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14. ABSTRACT Following a 108 dB SPL, 4 kHz noise exposure for 6 hours, animals were treated in a rescue paradigm with N-Acetyl L-cysteine, Ebselen, D-Methionine, Acetyl L-Carnitine or the Src protein tyrosine kinase inhibitor, KX1-004 and compared to untreated control subjects. Hearing loss, distortion product emissions and cochlear histology data were obtained in each animal. Statistical analyses indicated that there were no significant differences between the control and treated groups. The above five drugs were also applied in a rescue paradigm to groups of animals exposed to 10, 158 dB peak SPL blast waves. Data collection from two drug (L-NAC and D-MET) treated groups and one control group (N=20/group) is complete. Three groups (N=20/group) have been exposed and treated with ALCAR, Ebselen or Src Inh. Data collection and analysis on these groups is not complete. Variability in the response of animals to the blast exposures is a complicating factor. As a result of the variability in the blast wave control group, data on a second group (N=20) of noise only control subjects is being collected.					
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Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	1
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusion.....	6
References.....	6
Appendices (Figures 1 - 56)	7

INTRODUCTION:

The goal of this research was to compare, in the rescue mode of treatment, the effectiveness of the drugs identified below in reducing hearing and sensory cell loss as the result of blast injury to the auditory system. First, however, the following substances were used in a rescue mode to treat experimental groups of chinchillas following a high level (108 dB SPL), continuous 4 kHz octave band noise exposure: (1) L-N-acetylcysteine (L-NAC); (2) D-Methionine (D-MET); (3) Ebselen SPI-1005; (4) Acetyl-L-carnitine (ALCAR) and (5) Src-PTK inhibitor, KX1-004. The two drugs most effective in reducing hearing loss from the 4 kHz exposures were to then be used to rescue hearing following high level blast wave exposures produced by a shock tube. For the blast experiments two treatment schedules and three drug dosages were to be used. For both series of exposures appropriate controls were run. All animals were subjected to the following testing protocol. Evoked potential recordings from the inferior colliculus were used to estimate hearing thresholds at the 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 kHz test frequencies. Cubic distortion product otoacoustic emission (DPOAE) input/output functions at six frequencies were obtained along with DPOAEs as a function of frequency (DPEgram) at two primary intensities. Tympanograms were measured on Phase I blast-exposed animals to screen for conductive problems. At the completion of all testing the animals were killed, under anesthesia, for surface preparation histology from which a frequency specific estimate of sensory cell loss was obtained. As the research progressed implementation of the research design described above was changed as accumulating results dictated. Specifics are detailed in the following progress report.

BODY:

Year 2 Progress Report:

A. Phase II of the study has been completed.

Original Phase II SOW: In this phase of the study the following substances will be used in a rescue mode to treat the experimental groups of chinchillas that have been exposed to a 4.0 kHz octave band of noise for 6 hours at 105 dB SPL: (1) L-N-acetylcysteine (L-NAC); (2) D-Methionine (D-MET); (3) Ebselen SPI-1005; (4) Acetyl-L-carnitine (ALCAR) and (5) Src-PTK inhibitor, KX1-004. Five reference/replication experimental groups and 3 control groups will be run with 8 animals /group. The drug treatment protocol is as described in the original proposal and is similar to that used in several published studies. These 8 groups will serve three functions: (a) Partially replicate published work; (b) Establish a reference treatment outcome from a less traumatic exposure to which the AAT results can be compared; (c) Determine the most effective two of the five drugs in reducing hearing loss from an often-used test exposure.

For reasons explained in the previous annual report the 4 kHz octave band of noise was presented at 108 dB SPL and not at 105 dB as indicated in the above SOW.

(1) Phase II experimental control groups:

The entire experimental protocol (i.e., PTS, DPOAEs, & histology) was completed on four (4) noise and vehicle control groups. They were:

- (a) The noise only control group (N=8).

(b) The noise + saline control group (N = 7/8). The group mean PTS data are based on 7 animals. The evoked potential electrode on one animal came loose. All other mean data for this group are based on 8 animals.

(c) The noise + water + EDTA control group* (N = 6).

(d) The noise + saline + DMSO control group* (N = 6).

[*Note: Some of the published data using L-NAC treatment, actually used a commercial (Mucomyst) aqueous preparation containing EDTA. The Src and Ebselen were put into solution using DMSO. Both EDTA and DMSO have anti-oxidant/anti-inflammatory properties. As a result the SOW was modified to include control groups for both EDTA and DMSO. This required an additional 4 animals.]

Results for the four control groups:

(a) The noise only control group (N=8): Group mean data are shown in Figures 1 to 4. Permanent threshold shifts (PTS) varied from about 48 to 55 dB between the 2 and 16 kHz test frequencies. The corresponding outer hair cell (OHC) loss varied from 70 to 80 % while inner hair cell (IHC) loss peaked at about 35% at 8 kHz (Fig. 1). Distortion product otoacoustic emissions (DPOAE) were effectively absent from 2 kHz and above (Figs. 2 & 3). Individual cochleograms for this group are shown in Figure 4. The panels are arranged in descending order of severity based on the total number of missing OHCs.

(b) The noise + saline control group (N = 7/8): The group mean PTS data are based on 7 animals. The evoked potential electrode on one animal came loose. All other mean data are based on 8 animals. PTS varied from approximately 40 to 48 dB across the 2 to 16 kHz test frequency range. The corresponding OHC loss varied from 60 to 80% with about a 25 % IHC peak loss at 4 kHz. (Fig. 5). DPOAEs were absent across the entire test frequency range (Figs 6 & 7). Individual cochleograms for this group are shown in Figure 8. The panels are arranged in descending order of severity based on the total number of missing OHCs.

(c) The noise + water + EDTA control group (N = 6): PTS varied from approximately 48 to 55 dB across the 2 to 16 kHz test frequency range. The corresponding OHC loss varied from 65 to 85% with about a 29 % IHC peak loss at 8 kHz. (Fig. 9). DPOAEs were effectively absent from 2 kHz and above (Figs. 10 & 11). Individual cochleograms for this group are shown in Figure 12. The panels are arranged in descending order of severity based on the total number of missing OHCs.

(d) The noise + saline + DMSO control group (N = 6): PTS varied from approximately 33 to 48 dB across the 2 to 16 kHz test frequency range. The corresponding OHC loss varied from 60 to 72% with about a 37 % IHC peak loss at 8 kHz. (Fig.13). DPOAEs were effectively absent or severely depressed from 2 kHz and above (Figs. 14 & 15). Individual cochleograms for this group are shown in Figure 16. The panels are arranged in descending order of severity based on the total number of missing OHCs.

Statistical analysis: Evoked potential permanent threshold shifts and outer hair cell losses in octave-band lengths of the cochlea were compared among the groups of control and drug treated animals exposed to the 108 dB SPL, 4 kHz octave band of noise for 6 hours using a two-way mixed model analysis of variance (ANOVA) with repeated measures on one factor (frequency). The probability of a type I error was set at 0.05. Statistically significant main effects of frequency were expected and found in all of the following analyses because of the frequency-specific nature of the audibility curve of the chinchilla and the noise exposure stimulus. For this reason main effects of frequency are not addressed in

the following presentation of results. Post-hoc analyses (Tukey/Kramer test) were performed to establish any significant effects among the control and treatment groups.

The ANOVA analysis of the data from these 4 control groups indicated that there were no statistically significant differences among the 4 groups ($F = 0.737$, $df = 3$, $p = 0.5406$). Therefore the groups were collapsed into a single control group with $N = 28$ for use in comparisons with the drug treated groups. The result is shown in Figures 17 to 19.

2. The Phase II drug treatment of chinchillas in the rescue mode following an exposure to a 4.0 kHz octave band of noise for 6 hours at 108 dB SPL.

The 5 drugs used were: (1) L-N-acetylcysteine (L-NAC); (2) D-Methionine (D-MET); (3) Ebselen SPI-1005; (4) Acetyl-L-carnitine (ALCAR) and (5) Src-PTK inhibitor, KX1-004. All noise exposures began at 8 a.m. and terminated at 2 p.m. at which time the animals were given an I.P. drug injection. Eight (8) hours later a second injection was given. Over the next 4 days, injections were given at 8 a.m. and 4 p.m. Thirty (30) days following the exposure PTS and DPOAEs were collected and the animals were then euthanized for histology.

(a) L-N-acetylcysteine (L-NAC): L-NAC was dosed at 325 mg/kg from a 20% commercial preparation (Mucomyst, Hospira Inc.). This preparation contains 200 mg/ml L-NAC and 0.5 mg/ml EDTA, an antioxidant. The group ($N=8$) mean results of this treatment are shown in Figures 20-23. PTS in the 2 to 16 kHz regions varied from approximately 35 to 50 dB while OHC loss varied from about 48 to 70 %. At the lower primary intensities the DPOAEs were effectively eliminated across the entire test frequency range. In the 1 to 2 kHz range at the higher (e.g., $L_1 = 65$ dB) intensity the DPOAEs were severely depressed. Figure 23 shows the cochleogram for each individual animal in the group. The figure gives some appreciation of the variability within the group.

(b) Acetyl-L-Carnitine (ALCAR): ALCAR (Sigma-Aldrich, Cat. #A-1509-1G) was dosed at 100 mg/kg from a 25mg/ml solution (sterile normal saline). The group ($N=8$) mean results of this treatment are shown in Figures 24-27. The mean PTS varied from approximately 40 to 55 dB across the 2 to 16 kHz ranges of test frequencies. OHC loss varied from 55% at 2 kHz to a maximum of 95% at 4 kHz. DPOAEs were abolished or severely reduced across the entire range of test frequencies. The individual animal cochleograms are shown in Figure 27.

(c) D-Methionine (D-MET): D-MET (Sigma-Aldrich, Cat. #M-9375) was dosed at 200 mg/kg from a 30 mg/ml solution (sterile normal saline). The group ($N=8$) mean results of this treatment are shown in Figures 28-31. The mean PTS varied from approximately 50 to 58 dB across the 2 to 16 kHz ranges of test frequencies with 20 to 30 dB PTS at 0.5 and 1.0 kHz. OHC loss at 2 kHz and above was 100% with up to 60% loss at 1.0 kHz and the lower frequencies. DPOAEs were abolished across the entire range of test frequencies. The individual animal cochleograms are shown in Figure 31.

(d) Ebselen SPI-1005: Ebselen (Sigma-Aldrich, Cat. #E-3520) was dosed at 16 mg/kg from a 50mg/ml solution (DMSO). The group ($N=8$) mean results of this treatment are shown in Figures 32-35. The mean PTS varied from approximately 37 to 42 dB across the 2 to 16 kHz ranges of test frequencies. OHC loss varied from approximately 40 to 65% across the 2 to 16 kHz region of the cochlea. DPOAEs were severely reduced between 2 and 10 kHz especially at the lower intensities. Below 2 kHz the

decrement in the DPOAEs was less reflecting the OHC profile of loss. The individual animal cochleograms are shown in Figure 35.

(e) Src-PTK inhibitor, KX1-004: The Src inhibitor (Kinex Pharmaceuticals) was dosed at 50 mg/kg from a 100mg/ml solution (DMSO). The group (N=9) mean results of this treatment are shown in Figures 36-39. The mean PTS varied from approximately 35 to 45 dB across the 2 to 16 kHz ranges of test frequencies. OHC loss varied from approximately 55 to 70% across the 2 to 16 kHz region of the cochlea. DPOAEs were severely reduced between 2 and 10 kHz especially at the lower intensities. Below 2 kHz the decrement in the DPOAEs was less reflecting the OHC profile of loss. The individual animal cochleograms are shown in Figure 39.

In summary, for all 5 drug treated groups the mean effect of the noise was quite similar, i.e., large PTS at and above 2 kHz with much less PTS at 0.5 and 1.0 kHz. DPOAEs were typically reduced or eliminated across all frequencies with a profile of loss that generally reflected the loss of outer sensory cells although the complete loss of DPOAEs with less than total loss of OHCs should be recognized. The sensory cell loss generally began to increase at 2 kHz and either reached a maximum at 4 kHz and decreased at the higher frequencies or remained high at frequencies above 2 kHz.

Results of the statistical analysis:

A graphical comparison of the frequency specific magnitudes of the group mean PTS, OHC loss and IHC loss for the control and drug treated groups is shown in Figures 40 to 42. The ANOVA analysis indicated that there was no statistically significant ($p < 0.05$) main effect of group for PTS ($F = 1.54$, $df = 5$, $p = 0.190$); OHC loss ($F = 2.29$, $df = 5$, $p = 0.057$); or IHC loss ($F = 2.09$, $df = 5$, $p = 0.785$). That is, there was no significant difference in the hearing and sensory cell loss among the control group and the various rescue mode drug treatment groups exposed to the 4 kHz, OBN noise exposure at 108 dB SPL.

Post-hoc pair wise comparisons were made using the Tukey/Kramer test. There was a significant difference in the PTS between the D-Met group and the control, Ebselen, L-NAC, and Src groups. The group treated with D-Met exhibited the most PTS. There was a significant difference in the OHC loss between the D-Met group and the control, Ebselen, L-NAC, ALCAR, and Src groups. The group treated with D-Met exhibited the most OHC loss. There were no significant differences in IHC loss for the paired comparisons.

The final analysis of the Phase II data set looked at the total number of missing IHC and OHC over the entire length of the basilar membrane for each group. A graphical comparison of the group mean total number of missing sensory cells for the control group and the 5 drug treated groups is shown in Figure 43. ANOVA analysis for the OHC loss indicated no statistically significant difference among the groups ($F = 2.28$, $df = 5$, $p = 0.0571$). There was also no statistically significant difference in the IHC loss ($F = 2.0327$, $df = 5$, $p = 0.0871$). In the above frequency specific as well as total analyses of the OHC loss, the ANOVA approached significance at the 0.05 level possibly as a result of the relatively larger OHC loss seen in the group treated with D-MET.

B. The Phase III experiments are in progress.

A revision of the original Phase III SOW was required since there were no two drugs in Phase II of this work that performed 'best' in a rescue mode of treatment. The revised and approved SOW follows:

Revised Phase III SOW: Each of the 5 drugs will be used to treat 5 groups of animals exposed to the 158 dB peak SPL impulses (blast waves), i.e., the peak SPL produced by discharging the shock tube at an 11psi charge pressure. Each group will consist of 20 subjects for a total of 100 drug treated animals. An additional 20 animals will serve as a noise only control group. These 6 groups will follow the same testing and treatment protocols as used in the Phase II 4 kHz octave band exposures, i.e., treatment will begin immediately post exposure (T=0). The single 'best performing' drug will then be administered to another group exposed to the same blast waves but treatment will begin 1- hour post exposure (T=1). The best treatment schedule (i.e., T=0 or T=1 hour post) will then be repeated with each of 2 different drug doses in two additional exposed groups of 20 animals. This will require 3 additional groups or 60 subjects. Thus the revised SOW will utilize data from 180 subjects that have completed the entire protocol.

Results to date:

(1) The noise only control group: Twenty (20) animals were exposed to 10, 158 dB peak SPL impulses (blast waves) produced by the discharge of a shock tube with a compression section charge pressure of 11 psi. In the free field this charge pressure produces a 165 dB peak SPL impulse. The exposures lasted an average of 1.2 minutes. Figure 44 shows the mean permanent threshold shifts (PTS) and the mean cochleogram for the group. PTS varied from approximately 10 to 25 dB across the range of AEP test frequencies. A broad profile of outer hair cell (OHC) loss between 1 and 8 kHz was recorded with a peak loss in the 2 kHz octave band of ~ 45 %. Inner hair cell (IHC) losses were considerably smaller. Across the entire cochlea there were approximately 1500 OHCs missing. The results of this control group are compared to the group of animals used for the Phase I shock tube lesion calibration in Figure 45. The lesion calibration animals were exposed in the same configuration and to the same blast waves as the control animals. There is a considerable difference in the severity of the lesion. Cochleograms for the individual animals comprising the Phase III noise only control group are shown in Figure 46. There is substantial variability in the response of the animals to the blast exposure despite stringent control of the exposure conditions. Nine (9) subjects showed little or no effect of the exposure. The group mean DPOAE data for the Phase III noise only control group reflect the AEP and histological results and are shown in Figures 47 and 48. It should be noted that there were no inconsistencies between the audiometric, emission and histological data for the individual animals. The output of the shock tube has been checked several times and has proven to be very consistent. Each animal is handled in the same way and each is checked to be certain that the external canal is clear at the time of exposure. The pinna is secured so that it cannot occlude the external canal. Middle ear effects can be eliminated as a contributing factor since the pre exposure emissions and AEP thresholds are normal and the Phase I tympanogram results showed no changes in middle ear function at the levels used for the controls as well as at higher levels.

(2) A second noise only control group (N=20) has been run in order to obtain a clearer picture of the extent of the group to group variability. The results of this repeat control group are not yet complete.

(3) Rescue treatment with L-NAC (Mucomyst) following the 158 dB peak SPL blast wave exposure: The group (N = 20) mean data set for these animals is shown in Figures 49 through 52. The

PTS varied between approximately 30 and 40 dB across the 1 to 8 kHz test frequencies with a corresponding 70 to 80% loss of outer hair cells (Fig. 49). Emissions were severely reduced but not eliminated at the higher frequencies and intensities. The mean histological data are similar to the Phase I lesion calibration data shown in Figure 45B but the permanent changes in thresholds, emissions and cell loss are much greater than in the noise only control group (Figure 44 & 45A). The rescue treatment with L-NAC did not reduce trauma from the blast exposure. Cochleograms from the individual animals in the L-NAC treated group are shown in Figure 52. These data clearly highlight a problem, not unexpected, with variability. Variability is a problem with all noise research. As a consequence another control group with N=20 subjects is suggested and a protocol revision will be forthcoming.

(4) Rescue treatment with D-MET following 158 dB peak SPL blast wave exposure: The group (N = 20) mean data set for these animals is shown in Figures 53 through 56. The PTS varied from approximately 18 dB at 0.5 and 16 kHz to 30 dB at 8 kHz. The frequency specific OHC losses, while somewhat greater, approximated the profile seen in the noise only control subjects discussed above (Fig. 44). The DPOAE data generally reflected the PTS and sensory cell loss results.

(5) Three groups of 20 animals/group were exposed to the 158 dB peak SPL shock wave (11 psi charge pressure) and treated with ALCAR, Ebselen or the Src inhibitor. Data from these three groups is not yet complete.

KEY RESEARCH ACCOMPLISHMENTS:

- (1) Phase II of the SOW was completed.
- (2) The blast wave exposures of Phase III have begun.

REPORTABLE OUTCOMES:

None

CONCLUSION:

(1) In animals exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours, there were no statistically significant differences between the control groups and the groups treated with L-NAC, D-MET, ALCAR, Ebselen or the Src inhibitor.

(2) Variability in the response of individual animals to the blast wave exposure may confound the drug treatment results.

REFERENCES:

None

APPENDICIES:

Figures 1- 56

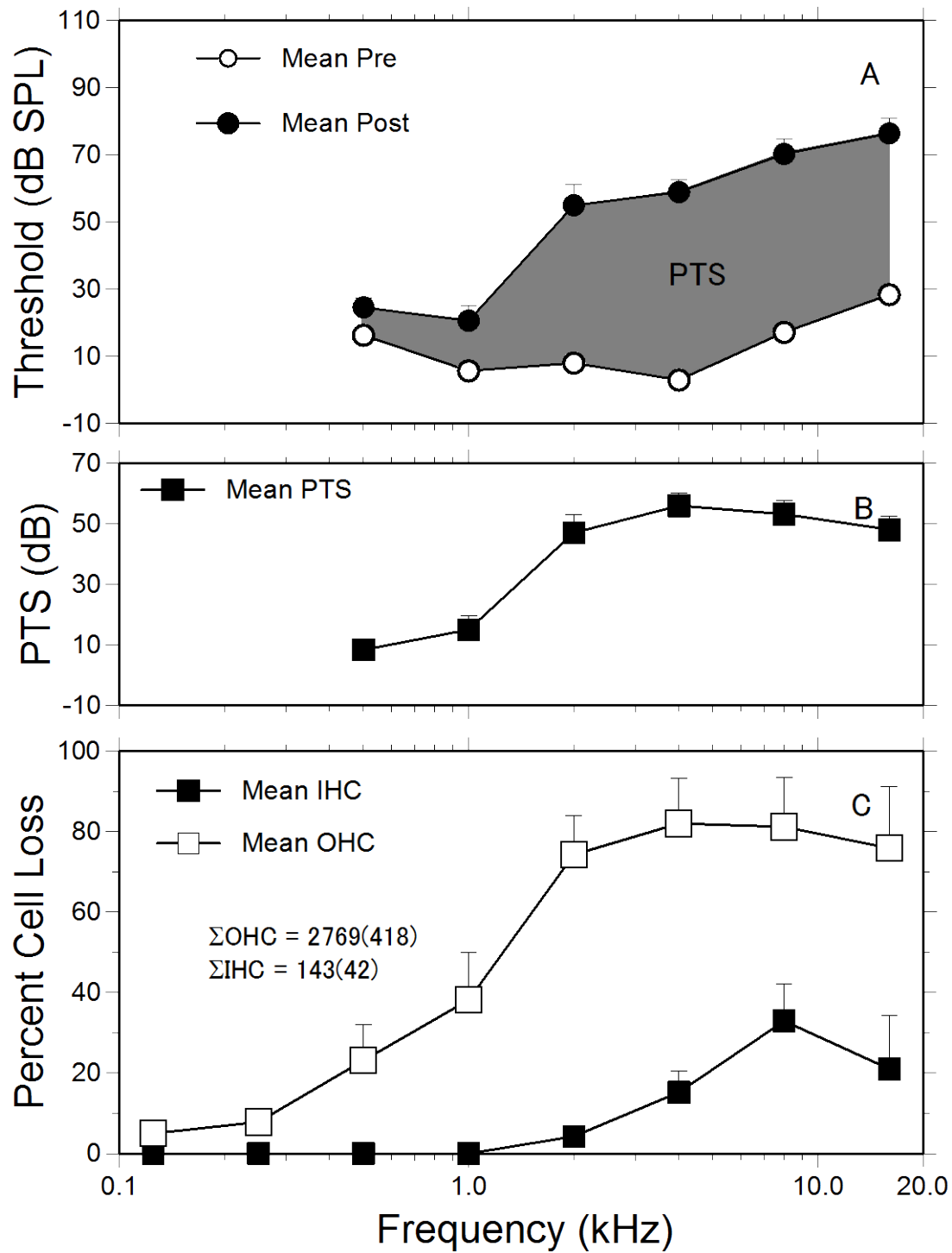


Figure 1. Phase II noise only control group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. $\Sigma\text{OHC} = 2769(418)$ and $\Sigma\text{IHC} = 143(42)$ = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (I = s.e.)

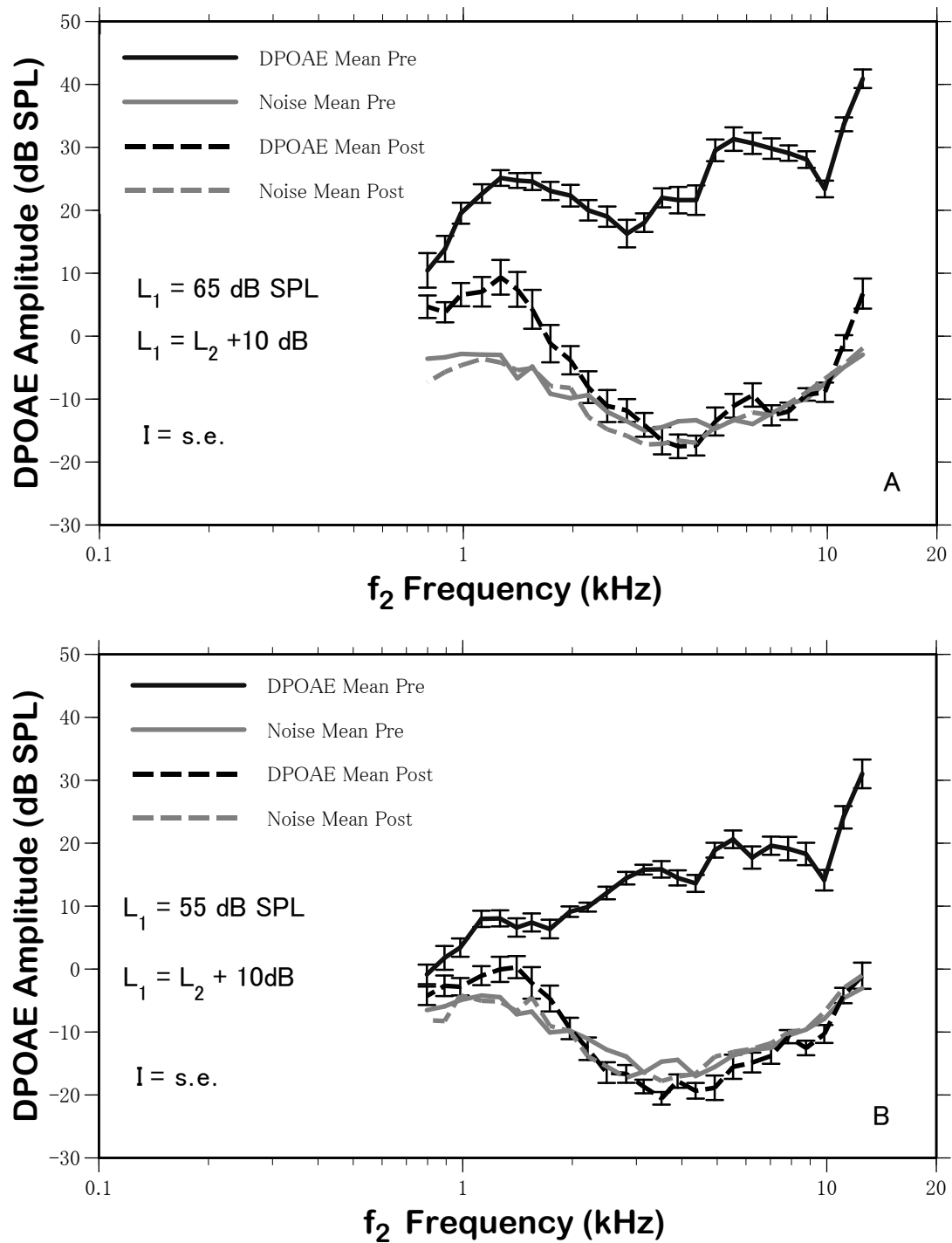


Figure 2. Phase II noise only control group mean ($N=8$) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

