred in response to the monaural baseline versus delayed binaural stimulation conditions. Thus, if the delayed presentations of the longer lasting primary-tonebursts did not affect either the emission level or the level of the f_1 -primary tone as measured in the ear canal, the function representing each response type would lie parallel to the x axis at “0” dB on the ordinate,

thus, representing a “no change” outcome. Then, negative values on difference plots would indicate sound-induced decrements in DPOAE levels from baseline, while positive values would designate post-exposure increases in emission magnitudes.

The relationships between the three indices representing average DPOAE adaptation as reflected by the MOC-R, MEM-R activity, and DPOAE loss were analyzed using commercial software (StatView v.4.5, Abacus Concepts Inc., Berkeley, CA) to determine various linear-regression coefficients and their associated p -values for each animal tested. When findings from both ears of an animal were present, the average value was used for statistical determinations, yet, individual values are plotted for each ear in the scatterplots below [see Figs. 2(D) and 3(D)]. A probability value of <0.05 represented the adopted level of statistical significance.

The University of Miami Miller School of Medicine’s and the VA Loma Linda Healthcare System’s Institutional Animal Care and Use Committees approved all procedures performed on the rabbit subjects.

FIG. 2. Sound-conditioning reduced DPOAE and MEM-R magnitudes, yet, DPOAE levels returned to pre-test conditions by 5 days post-conditioning. (A) Averaged standard DP-grams showing that at 1 day post-conditioning (open squares), a significant decrease in DPOAE levels from baseline measures (filled circles) was observed for all animals. (B) Average standard DP-grams for conditioned animals indicating that reduced DPOAEs returned essentially to pre-test conditions (solid circles) by 5 days post-conditioning (open circles). (C) Measures from conditioned rabbit R51R showing that the MEM-R as measured by a change in the f_1 level (L_1) difference with and without binaural activation of the f_1 and f_2 primaries, decreased generally in the alert rabbit with sound-conditioning. This difference was averaged over a truncated frequency region as shown by gray shading. (D) Scatterplot for conditioned rabbits similar to DPOAEs observed in (A) showing that the post-conditioned change in the averaged MEM-R was most pronounced at 1 day (open shaded squares) after conditioning, yet, still below unity for some animals by 5 days post-conditioning (open circles). Note that symbols representing data points for some animals overlapped, resulting in the number of plotted data points differing from the actual number of animals tested.

FIG. 3. Relationship between DPOAE adaptation and DPOAE loss for control animals was inversely related, i.e., lower adaptation indices were associated with greater DPOAE-loss indices, but this relationship was not observed for sound-conditioned animals. (A) Left (*L*) ear for control, non-conditioned rabbit R44L showed an appreciable DPOAE-loss index of $L = 22.9$ dB between pre- (line without symbols) and post-exposure (shaded circles) functions. Shading shows the truncated frequency region over which DPOAE losses were computed. (B) In contrast, for the right (*R*) ear of experimental rabbit R51R, sound-conditioning was effective in that the DPOAE-loss index of $L = 9.5$ dB provided ~ 5 – 10 dB protection from the OBN exposure. However, in most instances, conditioned DPOAE adaptation was lower than DPOAE adaptation before sound-conditioning. (C) Average DP-grams ± 1 SEM for non-conditioned control (solid circles) and conditioned experimental rabbits (open squares) showing the greater noise-induced reductions in DPOAEs for the former animals. (D) Scatterplot describing, for individual ears, the relationship between the adaptation and loss indices for conditioned (open squares) and non-conditioned (solid circles) rabbits. Note that symbols representing data points for some animals overlapped, resulting in the number of plotted data points differing from the actual number of animals tested. The examples of (A) (circle) and (B) (square) are shown as shaded symbols in (D), as can be observed by the average DPOAE adaptation, which was similar between these two ears, yet, their average DPOAE losses differed by ~ 14 dB.