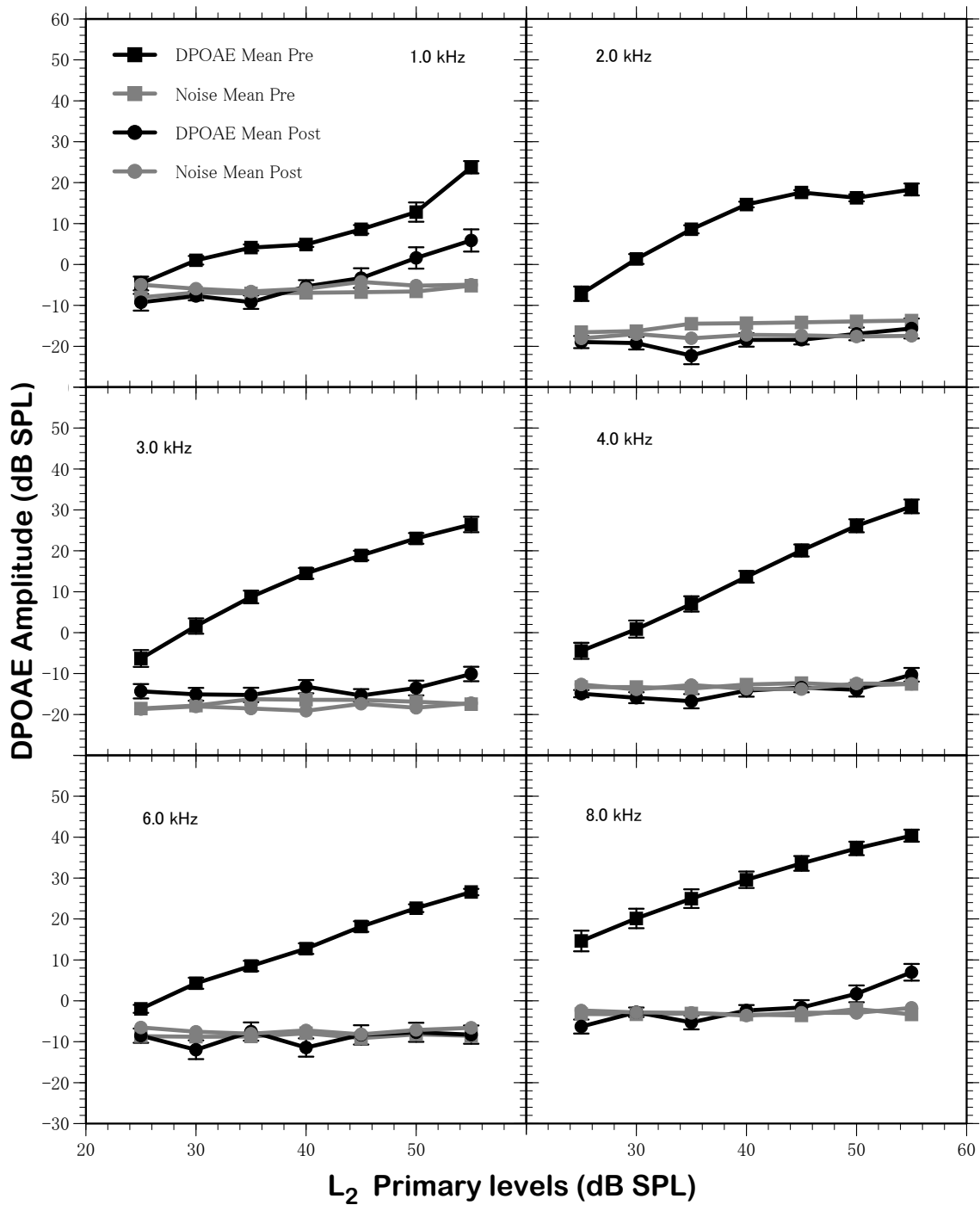


Figure 3. Phase II noise only control group mean (N=8) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

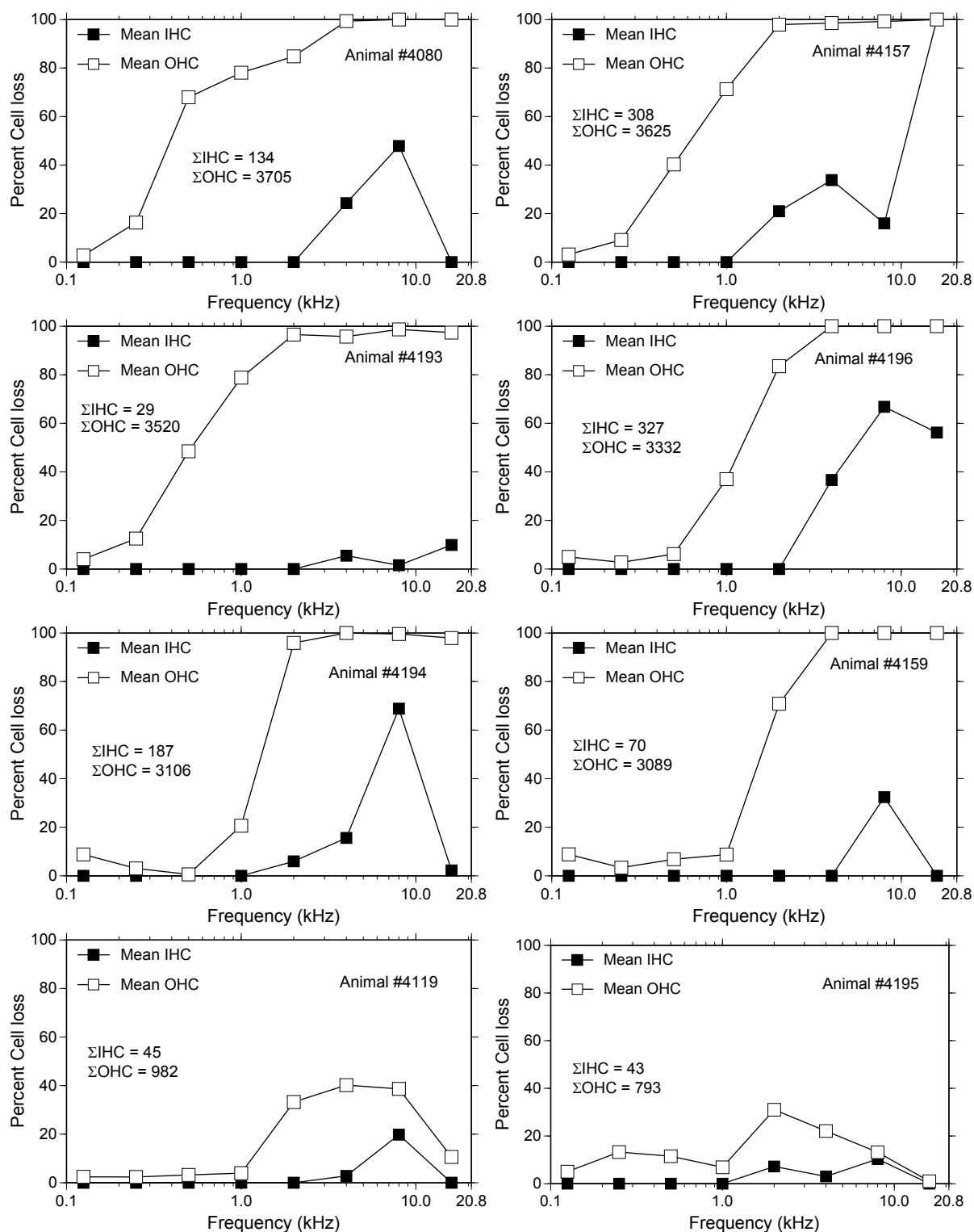


Figure 4. Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours (noise only control group). The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure. Σ IHC and Σ OHC = total number of missing inner and outer hair cells.

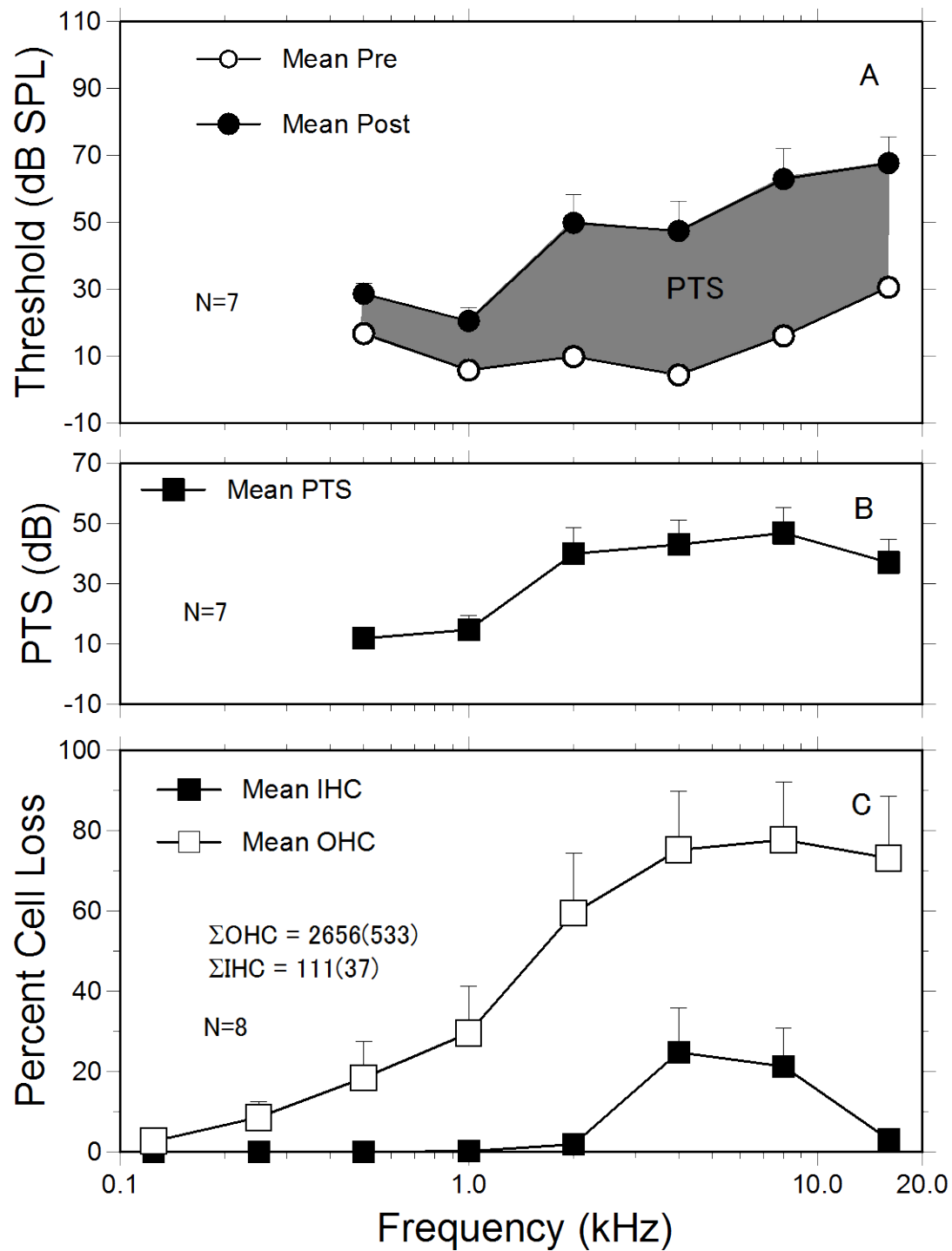


Figure 5. Phase II noise + saline control group (N=7/8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (I = s.e.)

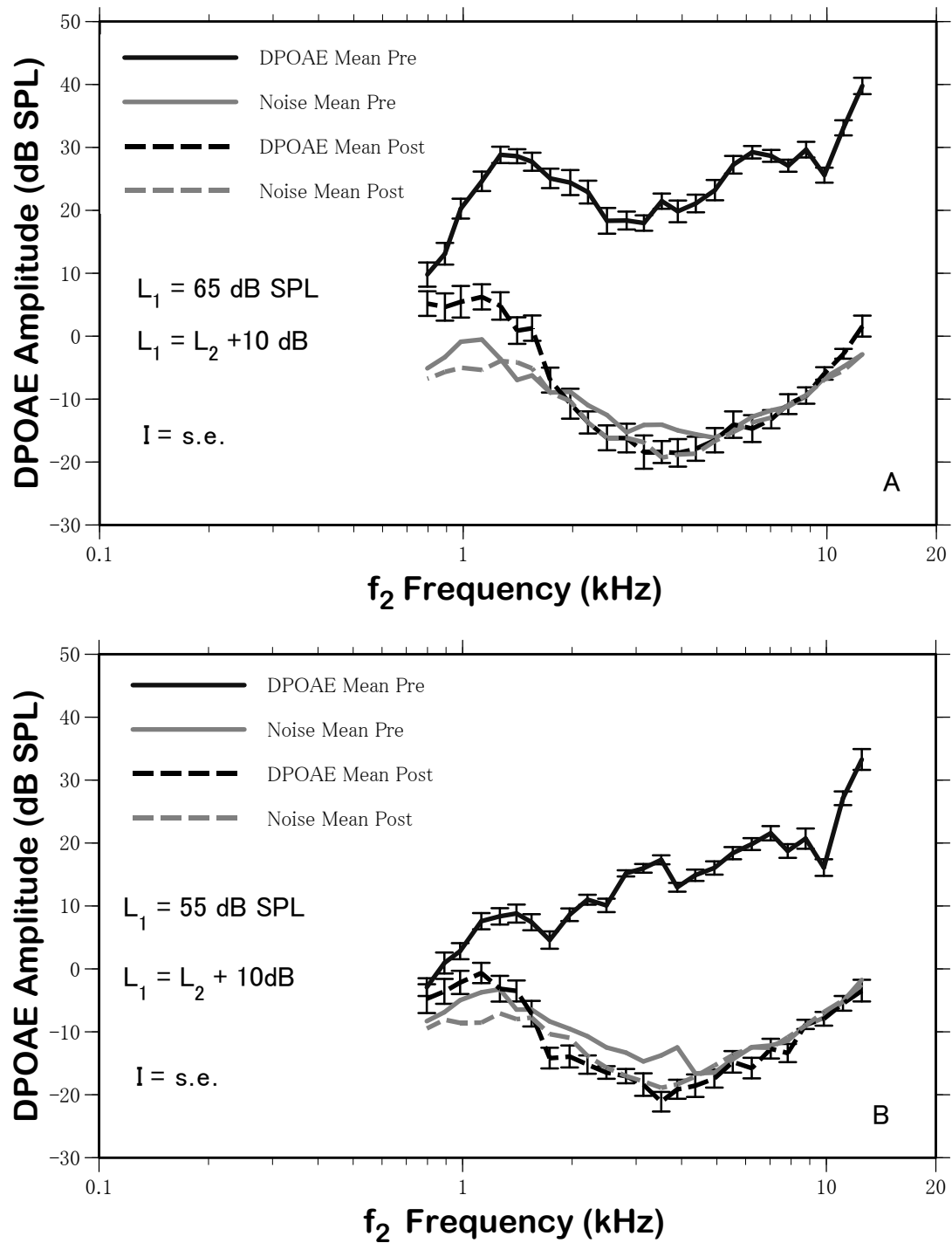


Figure 6. Phase II noise + saline control group mean (N=8) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

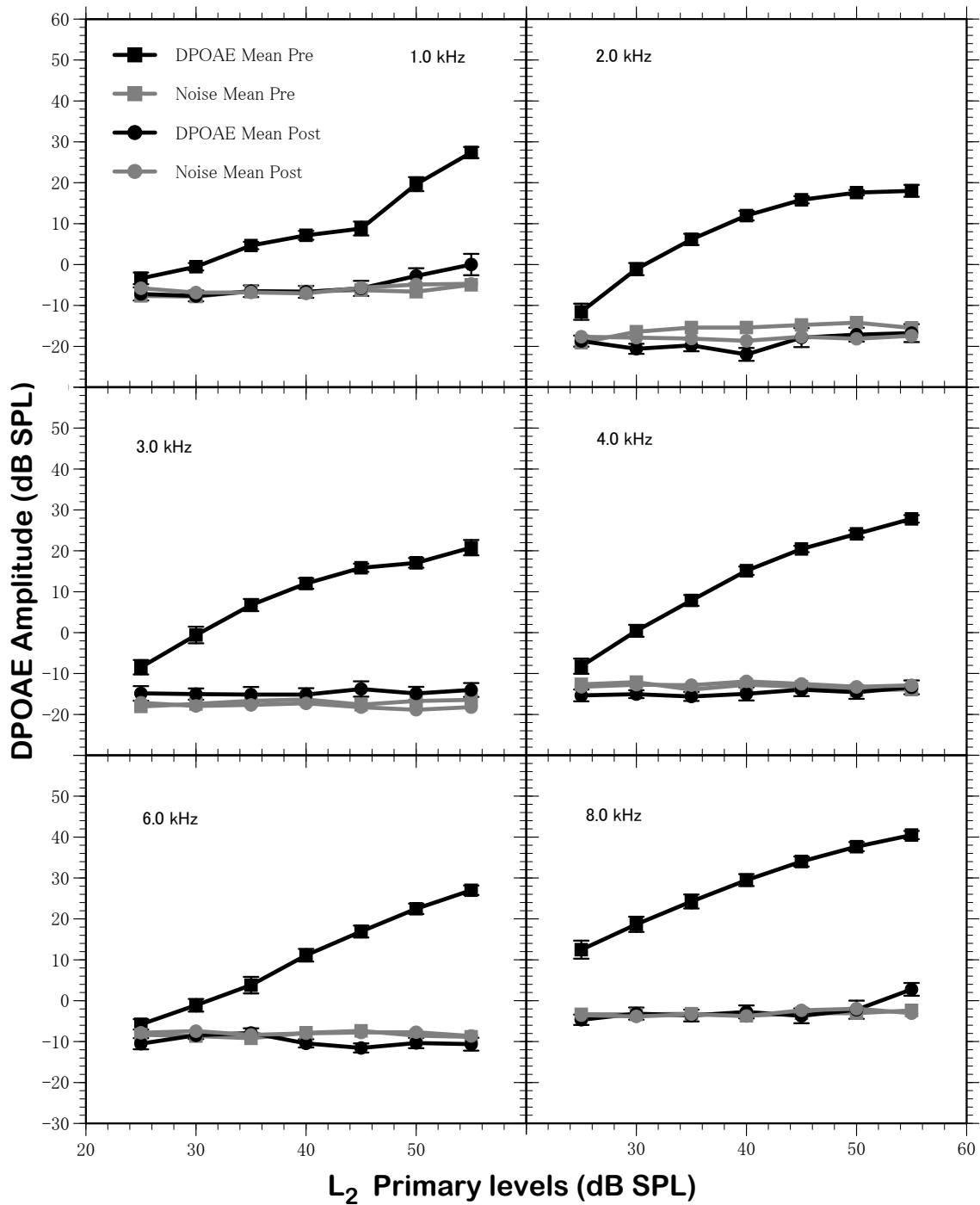


Figure 7. Phase II noise + saline control group mean (N=8) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

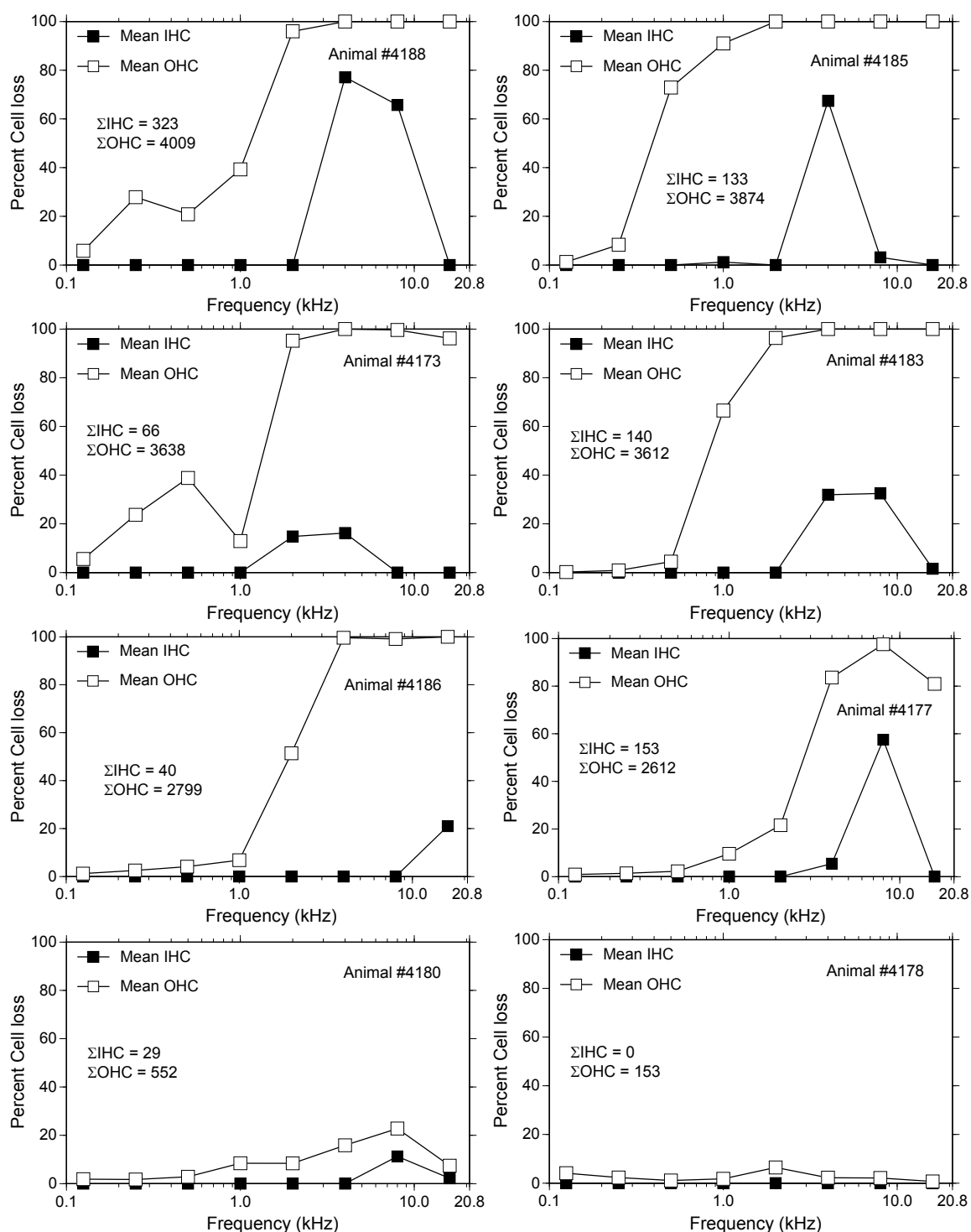


Figure 8. Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours (noise + saline control group). The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure. Σ IHC and Σ OHC = total number of missing inner and outer hair cells.

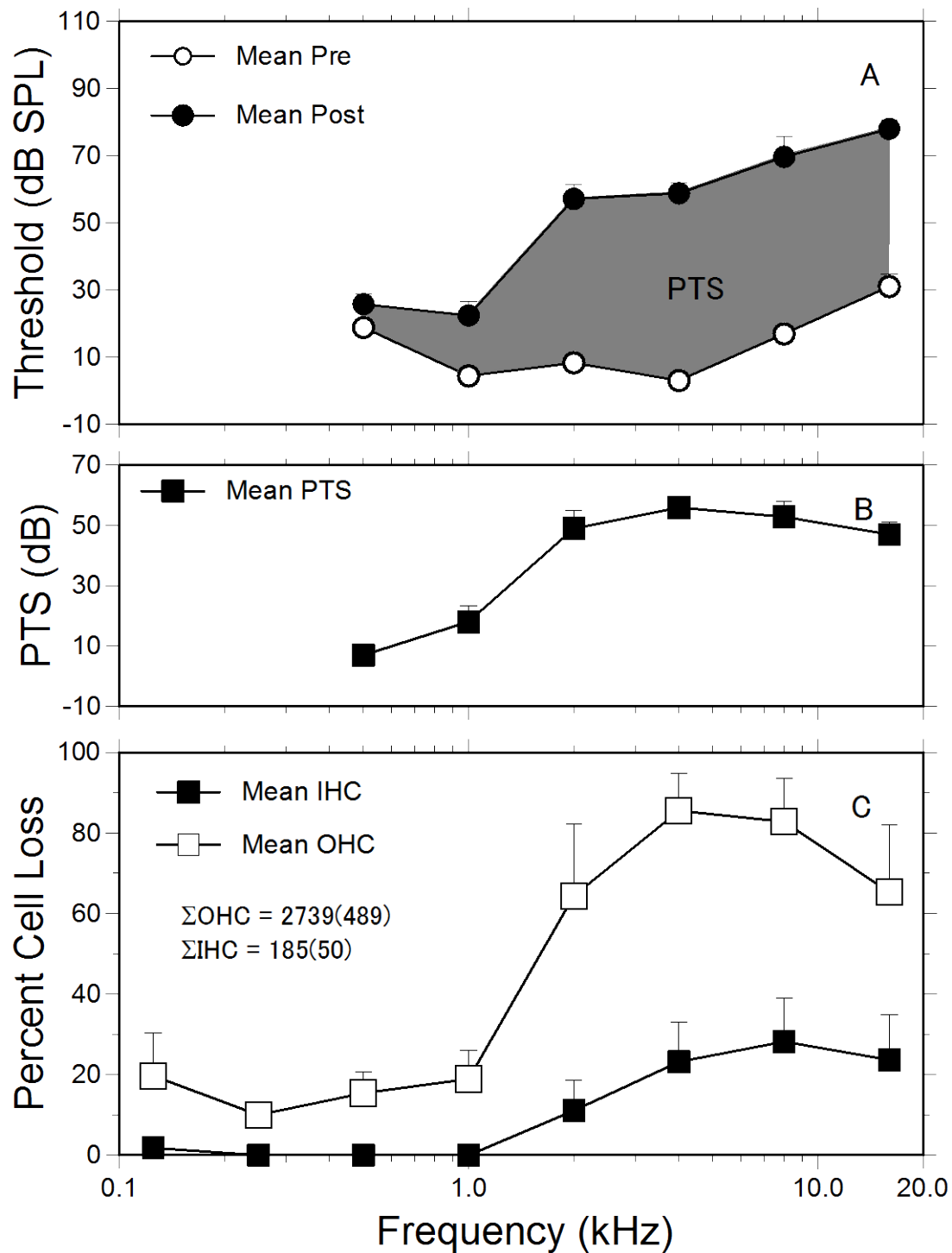


Figure 9. Phase II noise+ water + EDTA control group (N=6) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (I = s.e.)

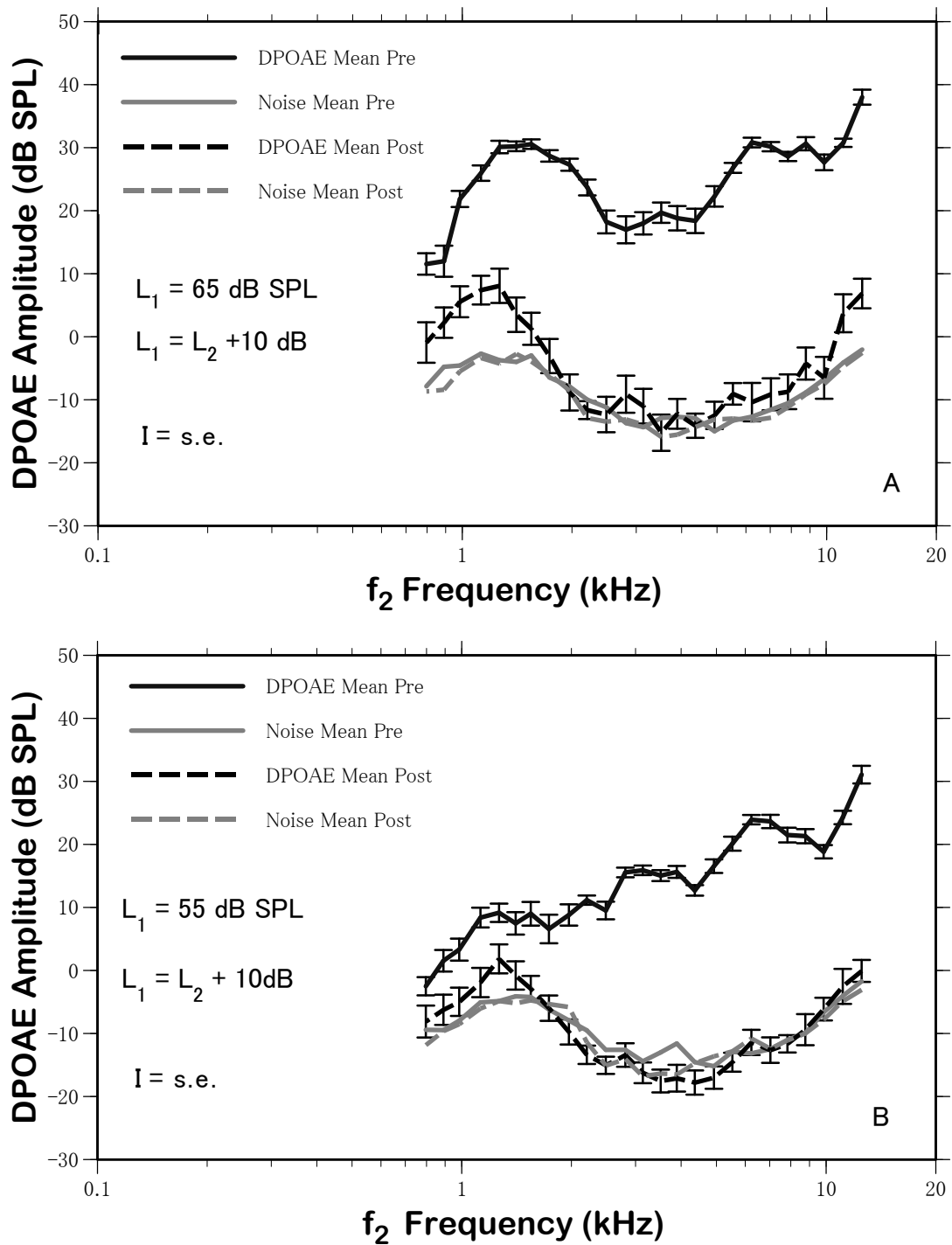


Figure 10. Phase II noise + water + EDTA control group mean ($N=6$) pre and post exposure cubic ($2f_1 - f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. ($I = \text{s.e.}$)

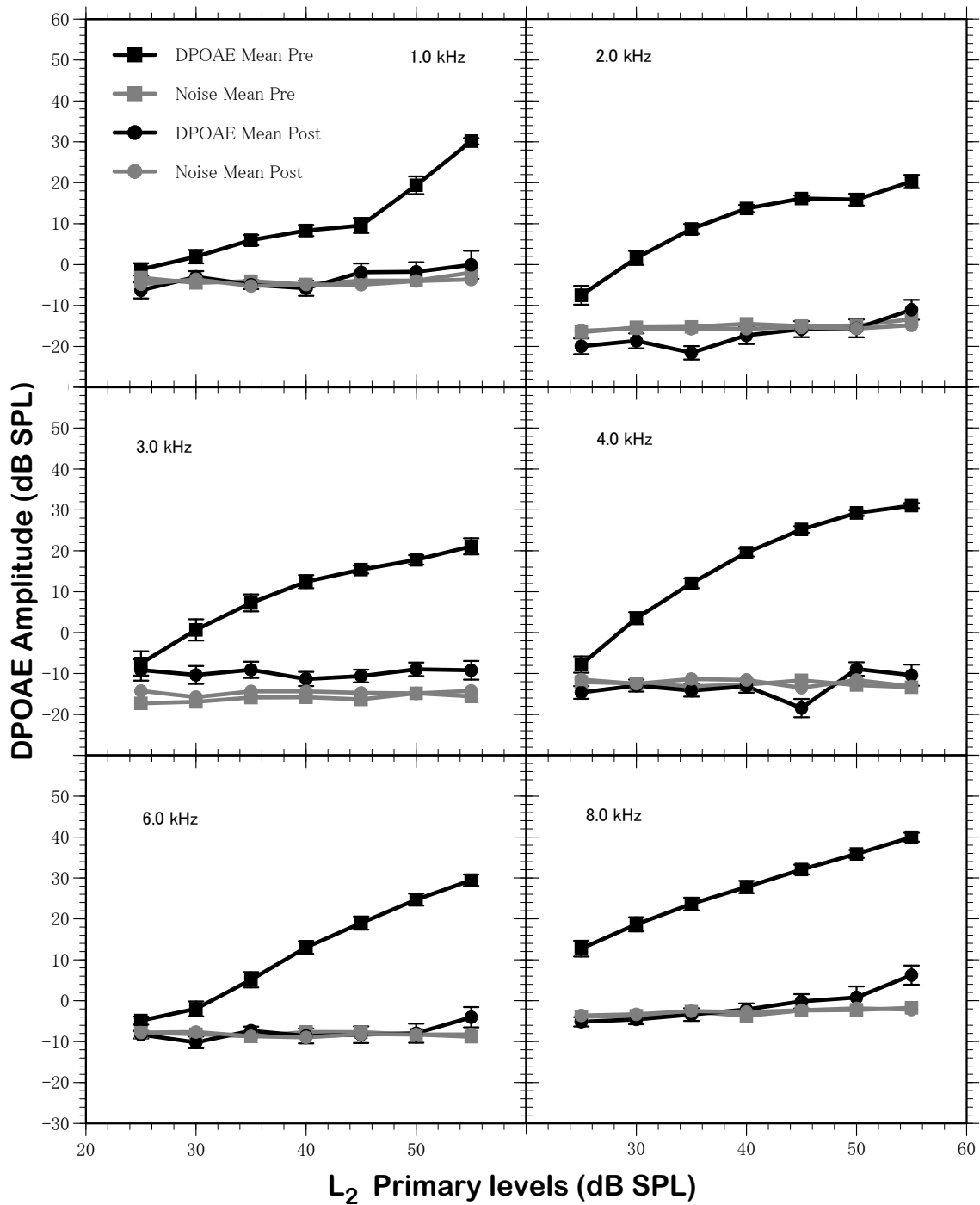


Figure 11. Phase II noise + water + EDTA control group mean (N=6) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

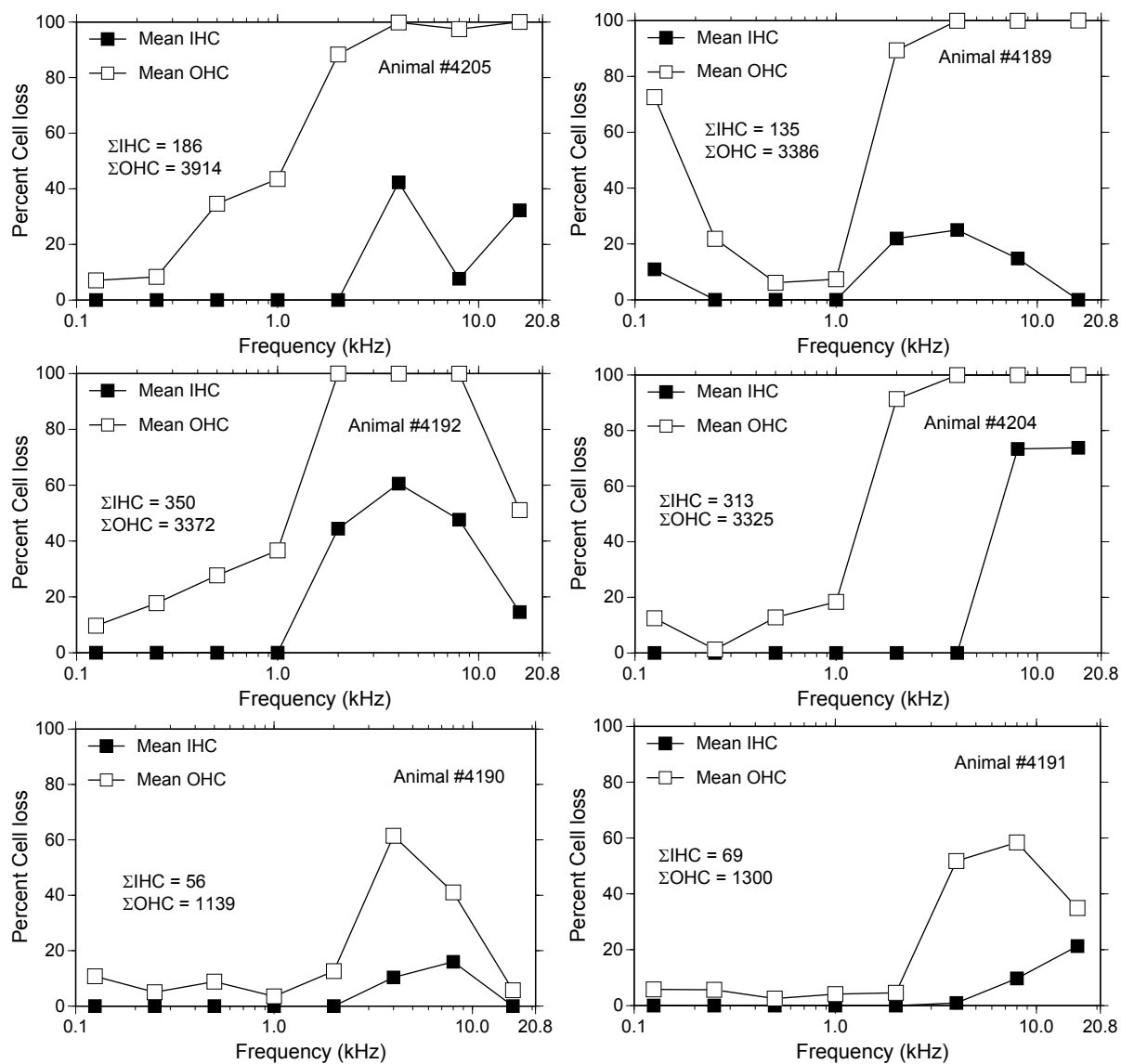


Figure 12. Individual cochleograms for the 6 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with saline + H₂O + EDTA injections BID for five days. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

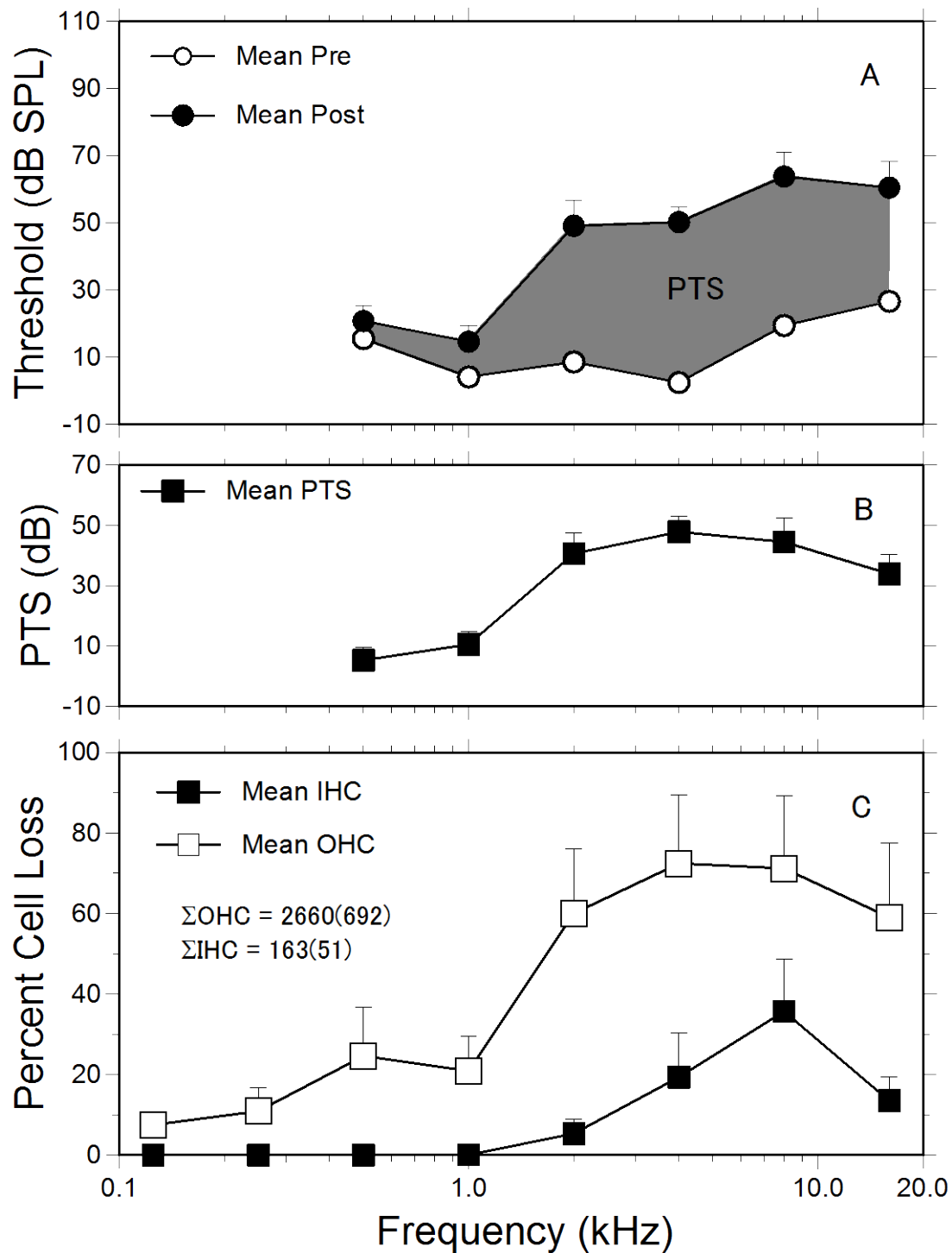


Figure 13. Phase II noise + saline + DMSO control group (N=6) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. Σ OHC and Σ IHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (I = s.e.)

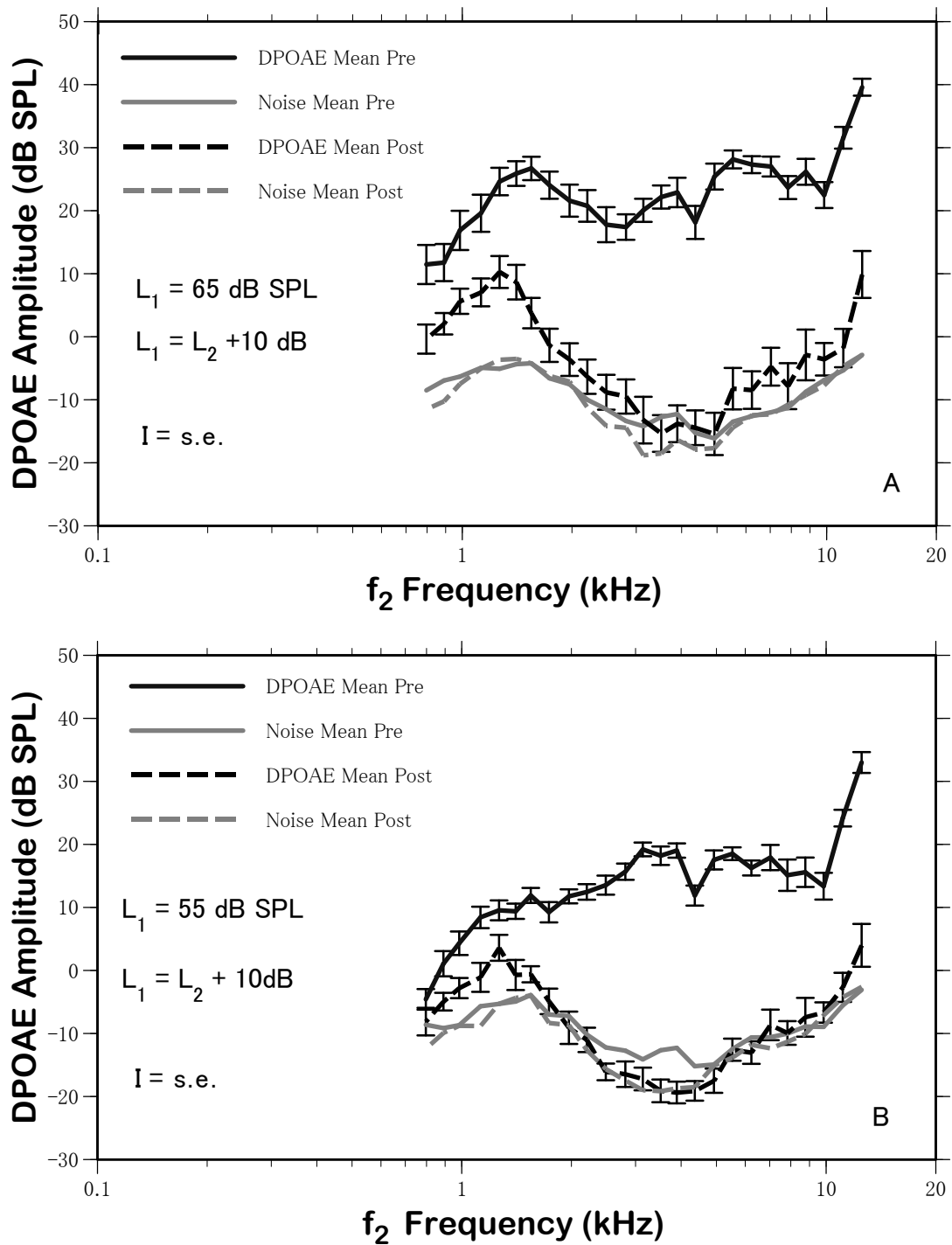


Figure 14. Phase II noise + saline + DMSO control group mean (N=6) pre and post exposure cubic ($2f_1$ - f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

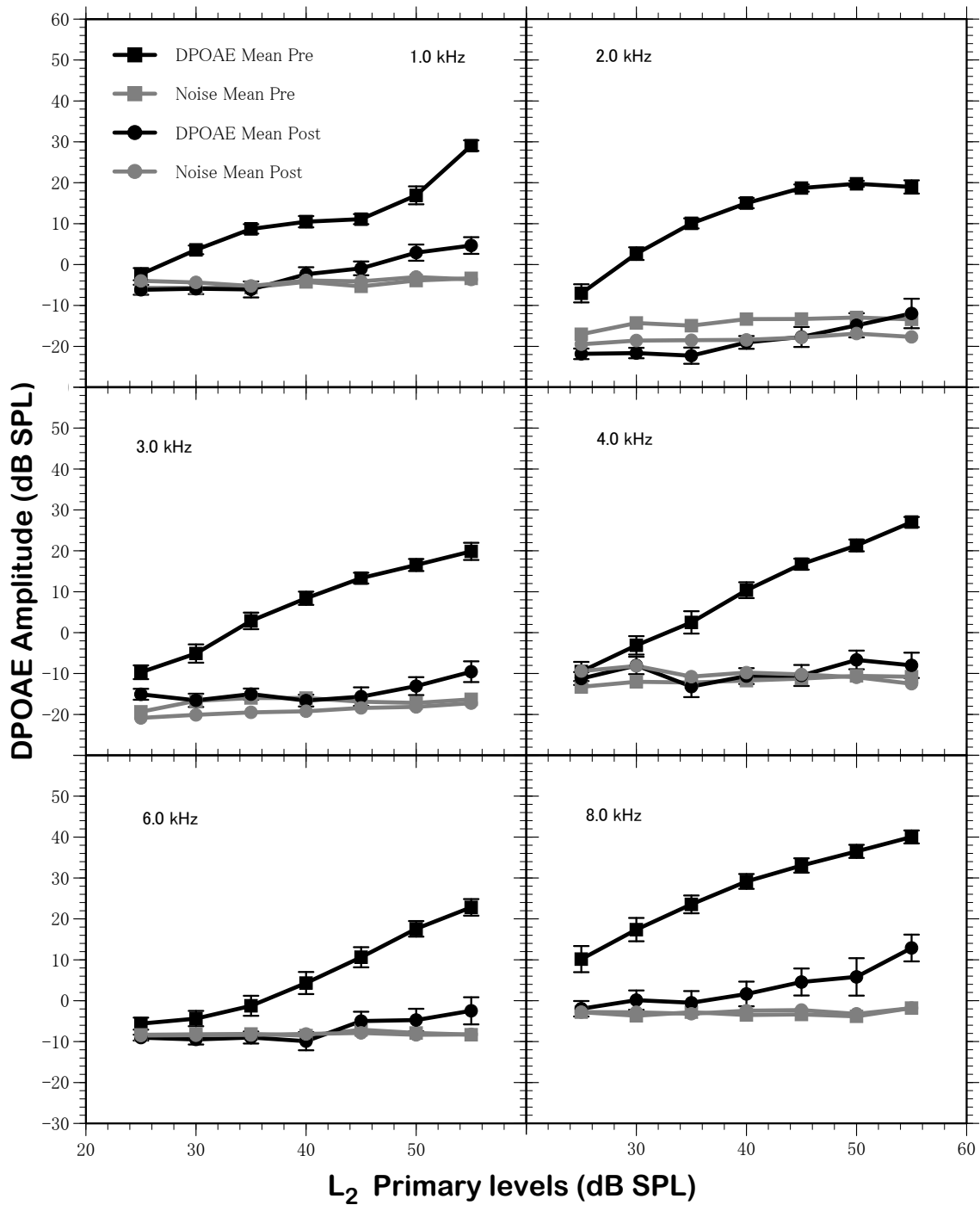


Figure 15. Phase II noise + saline + DMSO control group mean (N=6) pre and post exposure cubic ($2f_1$ - f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

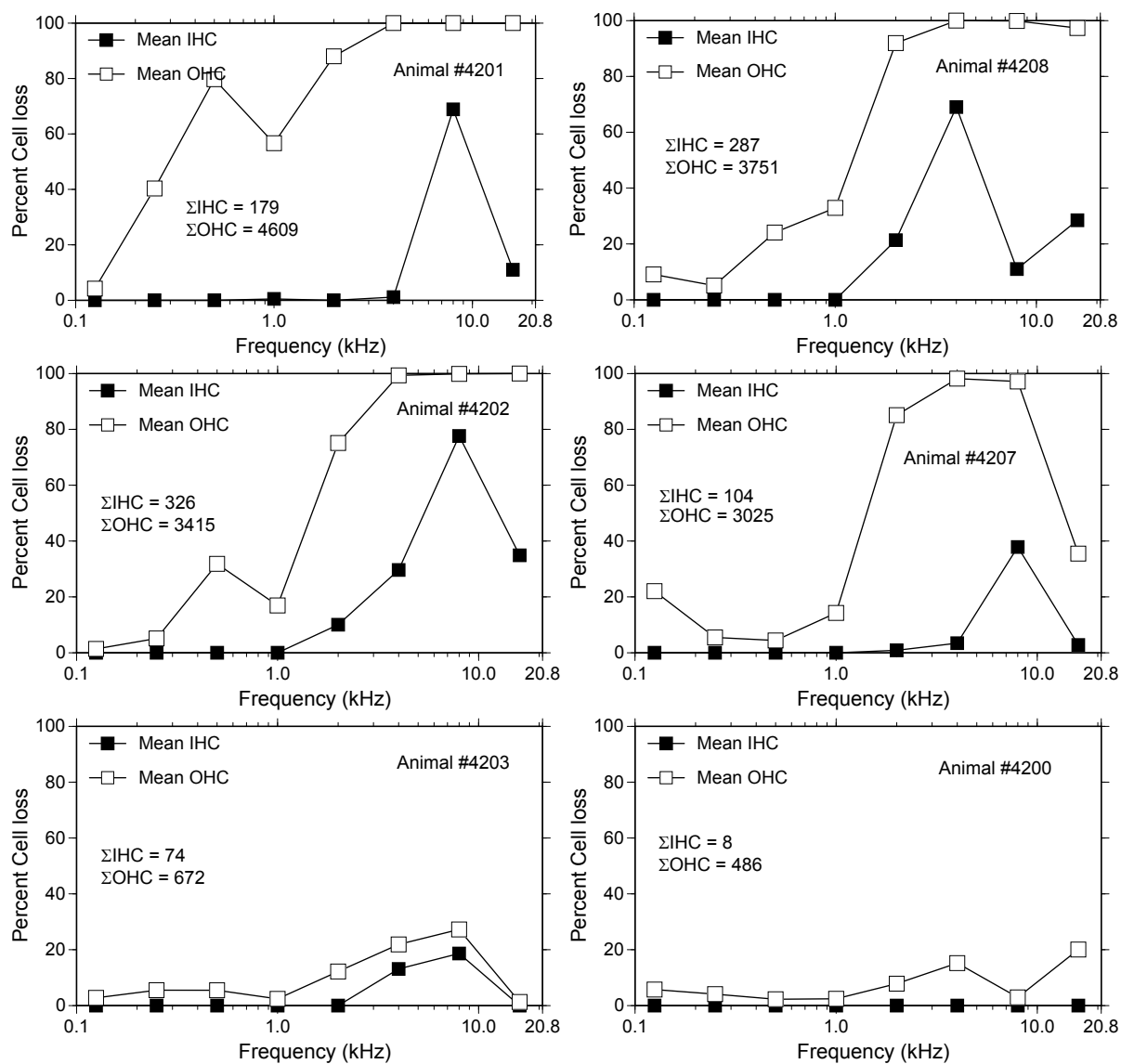


Figure 16. Individual cochleograms for the 6 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with saline plus DMSO injections BID for five days. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure. Σ IHC and Σ OHC = total number of missing inner and outer hair cells.