III. RESULTS

The present study in alert rabbits was designed to determine the role of sound-conditioning in effecting changes to DPOAE-adaptation processes. It was also of interest to determine if sound-conditioning produced a larger DPOAE-adaptation index, which would further reduce the effects of the OBN exposure on DPOAE levels. Thus, each conditioned animal's DPOAE responses were assessed at 1 day pre-, 1 day post-, and 5 days post-conditioning as diagrammed in Fig. 1(A). As shown in Figs. 1(A) and 1(B), both the conditioned and non-conditioned groups were tested before the OBN exposure interval, and again at 3 weeks post-exposure to determine the presence of permanent noise-induced reductions in DPOAEs.

Figures 2(A) and 2(B) present average DP-grams (± 1 standard error of the mean, SEM) elicited from

sound-conditioned rabbits with 55-dB SPL binaural primary tones during the pre-conditioning period (solid circles), and at 1 day [open squares in Fig. 2(A)] and 5 days [open circles in Fig. 2(B)] post-conditioning. Note, the reduced DPOAEs at 1 day post-conditioning in Fig. 2(A) of ~ 10 dB in the frequency band from 2.5–3.5 kHz, which was just above that of the 2-kHz OBN conditioning stimulus. However, as apparent in Fig. 2(B), all animals essentially recovered back to baseline levels by 5 days post-conditioning.

In the difference plot of Fig. 2(C) for the right (*R*) ear of representative rabbit R51R, alterations in the level of f_1 as a function of the f_2 frequency are shown for the pre-conditioning period (solid circles) and at post-conditioning days 1 (open shaded squares) and 5 (open circles). In this plot, the "0" line represents a "no change from baseline" condition, while the pre-conditioning solid circles show the f_1 -level difference that was elicited by the 55-dB SPL, longer binaural primary tones minus the f_1 level to the shorter-lasting

monaural primaries. This difference was averaged over the truncated frequency region, as shown by gray shading in Fig. 2(C), to represent a measure of the MEM-R for this particular ear as shown. It is clear that while the average pre-conditioned MEM-R at ~ 3.3 dB either decreased or increased the level of the f_1 primary, the conditioning experience tended to notably reduce the size of the MEM-R, which was ~ 0.65 and 1.3 dB at 1 and 5 days post-conditioning, respectively. Thus, the average MEM-R in this ear decreased after sound-conditioning.

Figure 2(D) shows a scatterplot of all the ears tested where the pre-conditioning MEM-R is plotted with respect to its post-conditioning counterpart at 1 (open shaded squares) or 5 days (open circles) post-training. Here, it is clear that while the MEM-R as assayed by the difference in pre- versus post- f_1 levels, decreased 1 day after sound-conditioning, about half of the rabbits returned to pre-conditioning levels by 5 days after sound-conditioning. It is noteworthy, however, that the MEM-R never increased after sound-conditioning in that none of the data points were significantly larger than the unity function (dashed-slanted line) of Fig. 2(D). Although not shown here, for non-conditioned control animals, the MEM-R did not change over time when tested and retested over a similar 15-day period.

The average DPOAE-loss index for each ear in all animals was established by determining the mean difference between the pre- versus post-standard DP-grams for f_2 frequency regions ranging from 2.8 to 11.3 kHz, i.e., for $n = 21$ of the entire 31-frequency span over the truncated frequency range. Representative examples are shown in Figs. 3(A) and 3(B) for non-conditioned and conditioned rabbits, respectively. In Fig. 3(A), the shading for the left (L) ear of non-conditioned control rabbit R44L indicates a DPOAE-loss index [L values on Figs. 3(A) and 3(B)] of 22.9 dB and, in Fig. 3(B) for the right (R) conditioned ear of rabbit R51R, shading signifies a DPOAE-loss index of $L = 9.6$ dB. Figure 3(C) shows the average DPOAE losses with SEM values for all the ears tested. As shown in Fig. 3(D), when the mean DPOAE losses were compared to their average DPOAE-adaptation values, a significant inverse correlation ($R^2 = 0.725$, $p < 0.01$) was apparent between the DPOAE-adaptation and DPOAE-loss indices for the non-conditioned control (solid circles) animals. Yet, this inverse relationship was not present after sound-conditioning as represented by the open-square symbols in Fig. 3(D) for the conditioned rabbits. That is, in general, although the control rabbits [solid circles in Fig. 3(D)] that did not experience the conditioning sound-training showed significantly ($r = 0.96$, $p < 0.01$) that their low DPOAE-adaptation index was associated with a higher DPOAE-loss index, the conditioned animals (open squares) did not exhibit this relationship between the two variables. The ears that are shown in Figs. 3(A) and 3(B) are shown as a shaded circle and shaded square, respectively, in the scatterplot of Fig. 3(D). Surprisingly, both rabbits exhibited similar average DPOAE-adaptation values of ~ 1.8 dB.