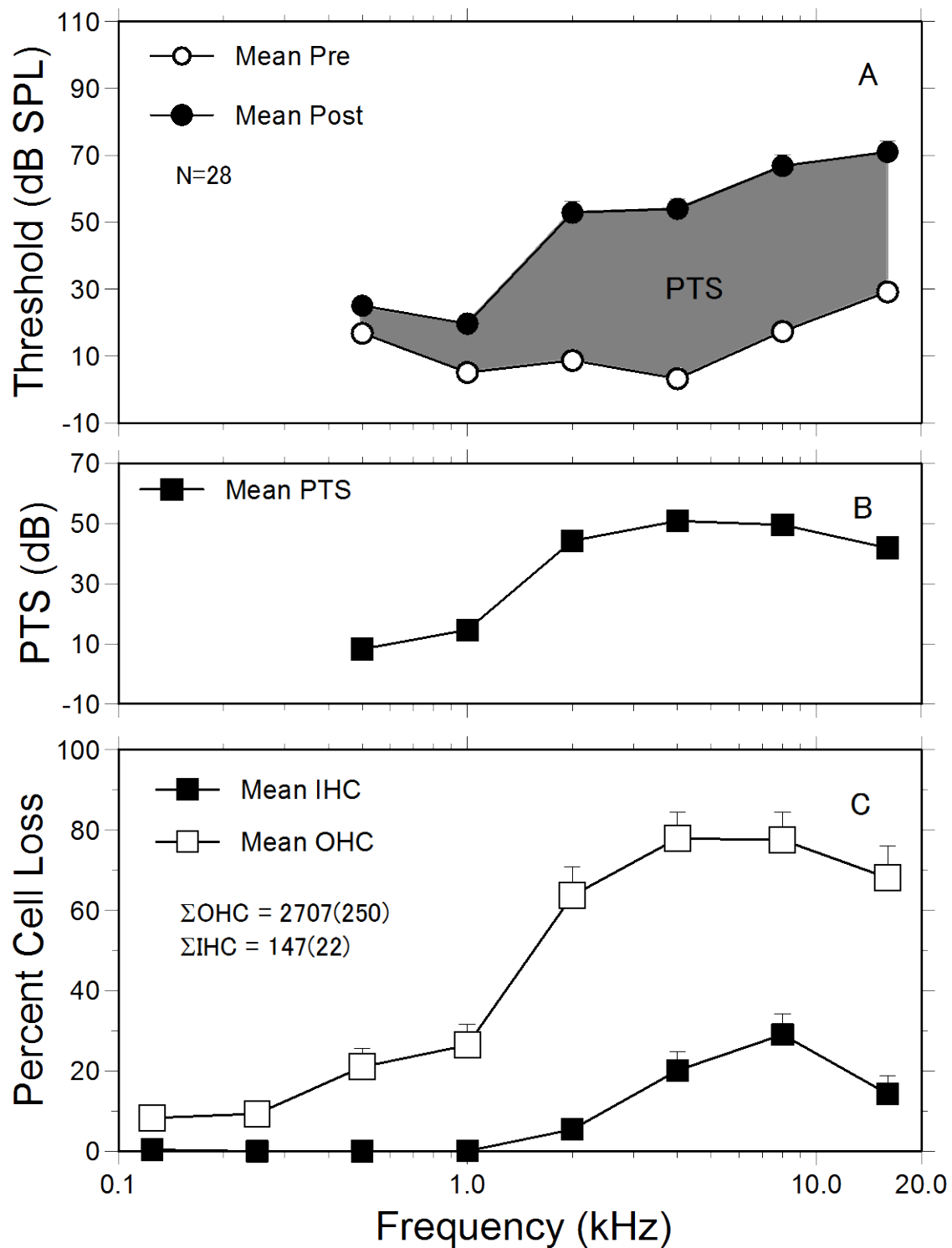


Figure 17. Phase II control group (N=28) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. Σ OHC and Σ IHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (I = s.e.)

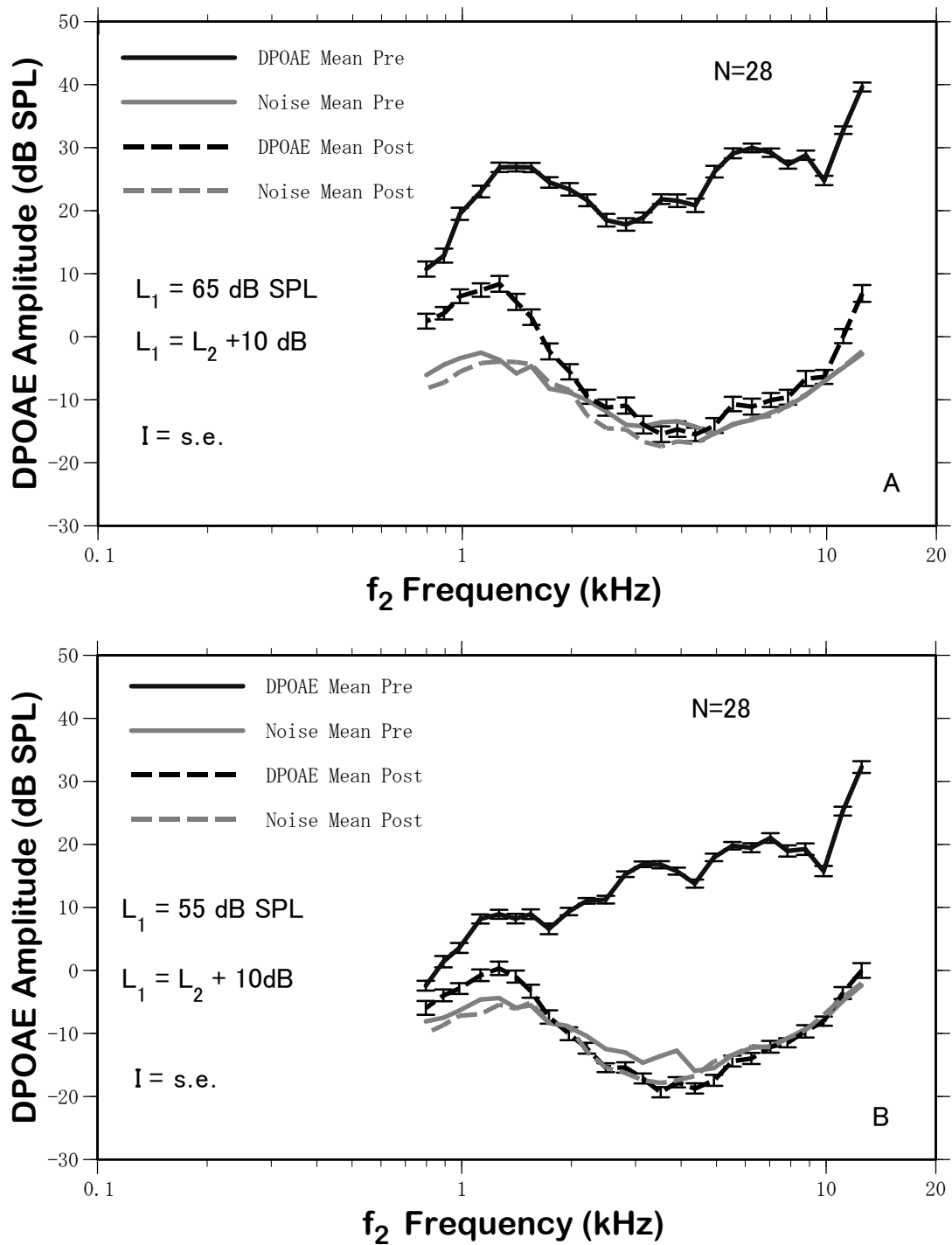


Figure 18. Phase II control group mean (N=28) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

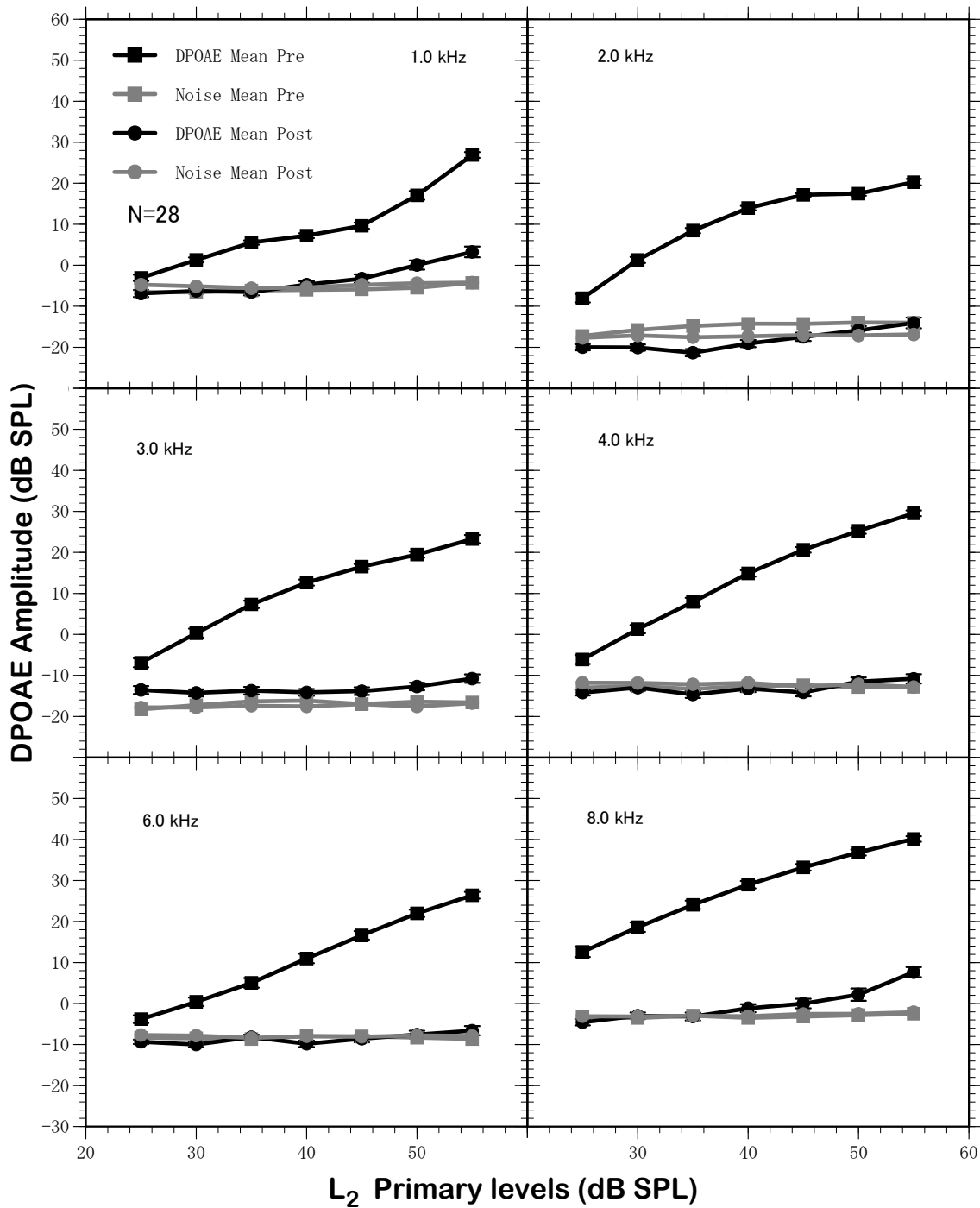


Figure 19. Phase II control group mean (N=28) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

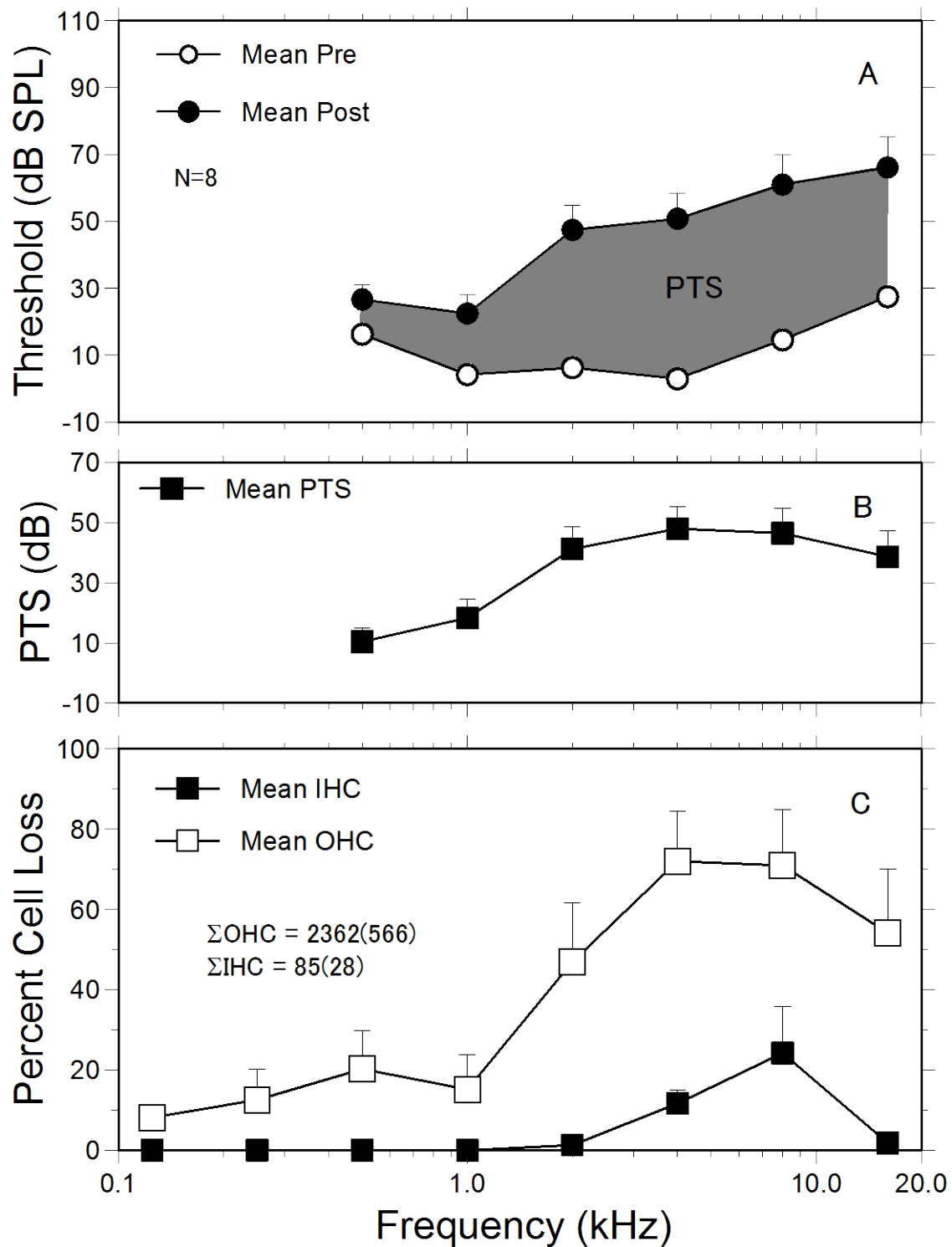


Figure 20. Phase II: L-NAC treated group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (I = s.e.)

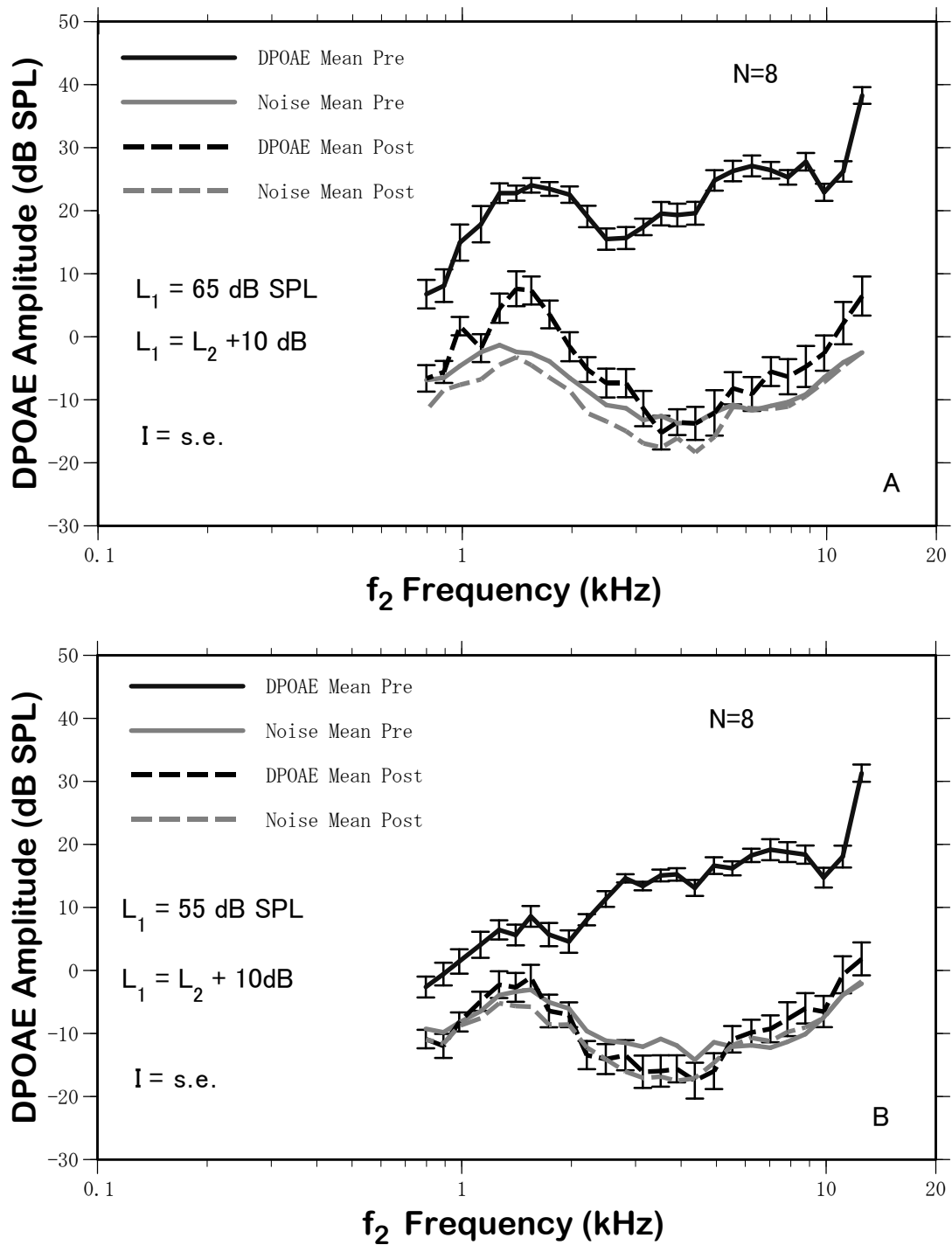


Figure 21. Phase II: L-NAC treated group mean (N=8) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

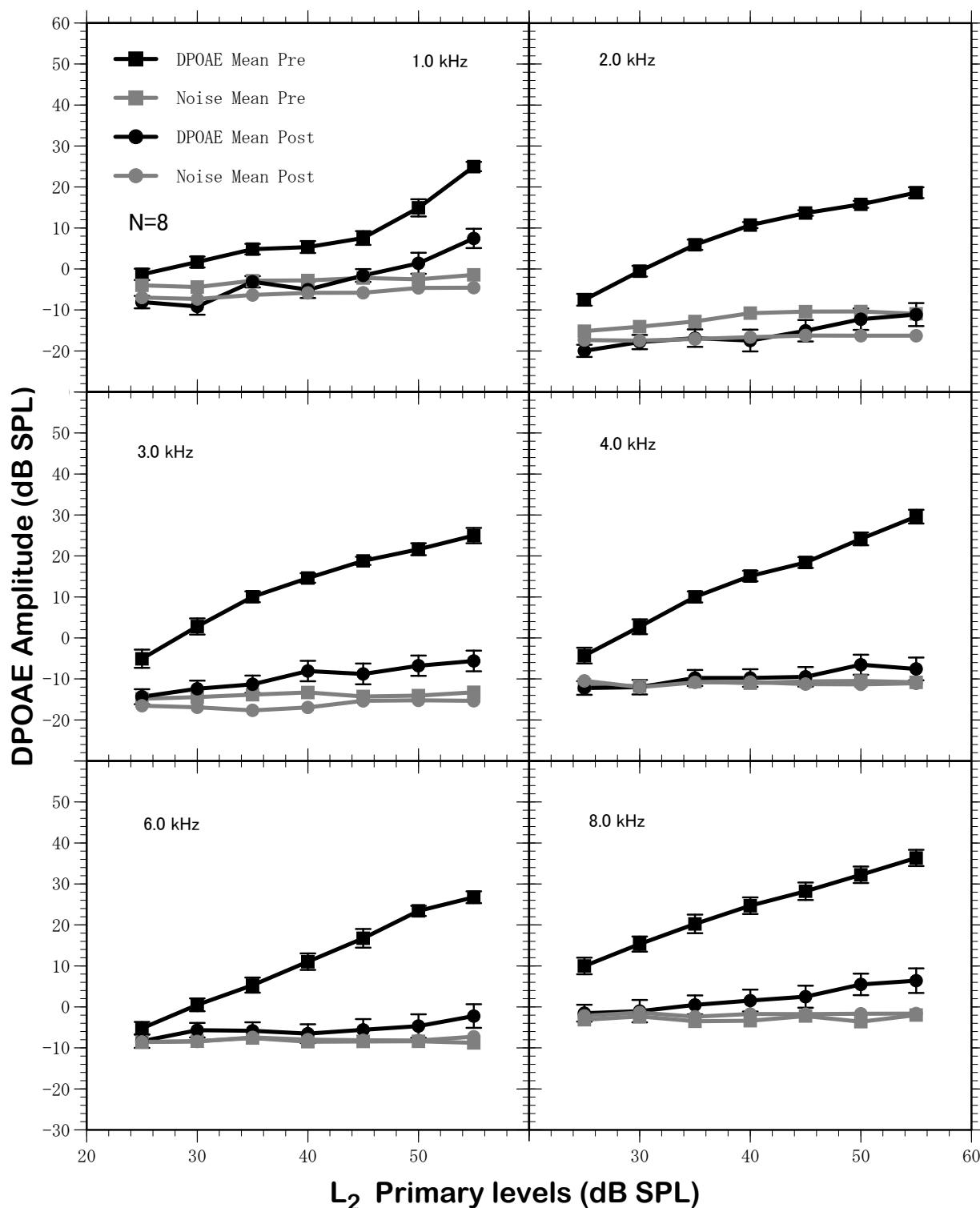


Figure 22. Phase II: L-NAC treated group mean (N=8) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

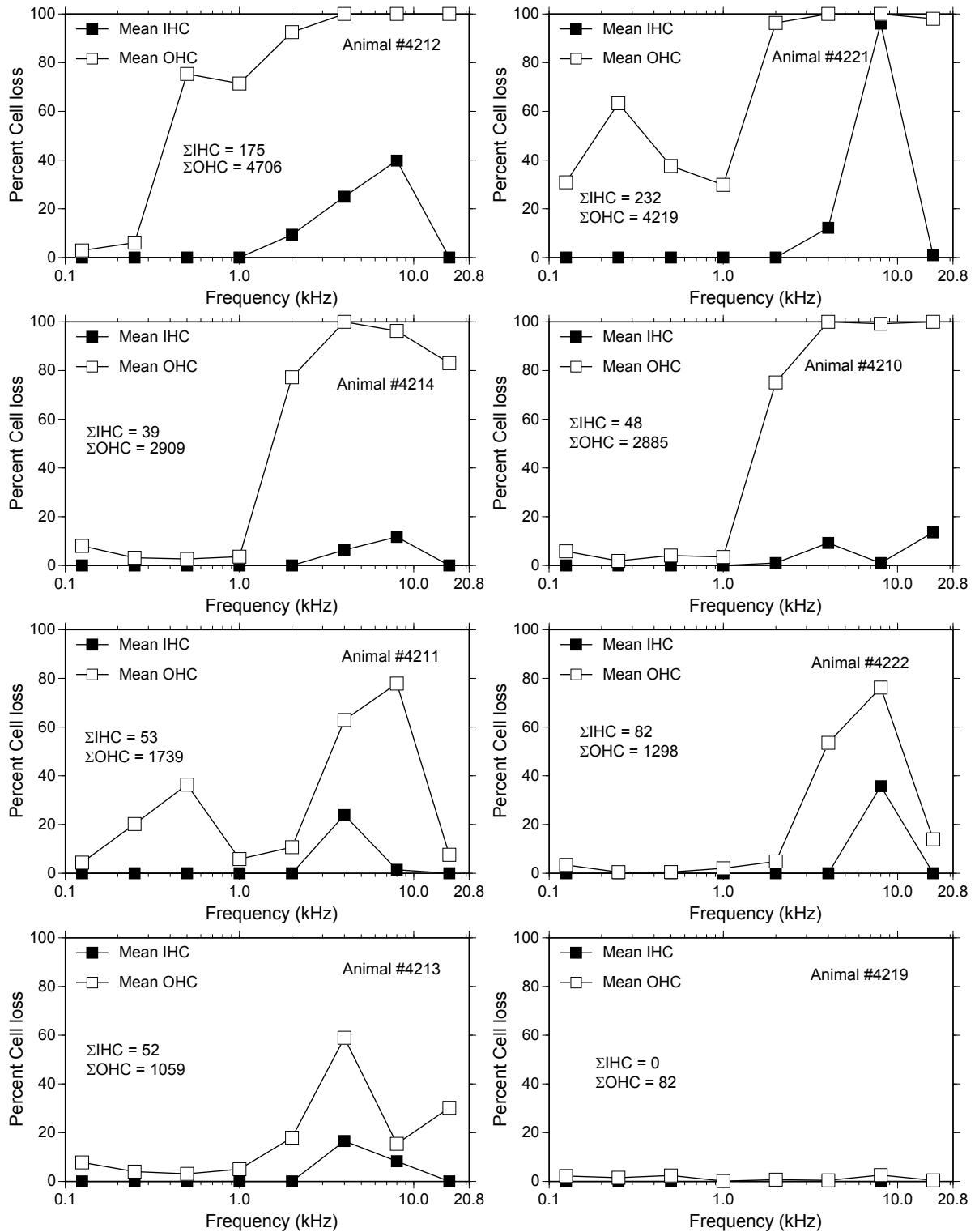


Figure 23. Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with L-NAC injections BID for five days post exposure. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days post exposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

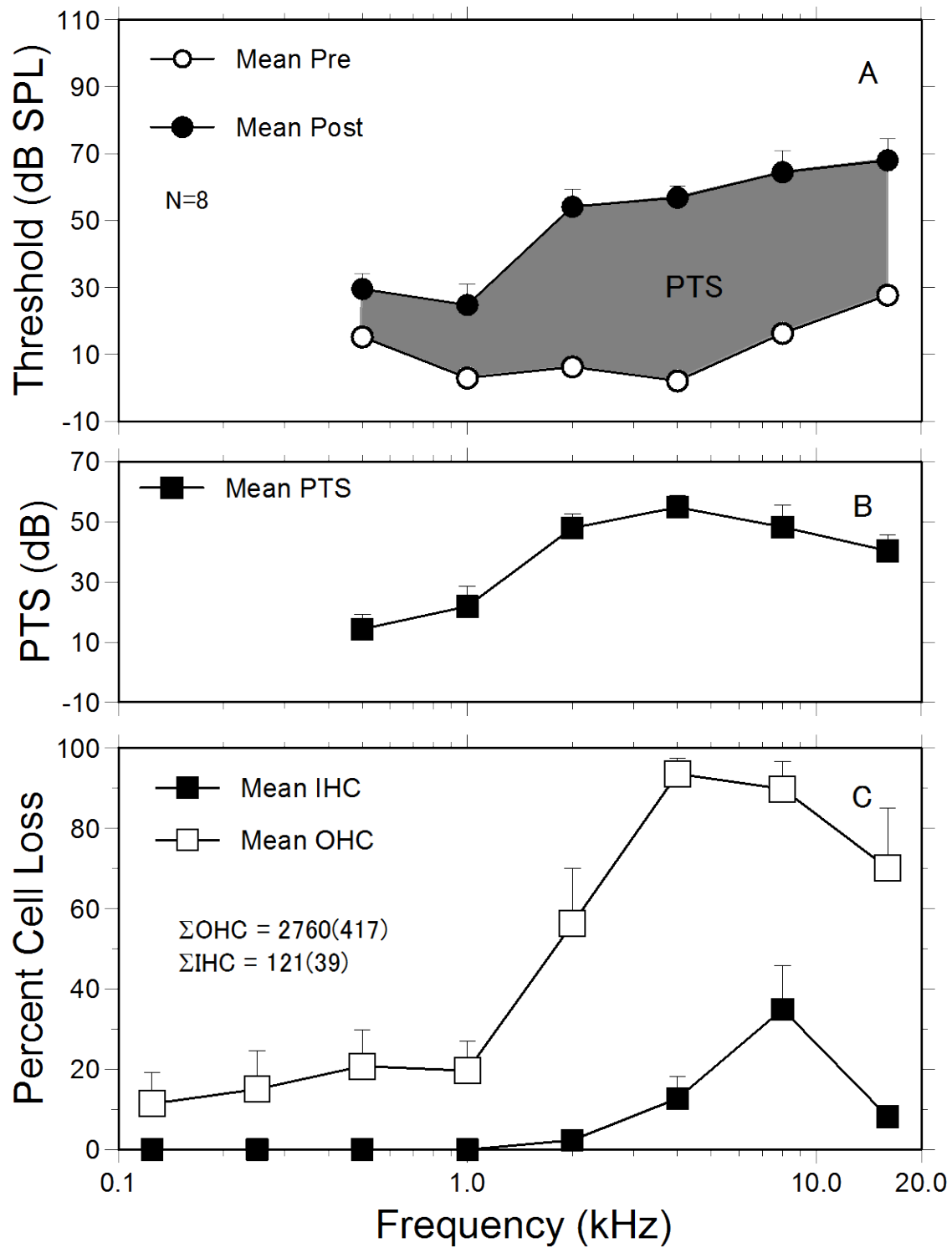


Figure 24. Phase II: ALCAR treated group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (I = s.e.)

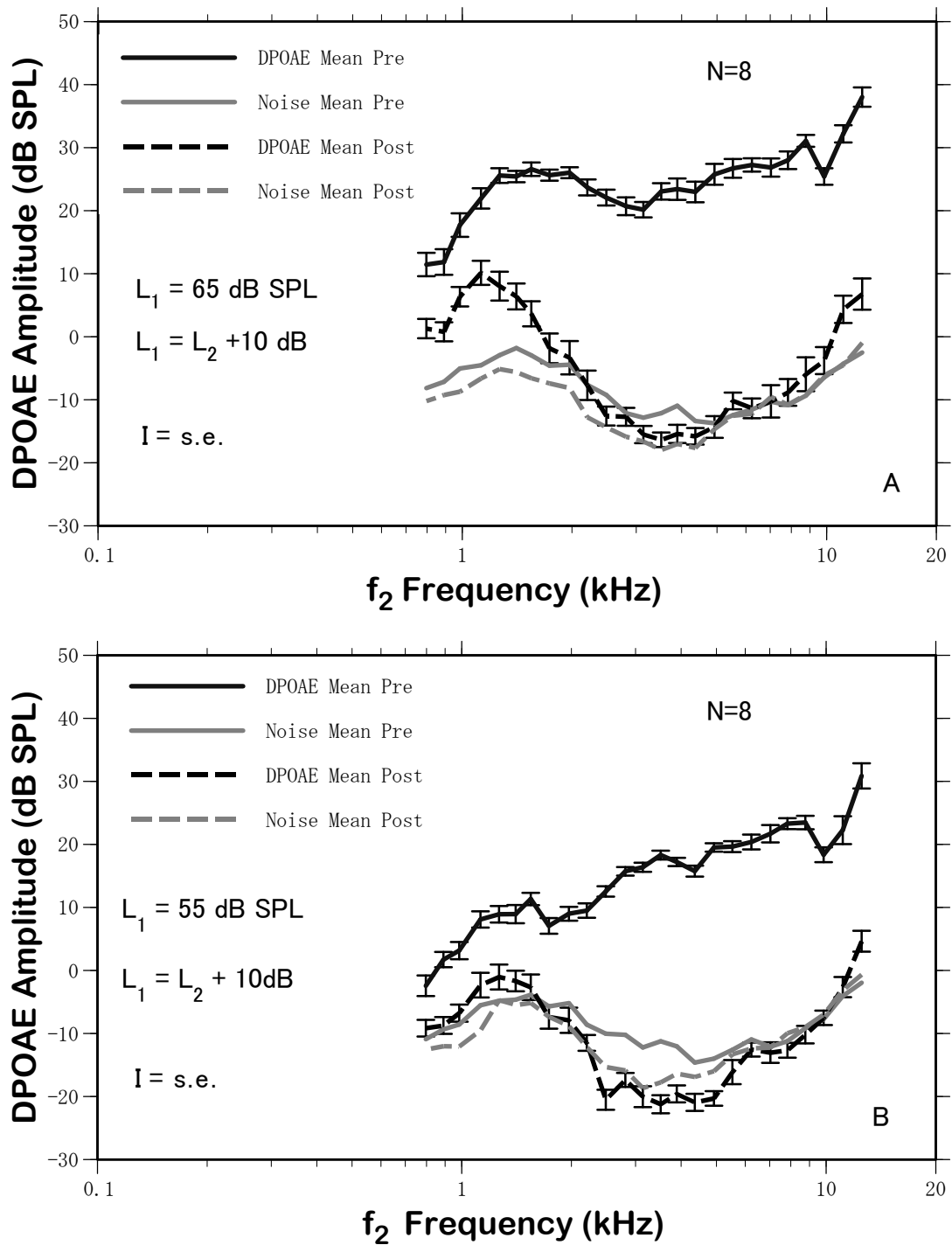


Figure 25. Phase II: ALCAR treated group mean (N=8) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. ($I = \text{s.e.}$)

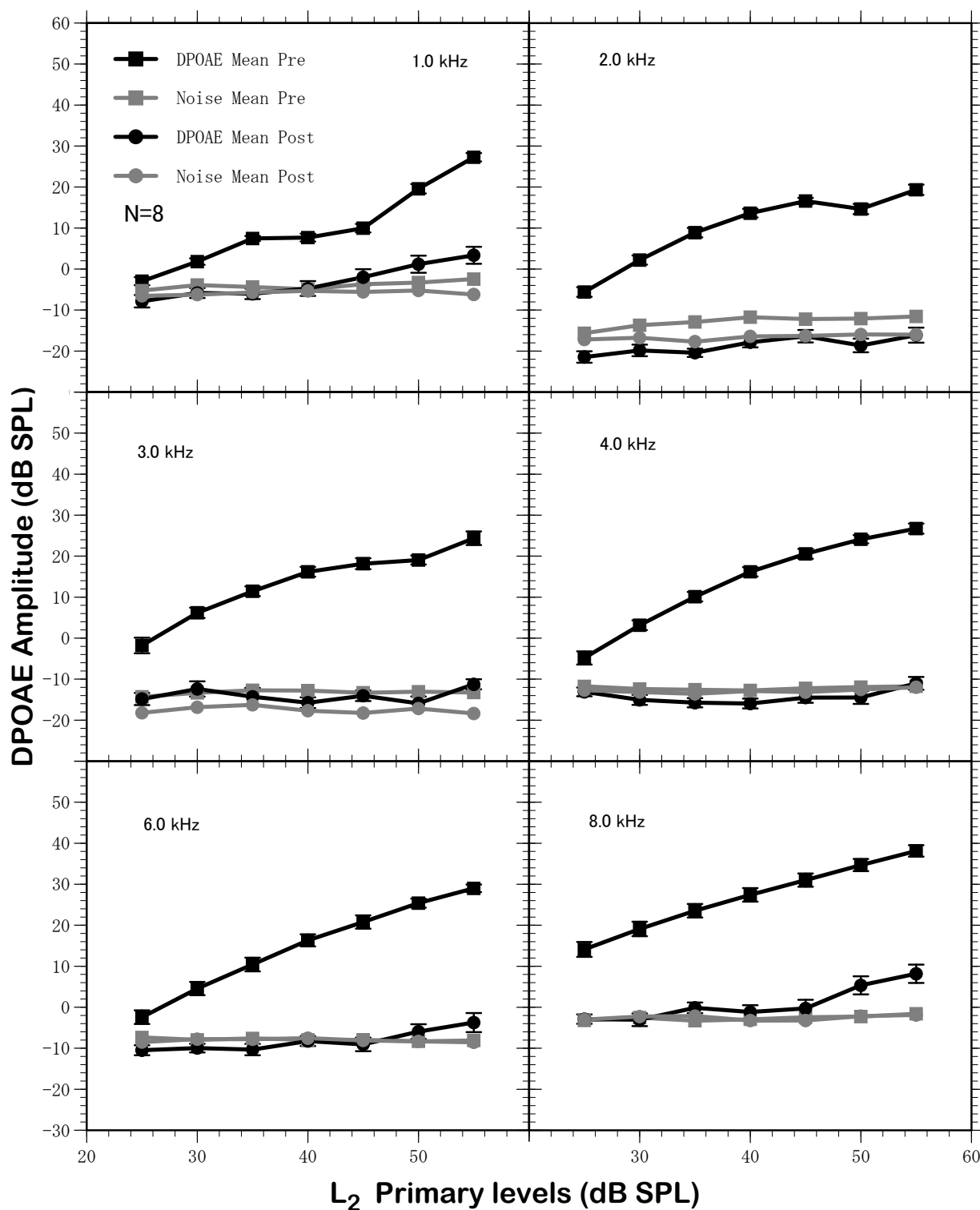


Figure 26. Phase II: ALCAR treated group mean (N=8) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

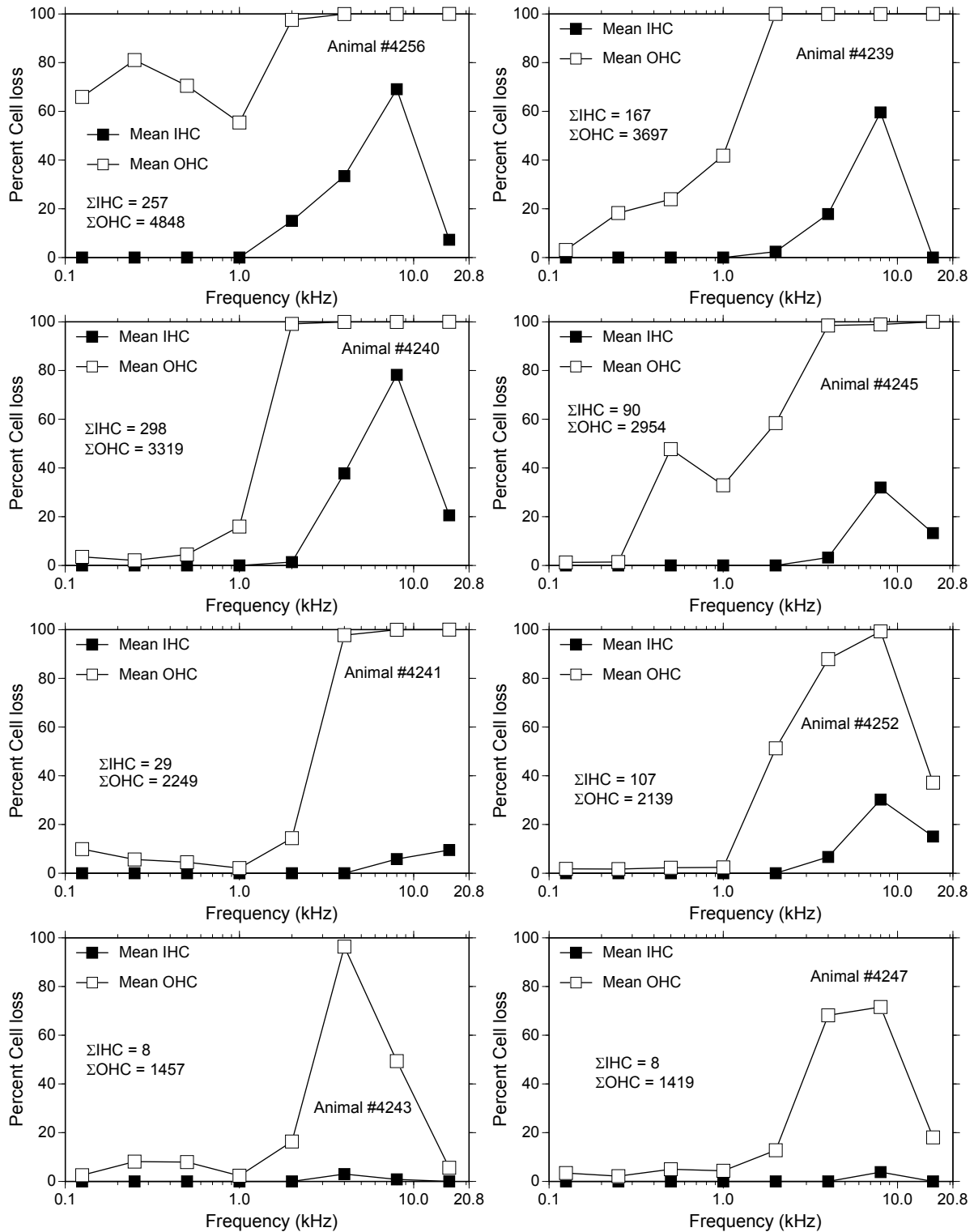


Figure 27. Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with ALCAR injections BID for five days post exposure. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days post exposure. Σ IHC and Σ OHC = total number of missing inner and outer hair cells.

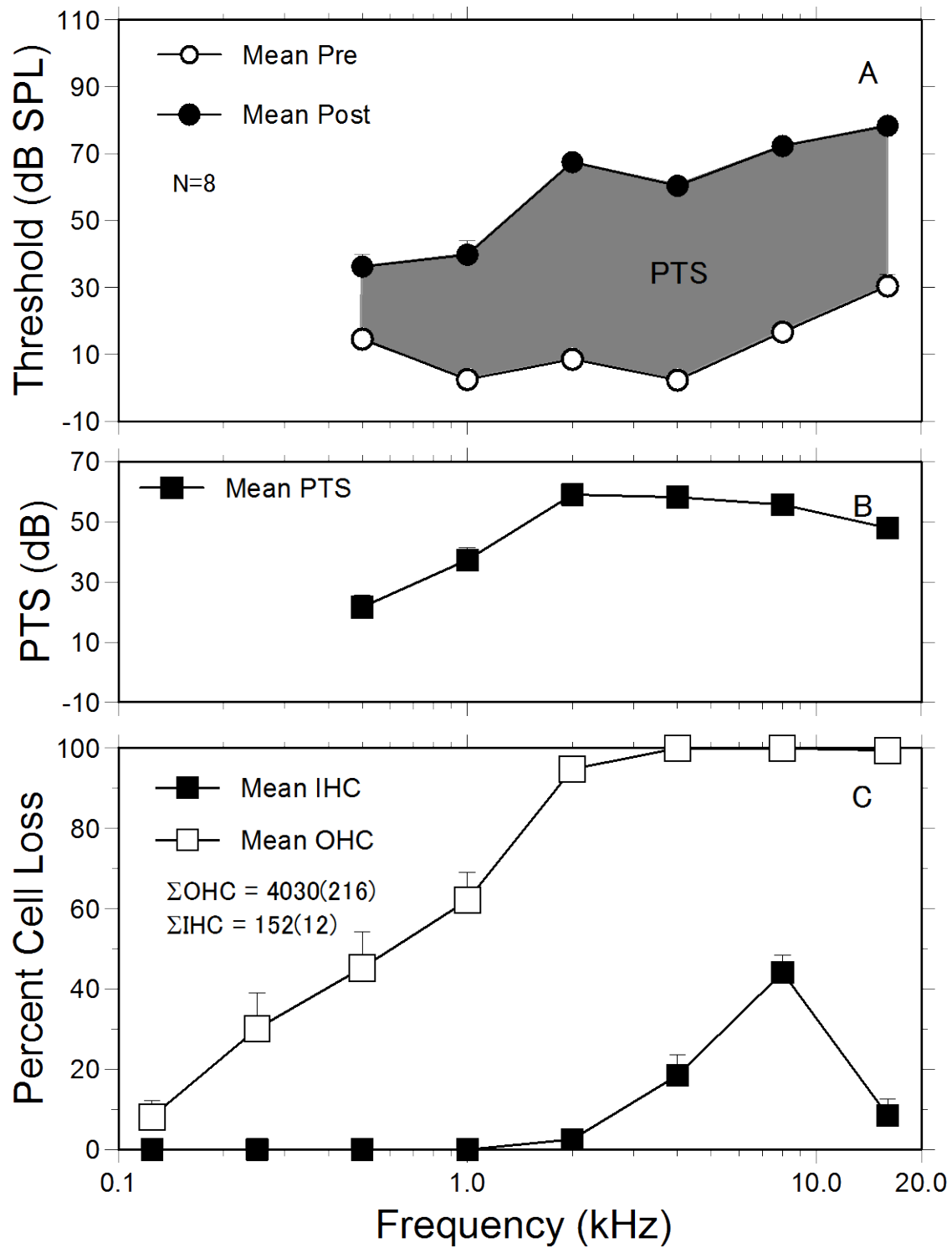


Figure 28. Phase II: D-MET treated group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (I = s.e.)

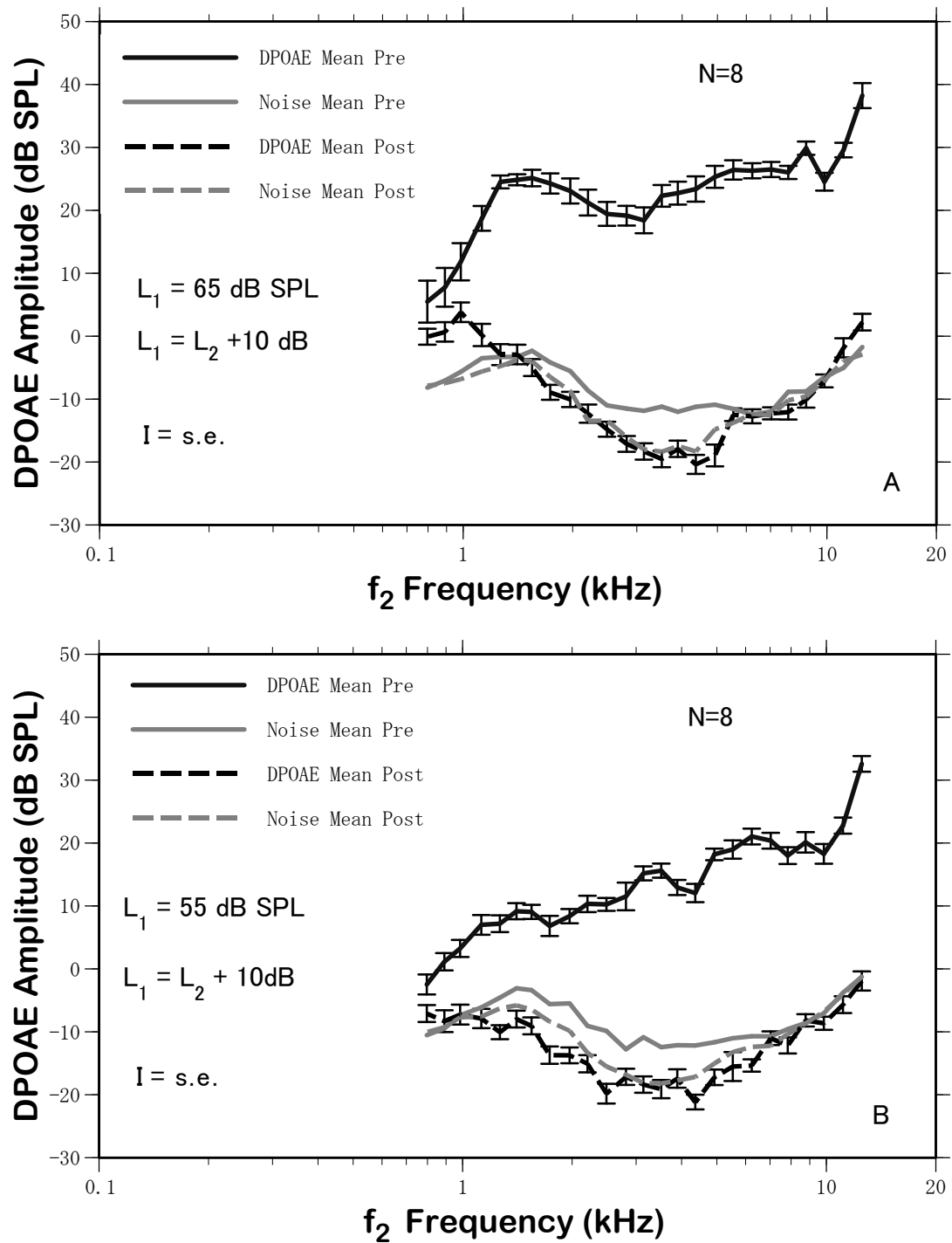


Figure 29. Phase II: D-MET treated group mean (N=8) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

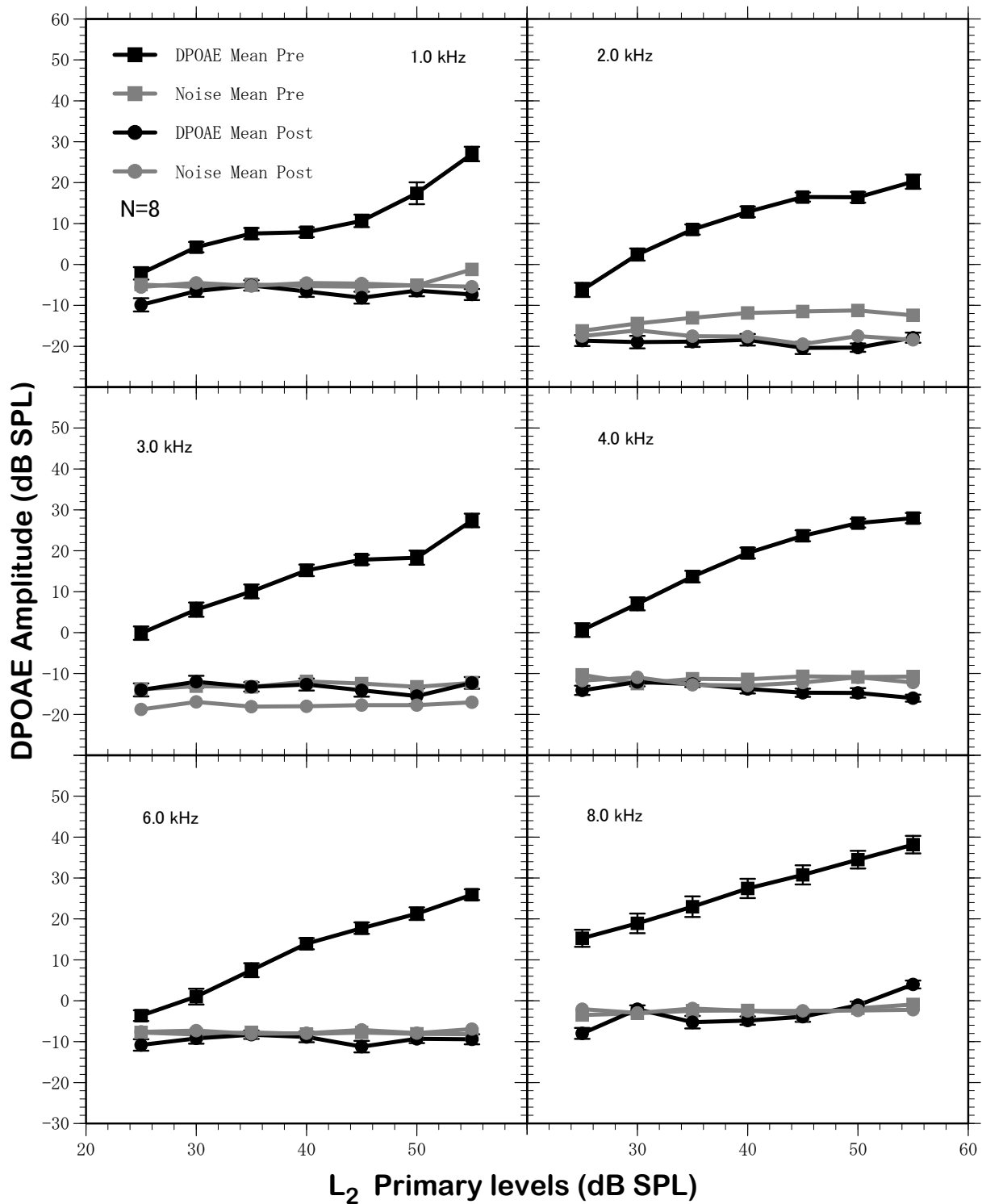


Figure 30. Phase II: D-MET treated group mean (N=8) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

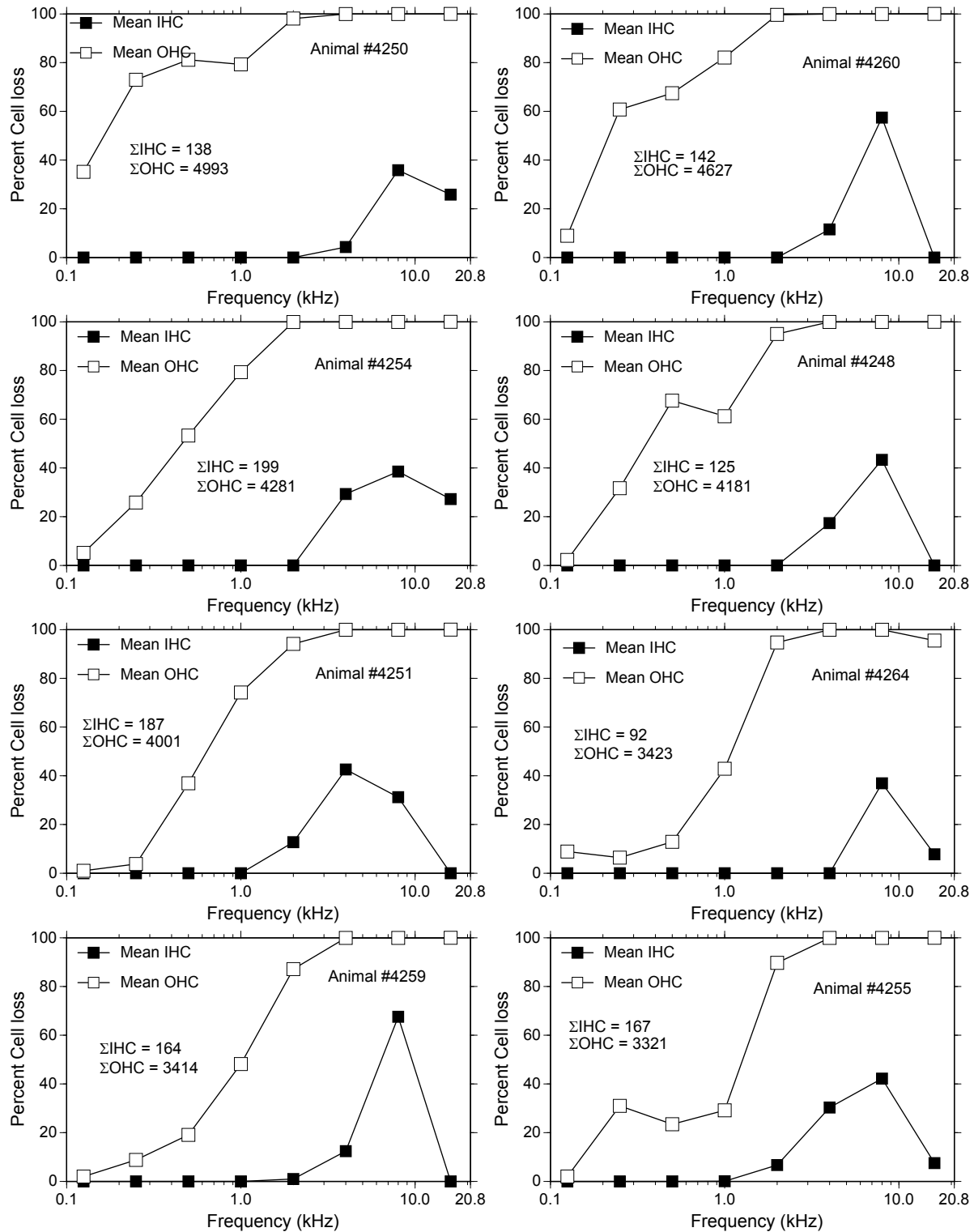


Figure 31. Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with D-MET injections BID for five days post exposure. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days post exposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