IV. DISCUSSION

The present results demonstrate that sound-conditioning can protect the ear from noise damage, however, the inverse

linear relationship between DPOAE adaptation (or MOC-R/MEM-R strength) and a noise-induced reduction in DPOAE levels was not present after sound-conditioning. A positive aspect of the present study was that MEM-R and MOC-R strengths were measured before and at 1 and 5 days after sound-conditioning in the same ears and animals for all conditioned rabbits. Moreover, DPOAEs and sound-based efferent-feedback strengths were tested in alert animals without sedation, thus, eliminating any effects of anesthesia on cochlear-efferent feedback activity (Boyev *et al.*, 2002). In addition, time periods of sound-conditioning and noise exposure at 6 h/day from ~ 9 a.m. to 3 p.m. were kept constant, and were administered at the same time of day for all animals. The timing factors for delivering the conditioning and noise-exposure protocols were carefully controlled as recent findings indicate that there is a relationship between the circadian clock of noise susceptibility and stress (Le Prell *et al.*, 2003). Consequently, in the present study, we controlled for these factors.

The conditioning experience showed that DPOAE levels in the alert rabbit were clearly decreased at 1 day post-conditioning, unlike the increased levels that were reported post-conditioning for alert guinea pigs (Peng *et al.*, 2007). Moreover, the MEM-R either decreased or remained the same after sound-conditioning, and was essentially unchanged by 5 days post-conditioning, hence, suggesting that sound-conditioning is not mediated by the MEM-R, as other investigators have also surmised through lesion studies (Dagli and Canlon, 1995). Although the MOC-R decreased after conditioning, it is not yet known how much of this decrement was due to the diminished MEM-R observed at 1 day post-conditioning. It is unlikely that this decrease was due to a lack of MOC efferent terminals because Canlon *et al.* (1999) reported earlier that sound-conditioning likely protected these nerve endings.

Brown *et al.* (1998) observed slight changes in MOC discharge rates in cells with best frequency responses about $\frac{1}{2}$ oct above the conditioning-frequency band, nevertheless, these changes in MOC discharge rates observed for sedated guinea pigs were not reflected in greater MOC-R strengths in either the alert rabbit of the current study, or the awake guinea pig (Peng *et al.*, 2007). However, while MOC-R strength was not significantly modified by sound-conditioning, earlier studies showed that the lateral olivocochlear (LOC) efferent pathway could be modified by sound-conditioning experience. For example, Canlon and colleagues (Niu and Canlon, 2002a,b; Niu *et al.*, 2004a; Niu *et al.*, 2004b,2007) showed that sound-conditioning upregulated tyrosine-hydroxylase in both LOC efferent neurons and lateral superior olivary cells in the brainstem, thus, suggesting that the conditioning experience may modify neural elements in the LOC pathway. The strength of the LOC neural tract has been difficult to assess using sound, yet, lesion studies have shown that this pathway is also needed for protection from acoustic trauma (Maison *et al.*, 2013).

A reasonable explanation concerning the present findings is that sound-conditioning causes a modification in cochlear-nerve fiber discharge activity, which likely is under LOC influence (Maison *et al.*, 2003). In keeping with this

notion, by 5 days after sound-conditioning, suprathreshold cochlear-nerve fiber activity was probably reduced, which would, then, cause a decrease in the neural-input drive to the MOC-R, thereby reducing its magnitude. In support of this view, [Mensh et al. \(1993\)](#) noted in rabbits undergoing recurrent bouts of acoustic trauma that, while the threshold for the acoustic reflex did not change with repeated overexposures, the magnitude of the reflex decreased over time, possibly, due to sound-induced changes in cochlear-nerve activity. However, because there is no sound-based assay of LOC strength, this hypothesis remains speculative. Fortunately, the relatively brief sound-conditioning protocols used in the present study did not cause a synaptopathy and, hence, would not have any long-lasting effects like those observed for the aged cochlea ([Fernandez et al., 2015](#)).

It is interesting that, to date, no otoacoustic emission (OAE)-based MOC-R strength measure has been successfully used to predict susceptibility to noise exposure in any sound-conditioned population ([VeUILlet et al., 2001](#); [Shupak et al., 2007](#)). Because most military personnel undergo extensive training in noisy environments, which could serve as a form of sound-conditioning, it is possible that the lack of a correlation with OAE-adaptation measures could be due to sound-conditioning. The present findings imply that using such DPOAE-based adaptation measures to predict susceptibility to noise exposure in the labor force has also suffered from the same confounding variable, i.e., habitual exposure to intense sounds potentially weakens the ability of the adaptive auditory efferent system to protect against the eventual harmful effects of acoustic over-exposure. It is probable, too, that other general populations, such as musicians or the over-users of personal listening devices may also be sound-conditioned, thus, confounding any attempts to predict noise-exposure sensitivities using adaptive cochlear measures.

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- Boyev, K. P., Liberman, M. C., and Brown, M. C. (2002). "Effects of anesthesia on efferent-mediated adaptation of the DPOAE," *J. Assoc. Res. Otolaryngol.* **3**, 362–373.
- Brown, M. C., Kujawa, S. G., and Liberman, M. C. (1998). "Single olivocochlear neurons in the guinea pig. II. Response plasticity due to noise conditioning," *J. Neurophysiol.* **79**, 3088–3097.
- Canlon, B., Borg, E., and Flock, A. (1988). "Protection against noise trauma by preexposure to a low level acoustic stimulus," *Hear. Res.* **34**, 197–200.
- Canlon, B., Fransson, A., and Viberg, A. (1999). "Medial olivocochlear efferent terminals are protected by sound conditioning," *Brain Res.* **850**, 253–260.
- Dagli, S., and Canlon, B. (1995). "Protection against noise trauma by sound conditioning in the guinea pig appears not to be mediated by the middle ear muscles," *Neurosci. Lett.* **194**, 57–60.