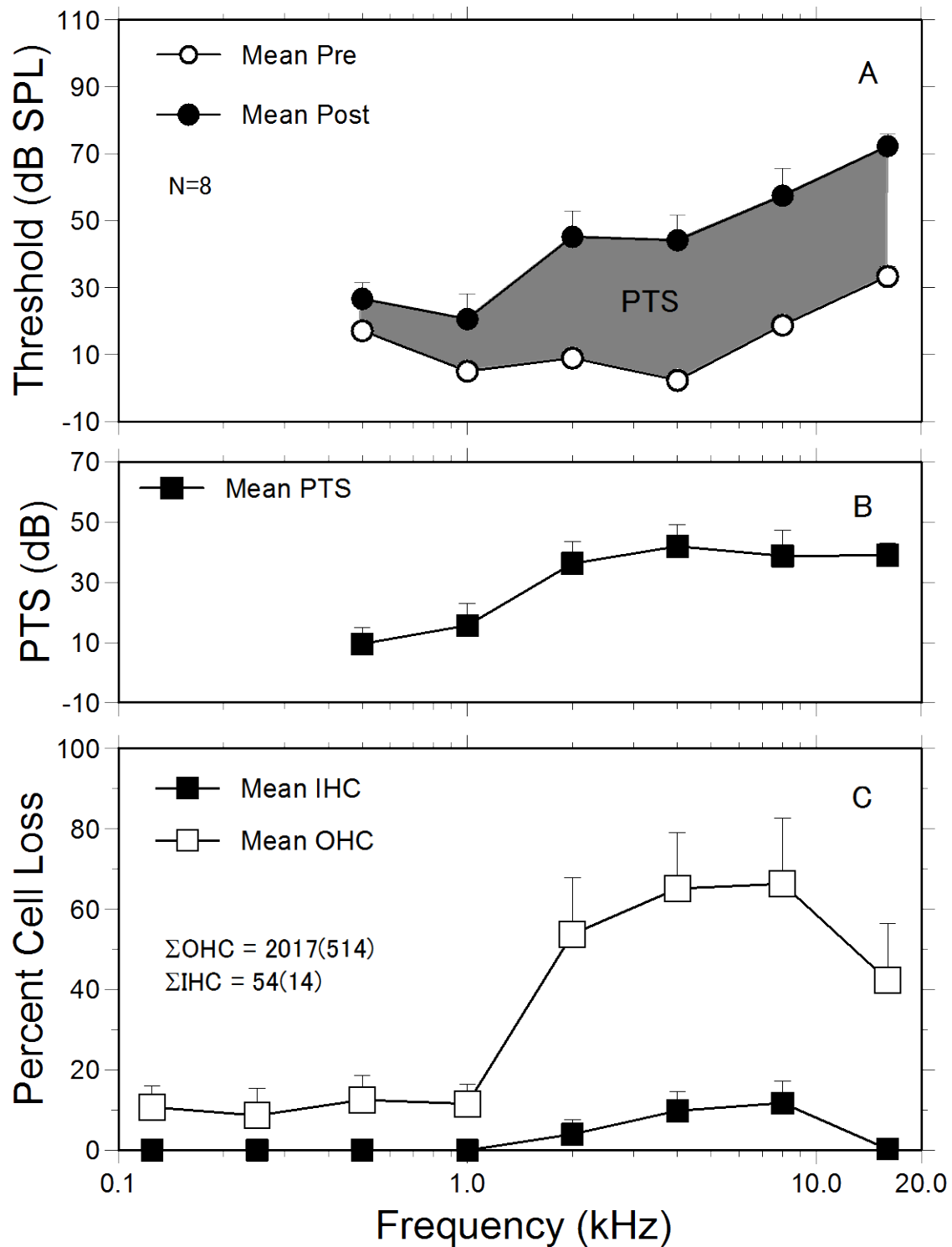


Figure 32. Phase II: Ebselen treated group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (I = s.e.)

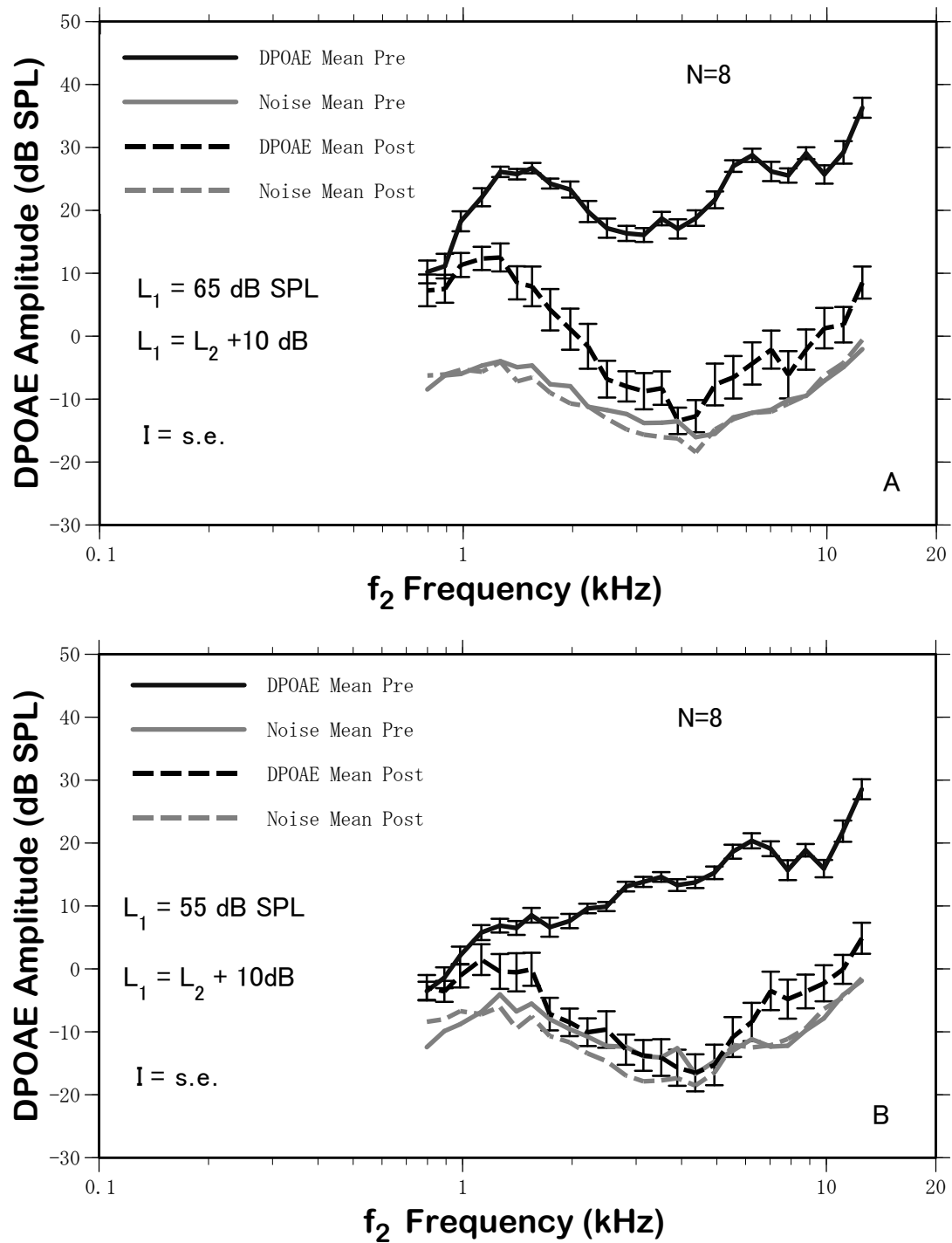


Figure 33. Phase II: Ebselen treated group mean ($N=8$) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. ($I = \text{s.e.}$)

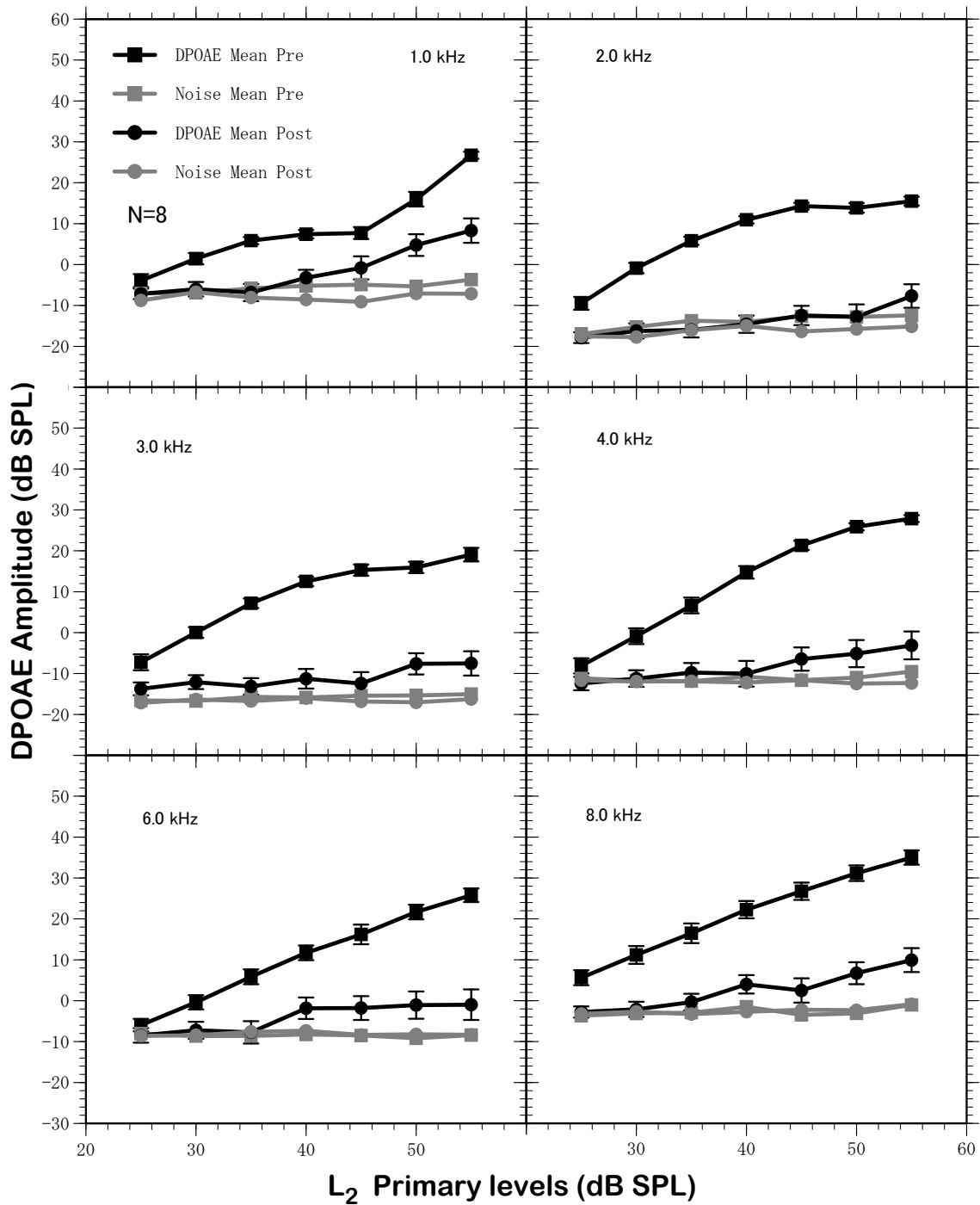


Figure 34. Phase II: Ebselen treated group mean (N=8) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

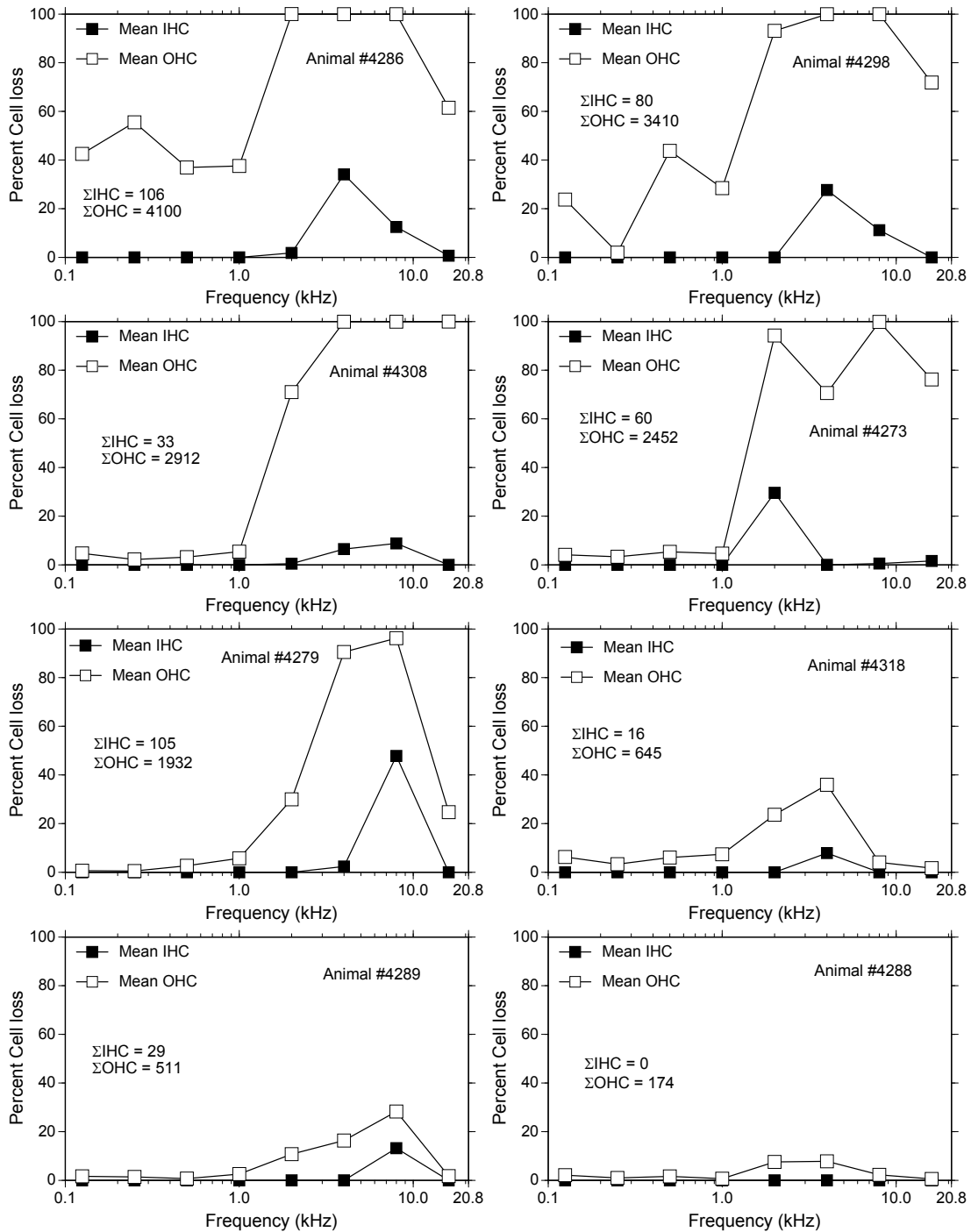


Figure 35. Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with Ebselen injections BID for five days post exposure. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days post exposure. Σ IHC and Σ OHC = total number of missing inner and outer hair cells.

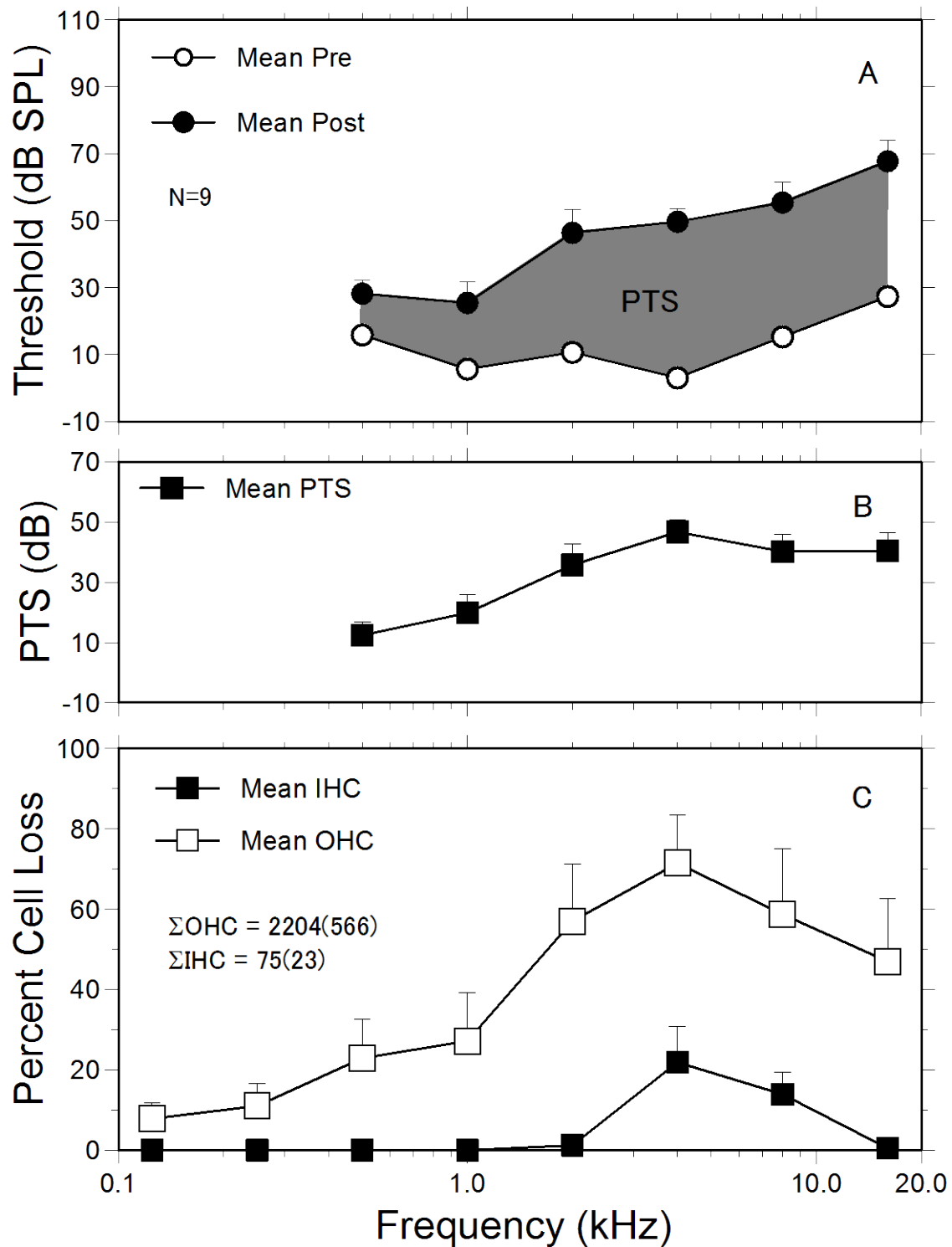


Figure 36. Phase II: Src Inh treated group (N=9) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. $\Sigma\text{OHC} = 2204(566)$ and $\Sigma\text{IHC} = 75(23)$ = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (I = s.e.)

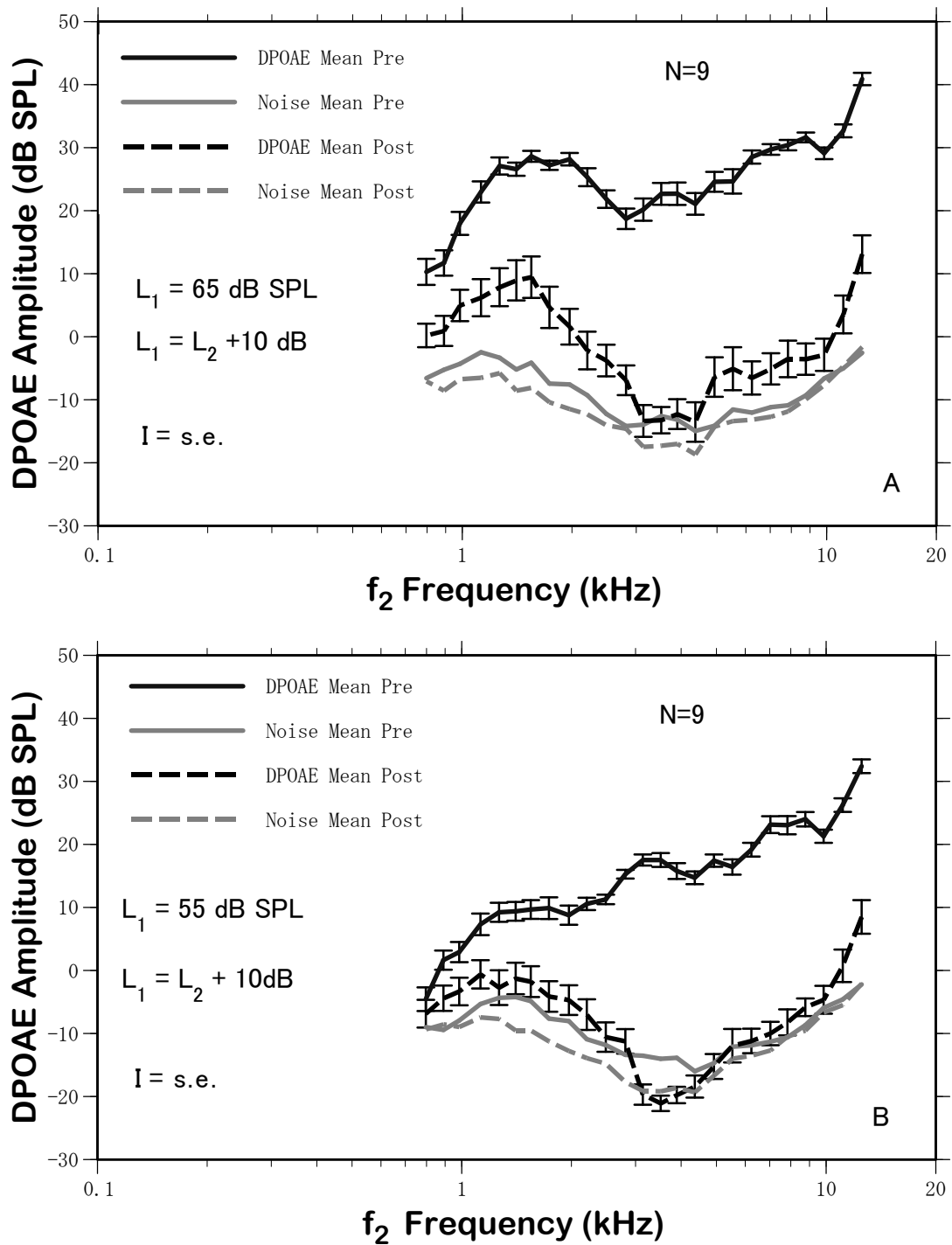


Figure 37. Phase II: Src Inh treated group mean ($N=9$) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. ($I = \text{s.e.}$)

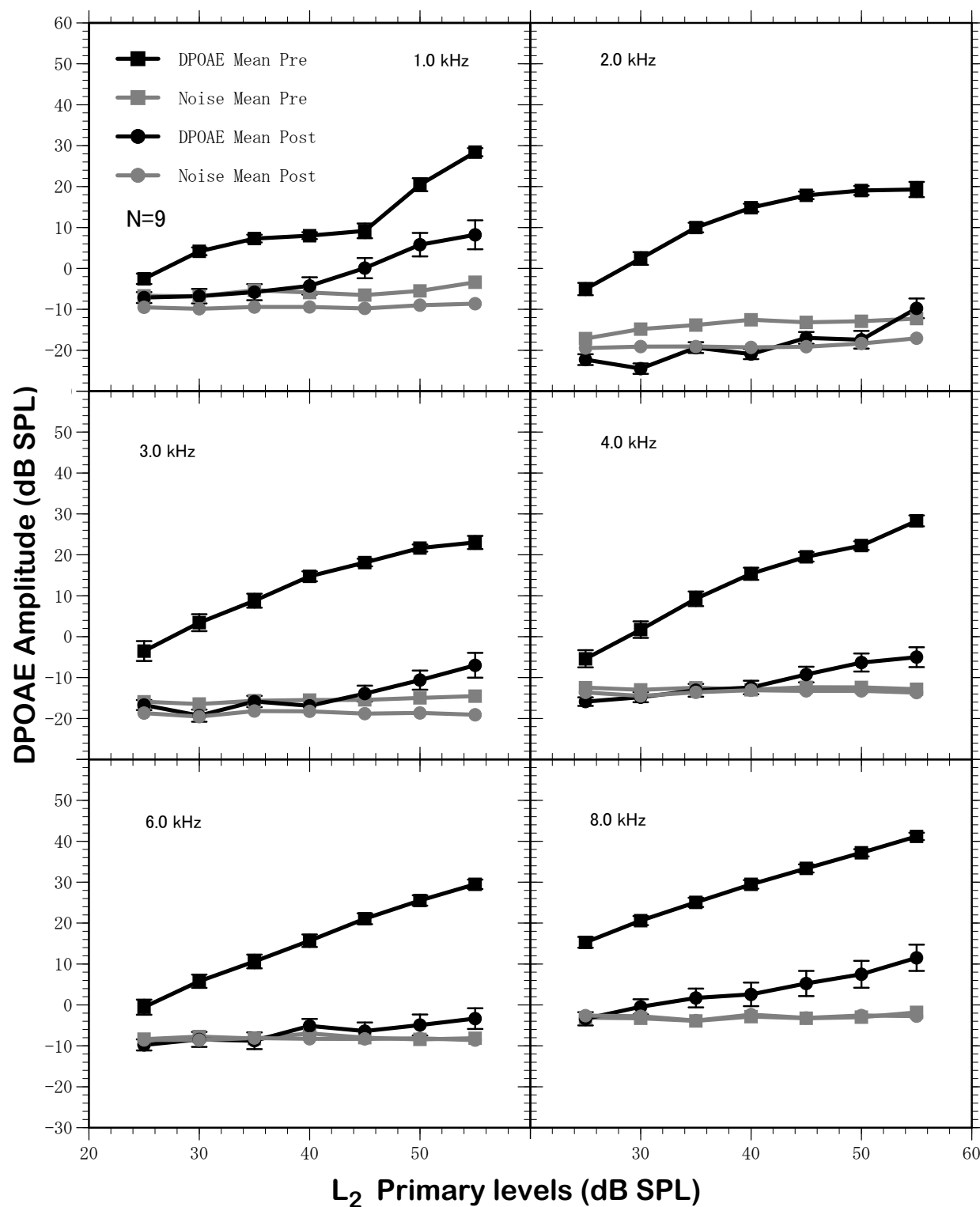


Figure 38. Phase II: Src Inh treated group mean (N=9) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

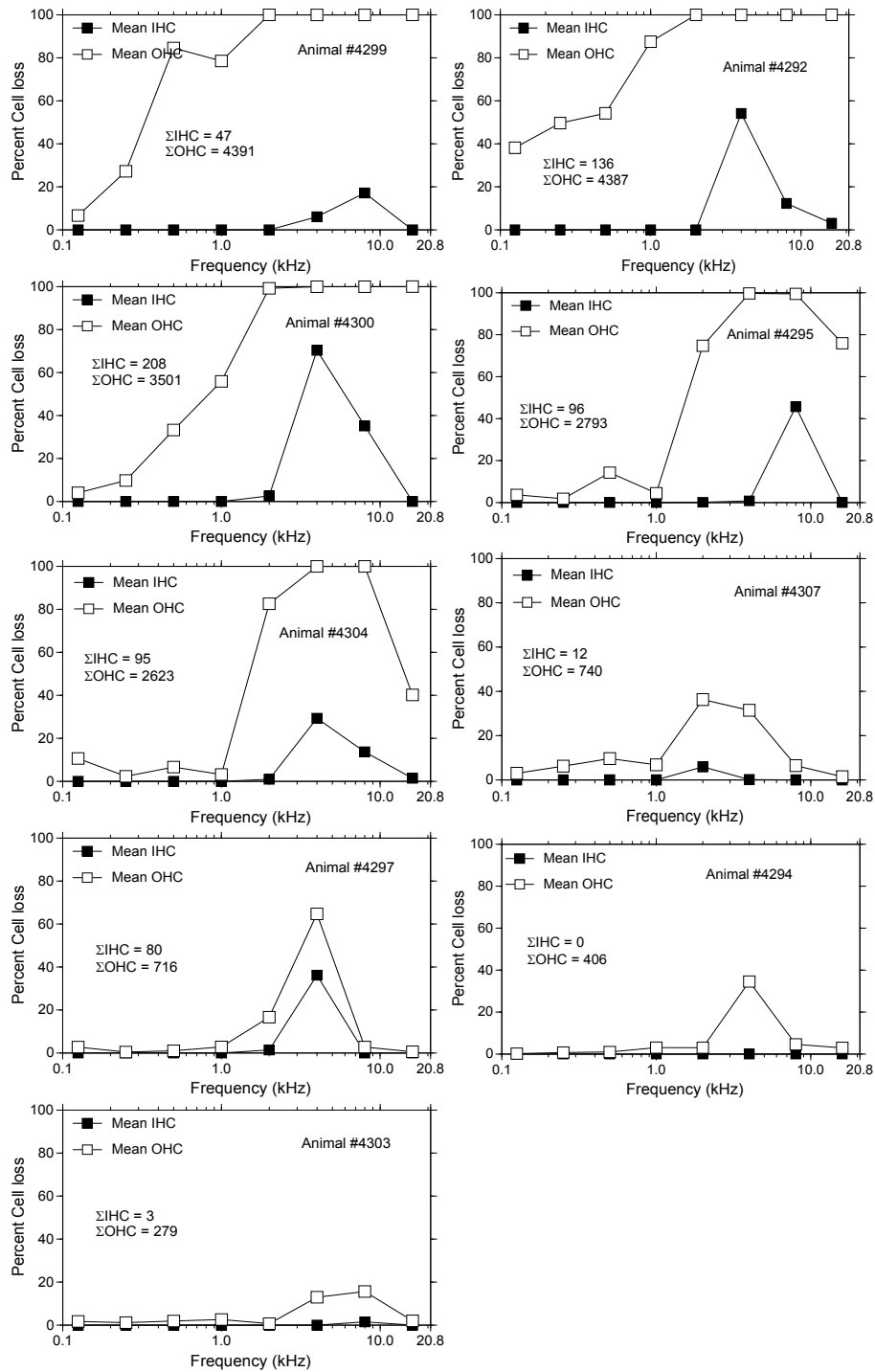


Figure 39. Individual cochleograms for the 9 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with Src Inh injections BID for five days post exposure. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days post exposure. Σ IHC and Σ OHC = total number of missing inner and outer hair cells.

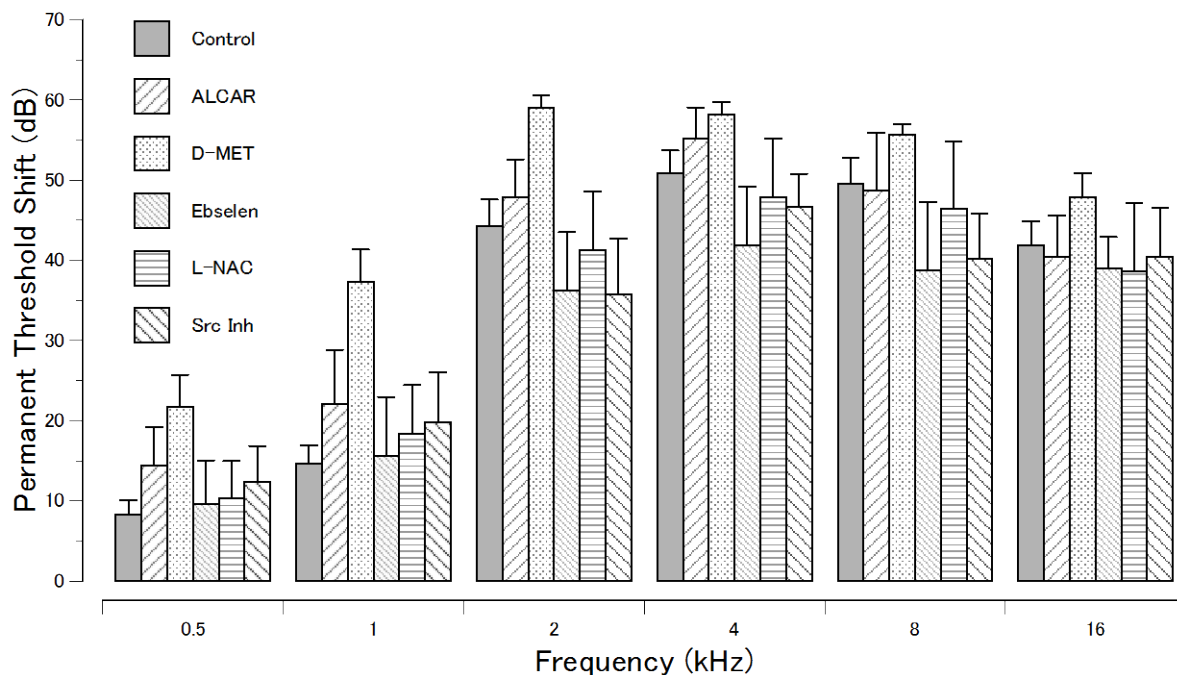


Figure 40. The mean permanent threshold shift for the control group and the five drug treated groups following exposure for 6 hours to a 4 kHz octave band of noise at 108 dB SPL. T = standard error of the mean.

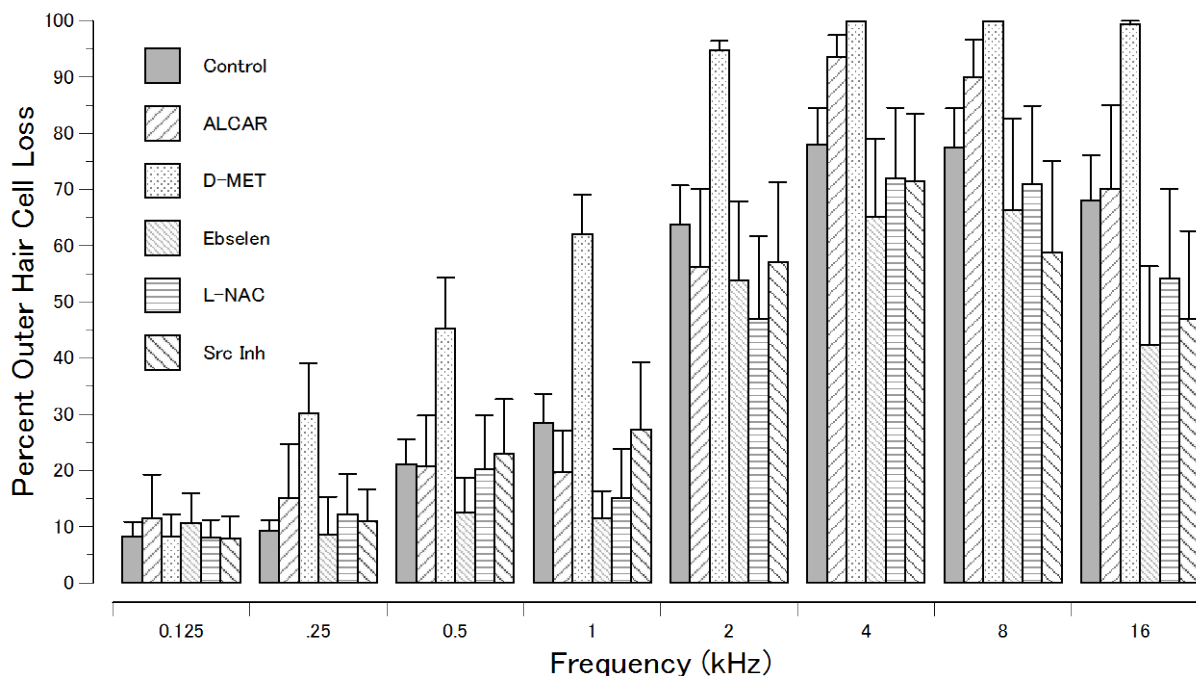


Figure 41. The mean outer hair cell loss in the indicated octave band length of the basilar membrane for the control group and the five drug treated groups following exposure for 6 hours to a 4 kHz octave band of noise at 108 dB SPL. T = standard error of the mean.

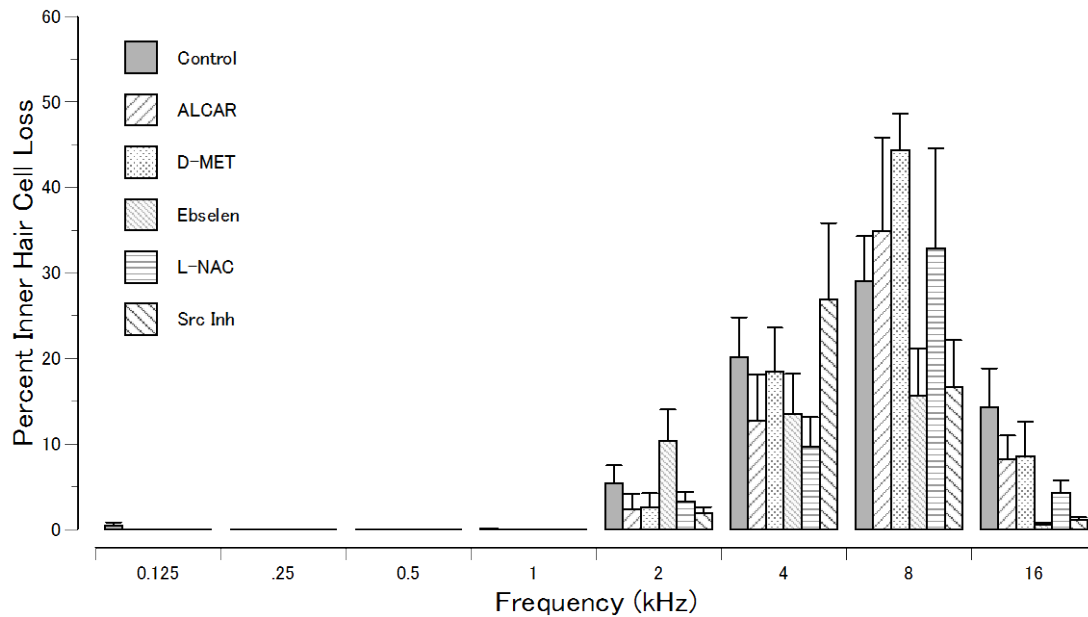


Figure 42. The mean inner hair cell loss in the indicated octave band length of the basilar membrane for the control group and the five drug treated groups following exposure for 6 hours to a 4 kHz octave band of noise at 108 dB SPL. T = standard error of the mean.

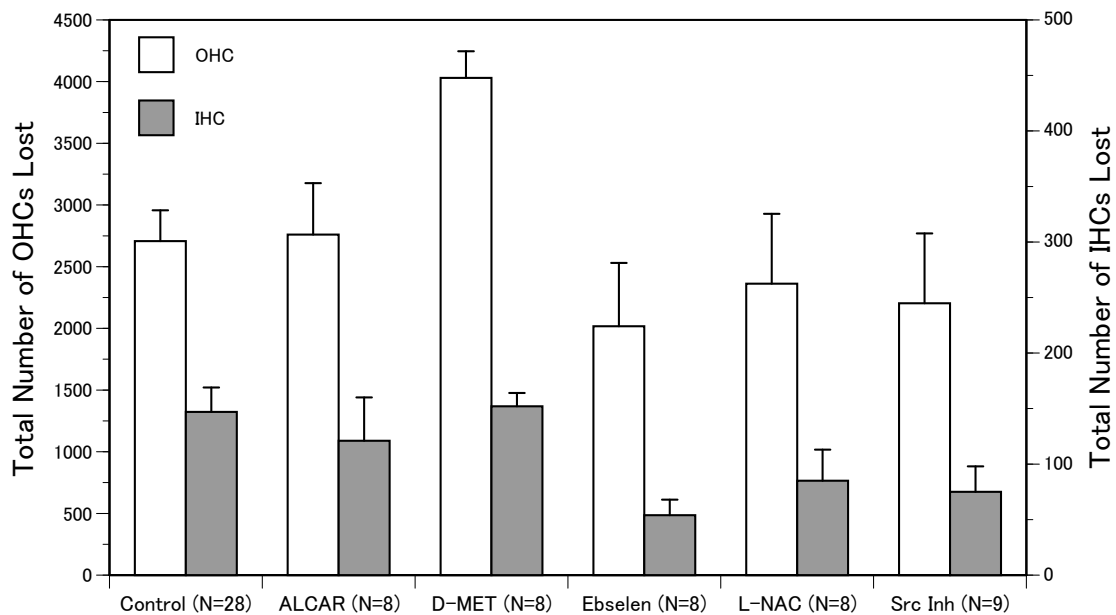


Figure 43. The group mean total number of missing outer hair cells (OHC) and inner hair cells (IHC) in the control and five drug treated groups that were exposed to the 4 kHz octave band of noise at 108 dB for 6 hours. T = standard error of the mean.

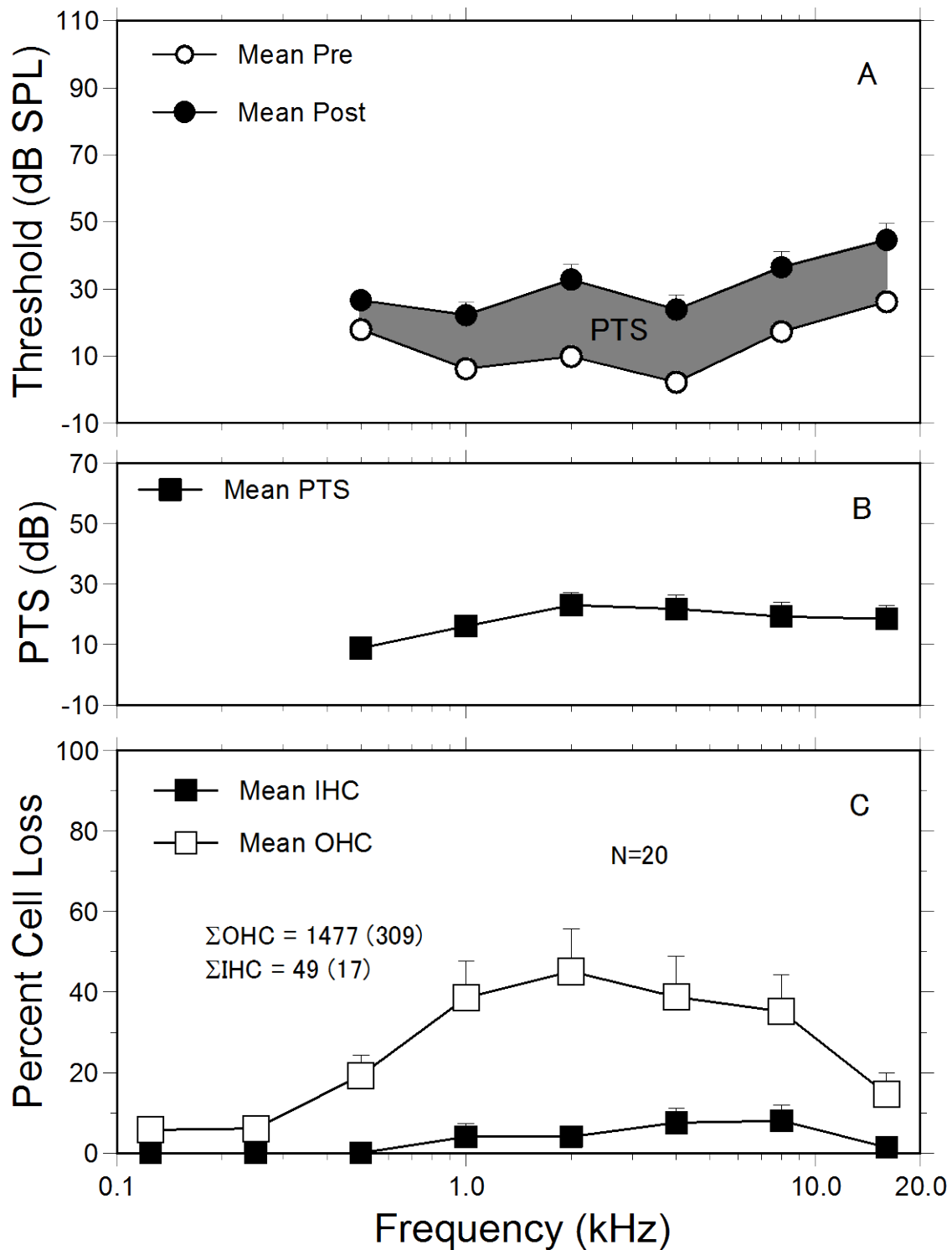


Figure 44. Phase III noise only control group (N=20) exposed to 10, 158 dB peak SPL impulses (shock tube controls 10x @ 11 psi charge pressure) within less than 2 min. Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) Permanent threshold shift and (C) percent inner and outer hair cell (IHC, OHC) loss.

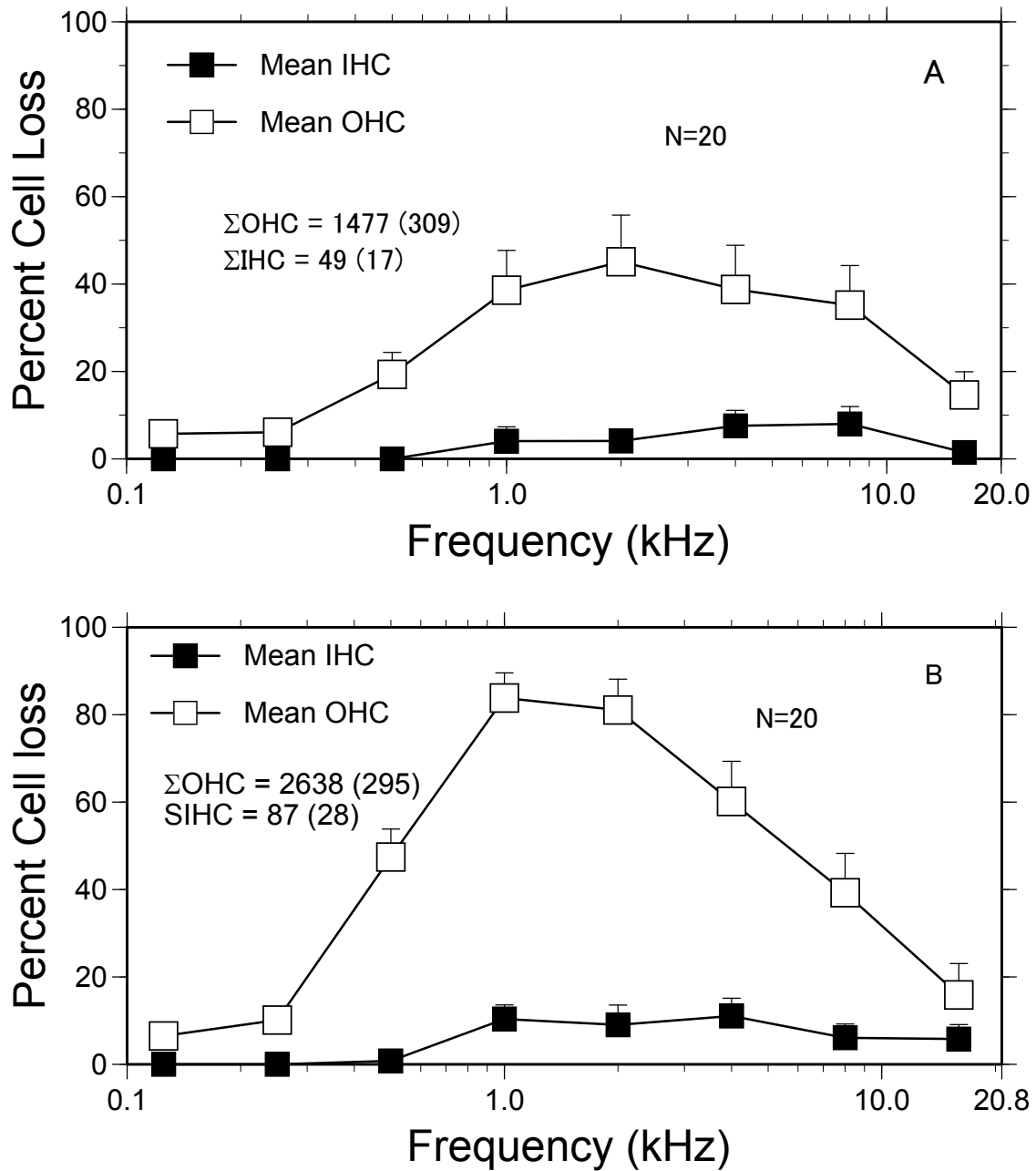


Figure 45. The group mean percent inner and outer hair cell (IHC, OHC) loss for chinchillas exposed to 10 impulses at 158 dB peak SPL. Each data point represents the mean cell loss estimated over a one octave band length of basilar membrane centered at the indicated frequencies. Animals were euthanized 30 days post exposure. (A) The group mean (N=20) cochleogram from the Phase III noise only control group. (B) The group mean (N=20) cochleogram from the Phase I lesion calibration. Both groups received the identical exposure.

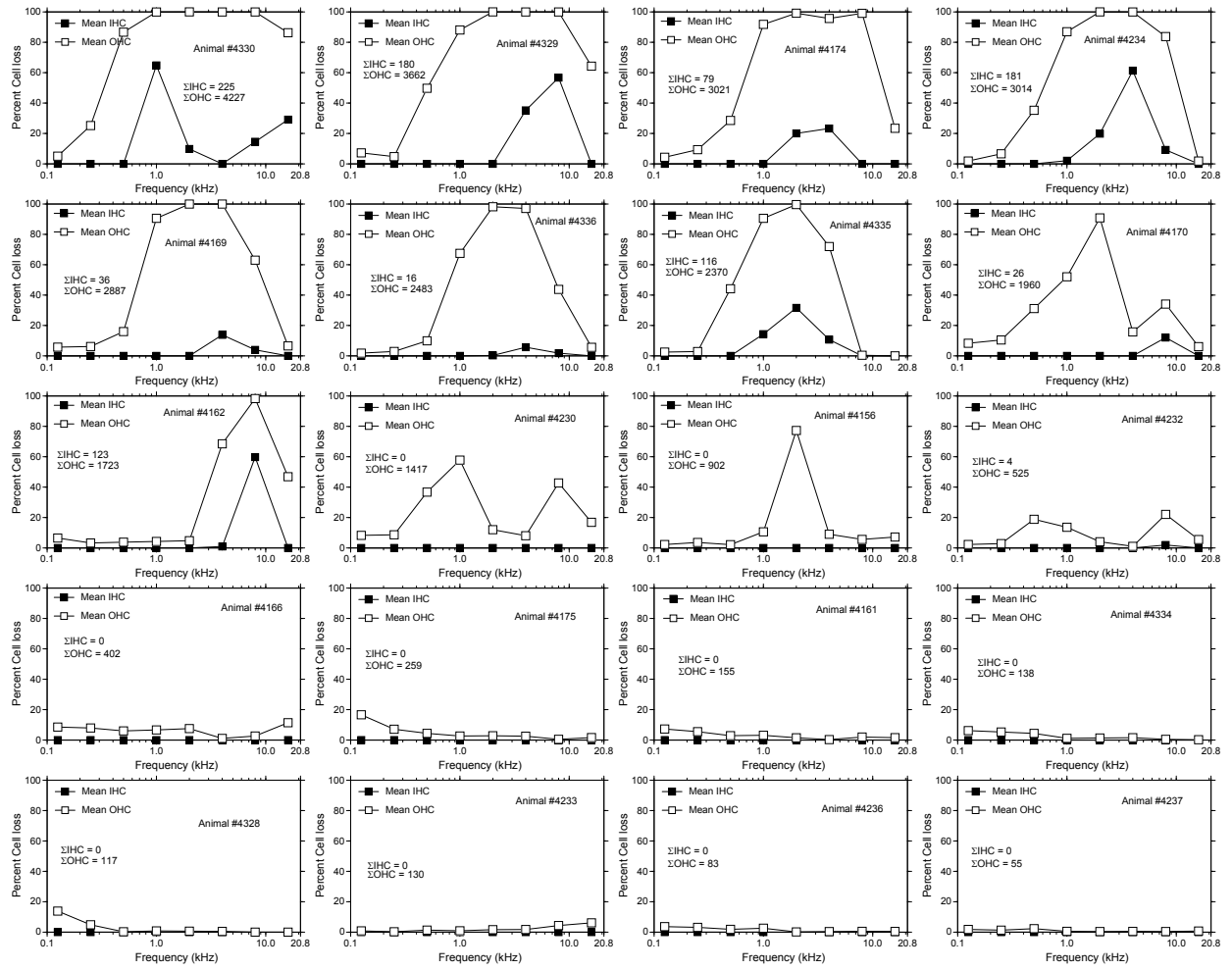


Figure 46. Phase III noise only control group (N=20) exposed to 10, 158 dB peak SPL impulses (shock tube controls 10x @ 11 psi charge pressure) within less than 2 min.