- Fernandez, K. A., Jeffers, P. W. C., Lall, K., Liberman, M. C., and Kujawa, S. G. (2015). "Aging after noise exposure: Acceleration of cochlear synaptopathy in 'recovered' ears," *J. Neurosci.* **35**, 7509–7520.
- Franklin, D. J., Lonsbury-Martin, B. L., Stagner, B. B., and Martin, G. K. (1991). "Altered susceptibility of $2f_1-f_2$ acoustic-distortion products to the effects of repeated noise exposure in rabbits," *Hear. Res.* **53**, 185–208.
- Kujawa, S. G., and Liberman, M. C. (1999). "Long-term sound conditioning enhances cochlear sensitivity," *J. Neurophysiol.* **82**, 863–873.
- Le Prell, C. G., Dolan, D. F., Schacht, J., Miller, J. M., Lomax, M. I., and Altschuler, R. A. (2003). "Pathways for protection from noise induced hearing loss," *Noise Health* **5**, 1–17.
- Liberman, M. C., Puria, S., and Guinan, J. J., Jr. (1996). "The ipsilaterally evoked olivocochlear reflex causes rapid adaptation of the $2f_1-f_2$ distortion product otoacoustic emission," *J. Acoust. Soc. Am.* **99**, 3572–3584.
- Luebke, A. E., Stagner, B. B., Martin, G. K., and Lonsbury-Martin, B. L. (2014). "Adaptation of distortion product otoacoustic emissions predicts susceptibility to acoustic over-exposure in alert rabbits," *J. Acoust. Soc. Am.* **135**, 1941–1949.
- Maison, S. F., Emeson, R. B., Adams, J. C., Luebke, A. E., and Liberman, M. C. (2003). "Loss of α CGRP reduces sound-evoked activity in the cochlear nerve," *J. Neurophysiol.* **90**, 2941–2949.
- Maison, S. F., Usubuchi, H., and Liberman, M. C. (2013). "Efferent feedback minimizes cochlear neuropathy from moderate noise exposure," *J. Neurosci.* **33**, 5542–5552.
- Martin, G. K., Jassir, D., Stagner, B. B., and Lonsbury-Martin, B. L. (1998). "Effects of loop diuretics on the suppression tuning of distortion-product otoacoustic emissions in rabbits," *J. Acoust. Soc. Am.* **104**, 972–983.
- McFadden, S. L., Henderson, D., and Shen, Y. H. (1997). "Low-frequency 'conditioning' provides long-term protection from noise-induced threshold shifts in chinchillas," *Hear. Res.* **103**, 142–150.
- Mensh, B. D., Patterson, M. C., Whitehead, M. L., Lonsbury-Martin, B. L., and Martin, G. K. (1993). "Distortion-product emissions in rabbit: I. Altered susceptibility to repeated pure-tone exposures," *Hear. Res.* **70**, 50–64.
- Miyakita, T., Hellstrom, P. A., Frimanson, E., and Axelsson, A. (1992). "Effect of low level acoustic stimulation on temporary threshold shift in young humans," *Hear. Res.* **60**, 149–155.
- Niu, X., Bogdanovic, N., and Canlon, B. (2004a). "The distribution and the modulation of tyrosine hydroxylase immunoreactivity in the lateral olivocochlear system of the guinea-pig," *Neuroscience* **125**, 725–733.
- Niu, X., and Canlon, B. (2002a). "Activation of tyrosine hydroxylase in the lateral efferent terminals by sound conditioning," *Hear. Res.* **174**, 124–132.
- Niu, X., and Canlon, B. (2002b). "Protective mechanisms of sound conditioning," *Adv. Otorhinolaryngol.* **59**, 96–105.
- Niu, X., Tahera, Y., and Canlon, B. (2004b). "Protection against acoustic trauma by forward and backward sound conditioning," *Audiol. Neurotol.* **9**, 265–273.
- Niu, X., Tahera, Y., and Canlon, B. (2007). "Environmental enrichment to sound activates dopaminergic pathways in the auditory system," *Physiol. Behav.* **92**, 34–39.
- Peng, J. H., Tao, Z. Z., and Huang, Z. W. (2007). "Long-term sound conditioning increases distortion product otoacoustic emission amplitudes and decreases olivocochlear efferent reflex strength," *Neuroreport* **18**, 1167–1170.
- Shupak, A., Tal, D., Sharoni, Z., Oren, M., Ravid, A., and Pratt, H. (2007). "Otoacoustic emissions in early noise-induced hearing loss," *Otol. Neurotol.* **28**, 745–752.
- VeUILlet, E., Martin, V., Suc, B., Vesson, J. F., Morgon, A., and Collet, L. (2001). "Otoacoustic emissions and medial olivocochlear suppression during auditory nerve recovery from acoustic trauma in humans," *Acta Otolaryngol.* **121**, 278–283.
- Whitehead, M. L., Martin, G. K., and Lonsbury-Martin, B. L. (1991). "Effects of the crossed acoustic reflex on distortion-product otoacoustic emissions in awake rabbits," *Hear. Res.* **51**, 55–72.