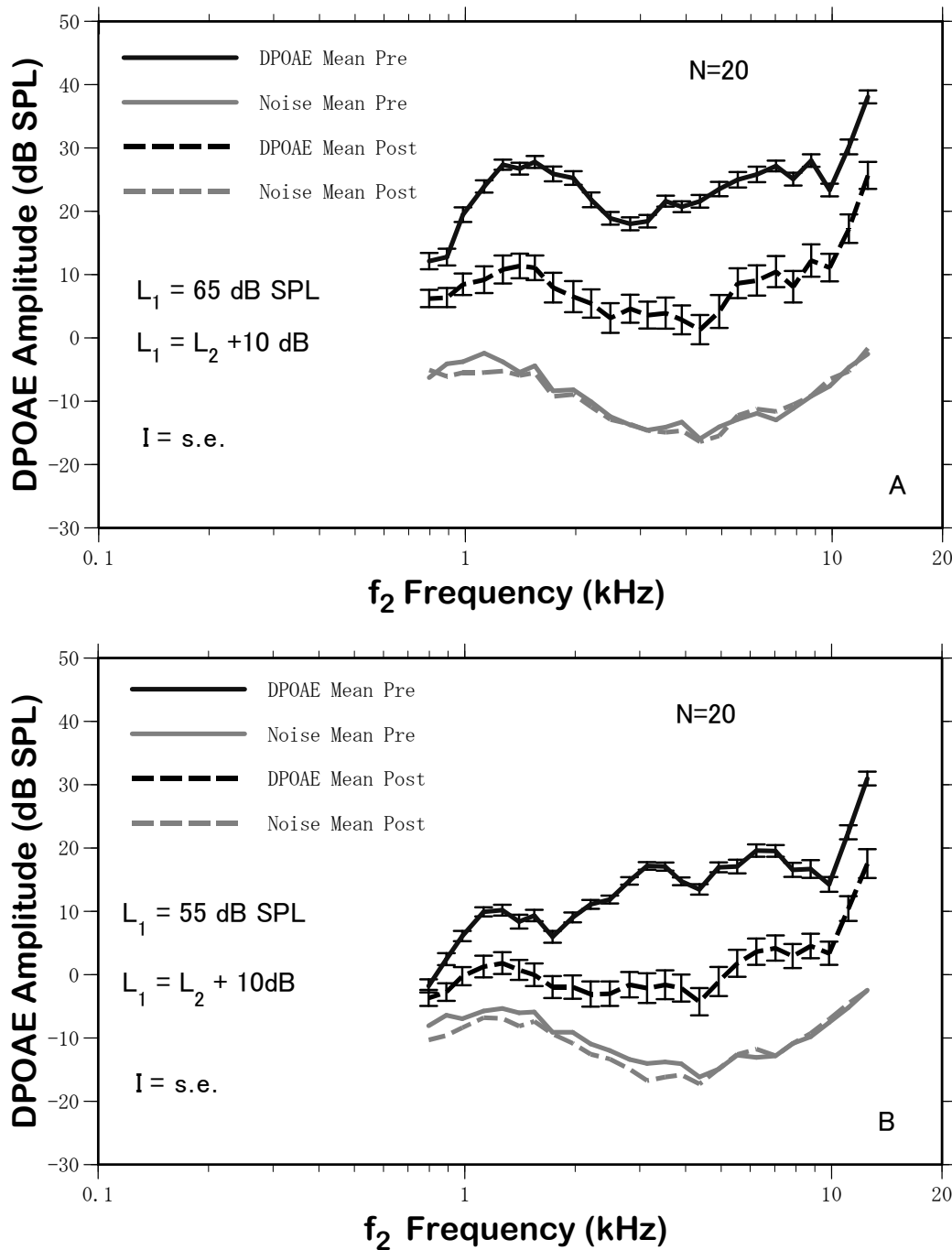


Figure 47. Phase III noise only control group mean (N=20) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 10, 158 dB peak SPL impulses (shock tube controls 10x @ 11 psi charge pressure) within less than 2 min. (I = s.e.)

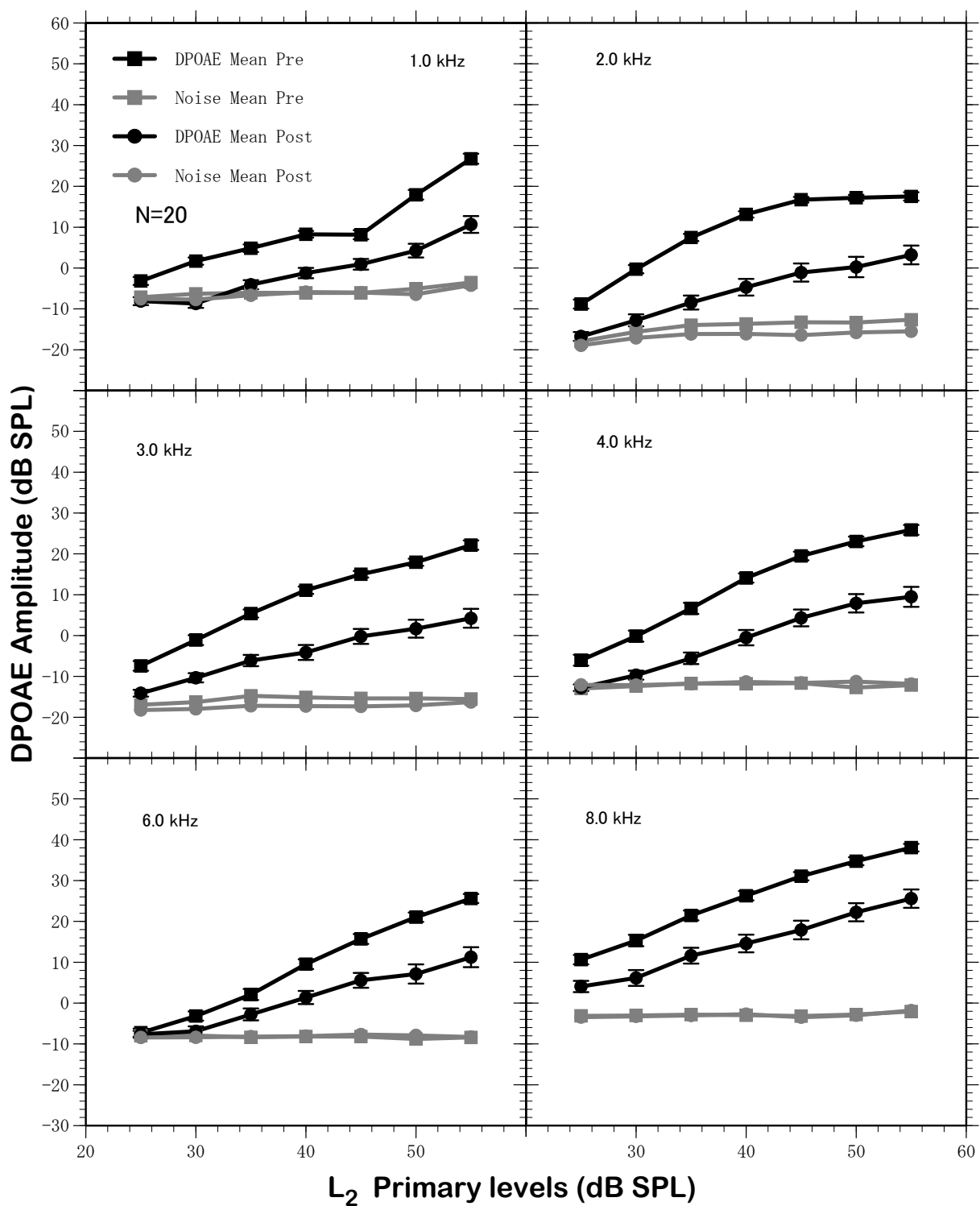


Figure 48. Phase III noise only control group mean (N=20) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 10, 158 dB peak SPL impulses (shock tube controls 10x @ 11 psi charge pressure) within less than 2 min. (I = s.e.)

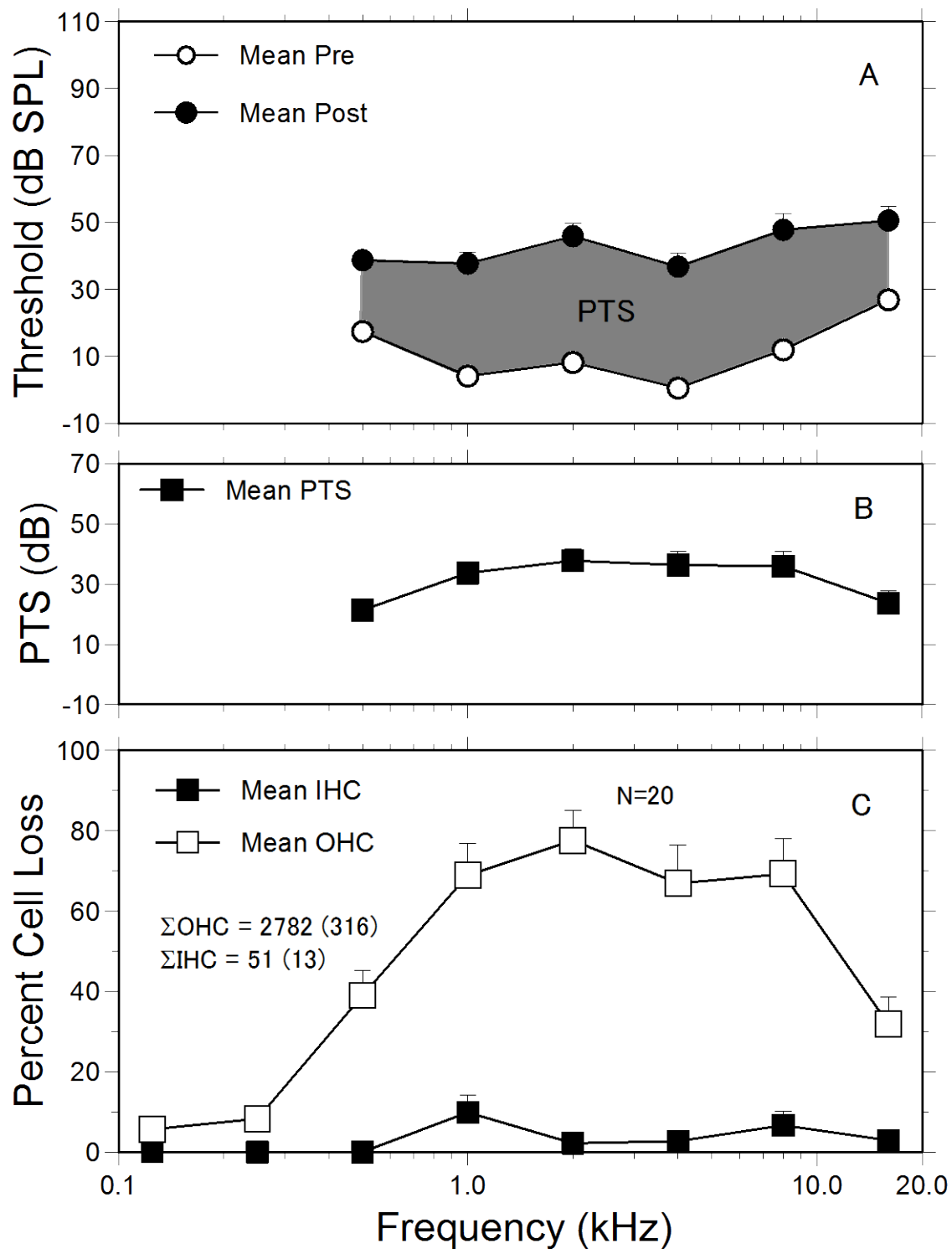


Figure 49. Phase III L-NAC group (N=20) exposed to 10, 158 dB peak SPL impulses (shock tube controls 10x @ 11 psi charge pressure) within less than 2 min. Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) Permanent threshold shift and (C) percent inner and outer hair cell (IHC, OHC) loss.

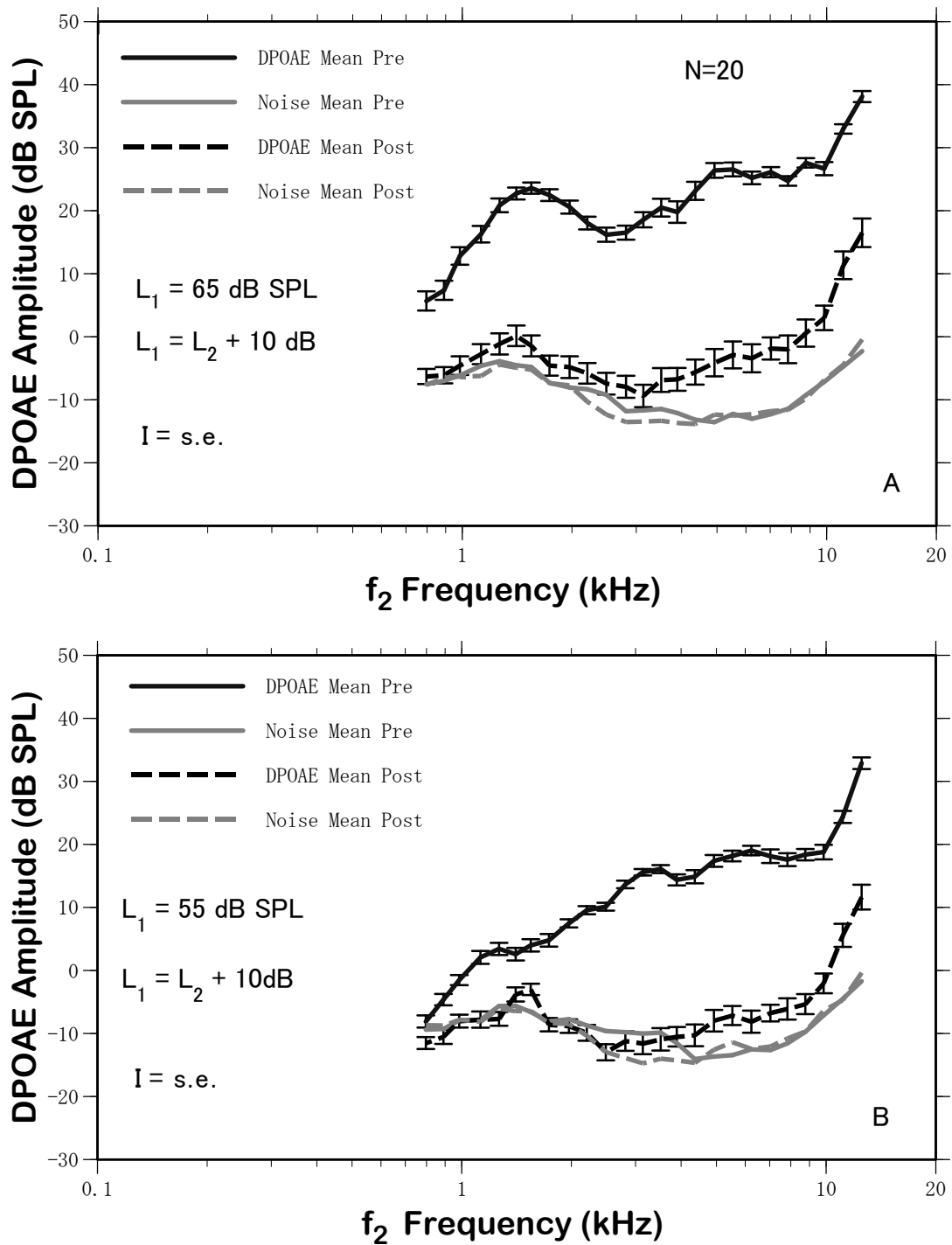


Figure 50. Phase III L-NAC group mean (N=20) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 10, 158 dB peak SPL impulses (shock tube controls 10x @ 11 psi charge pressure) within less than 2 min. (I = s.e.)

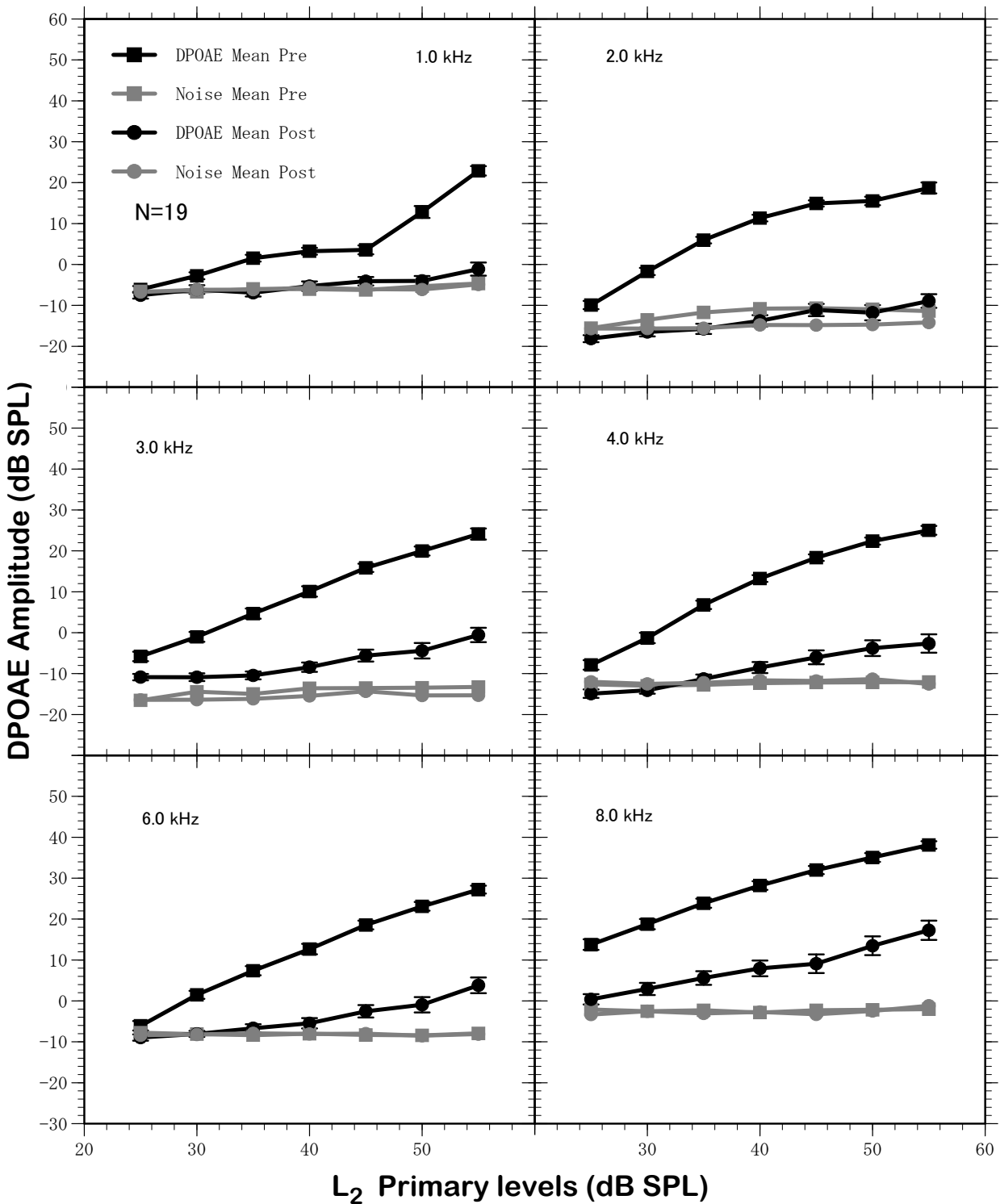


Figure 51. Phase III L-NAC group mean (N=20) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 10, 158 dB peak SPL impulses (shock tube controls 10x @ 11 psi charge pressure) within less than 2 min. (I = s.e.)

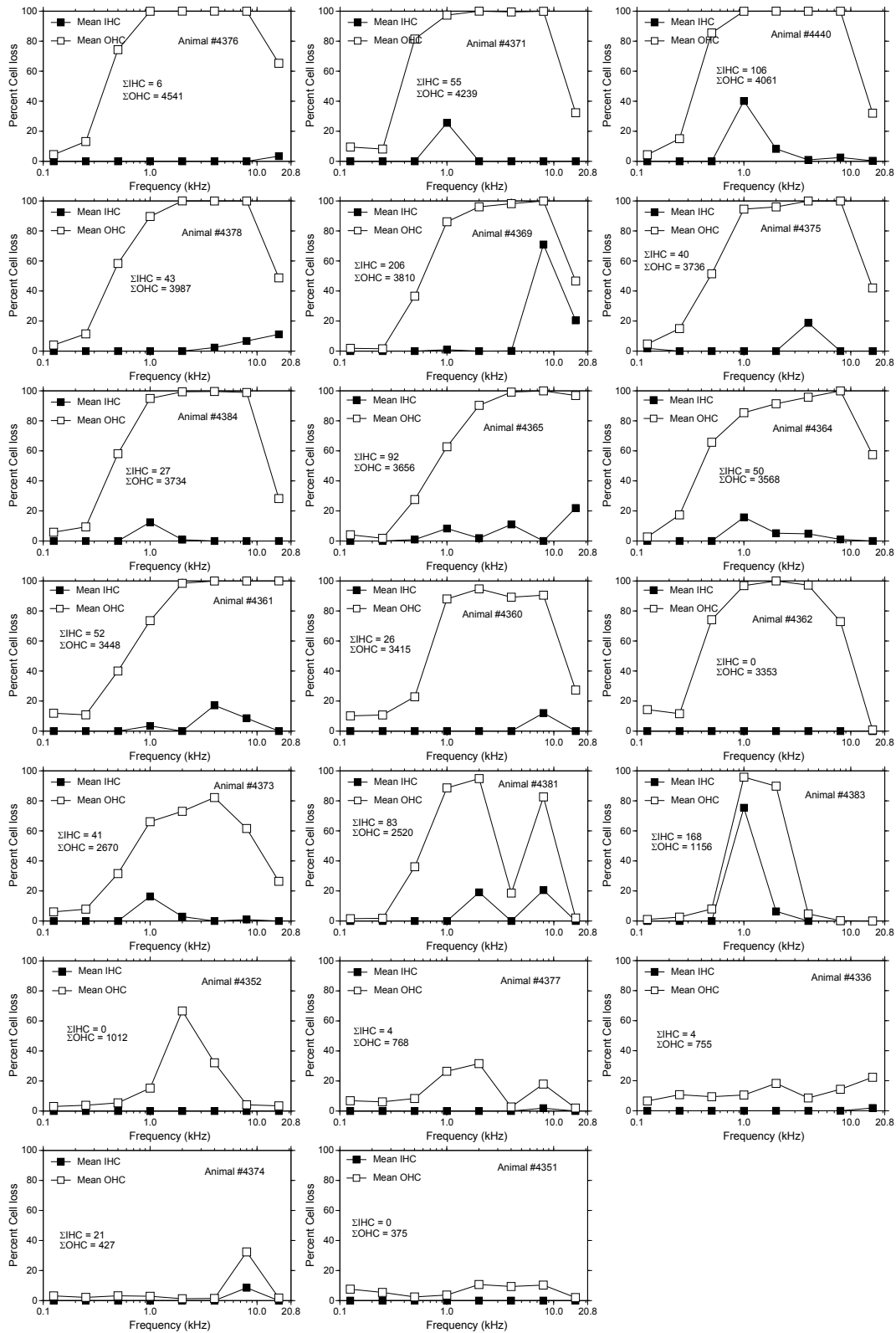


Figure 52. Phase III L-NAC individual cochleograms for the 20 animals exposed to the 158 dB peak SPL shock wave (11 psi charge pressure) that were subjected to pre and post exposure tympanometry.

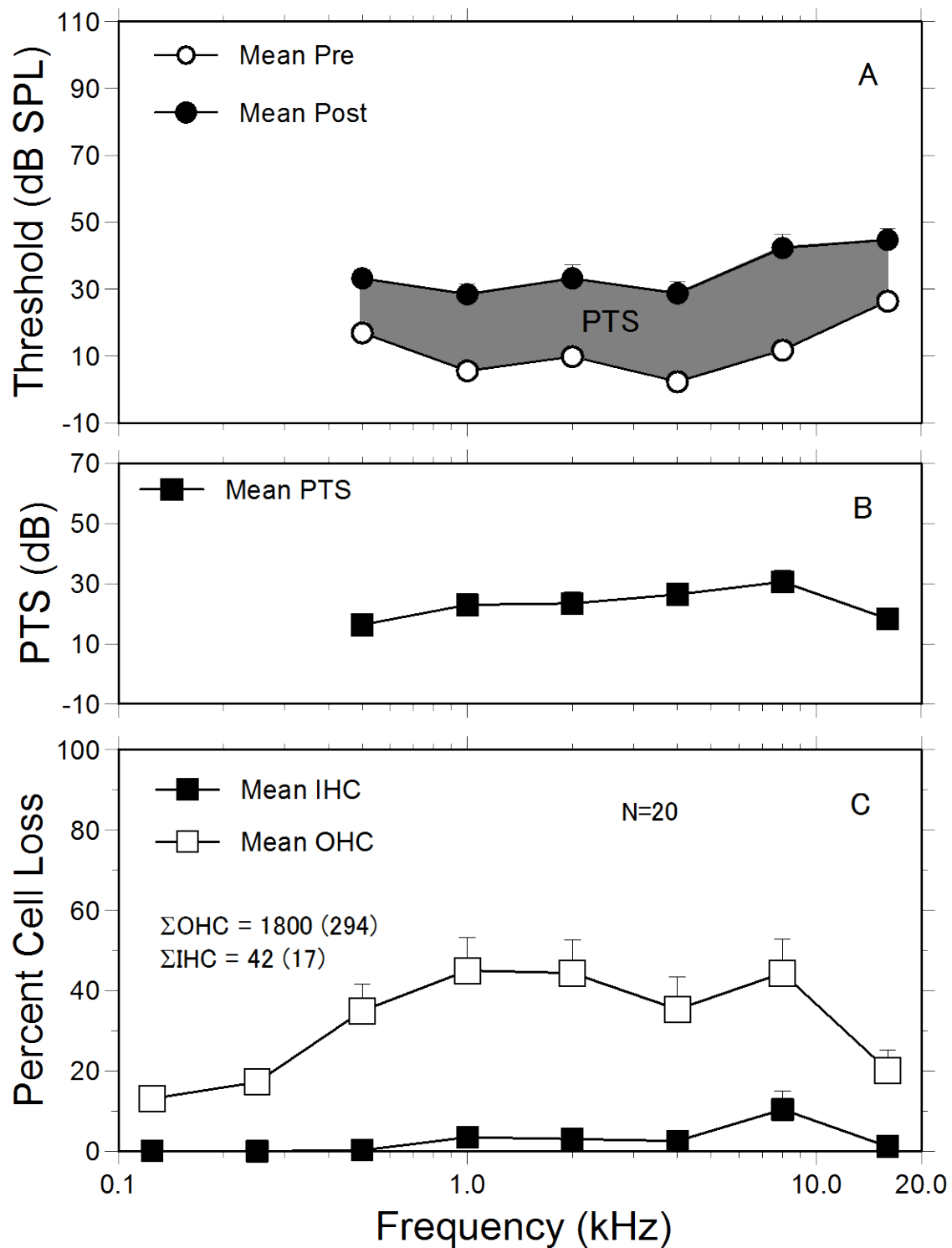


Figure 53. Phase III D-MET group (N=20) exposed to 10, 158 dB peak SPL impulses (shock tube controls 10x @ 11 psi charge pressure) within less than 2 min. Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) Permanent threshold shift and (C) percent inner and outer hair cell (IHC, OHC) loss.

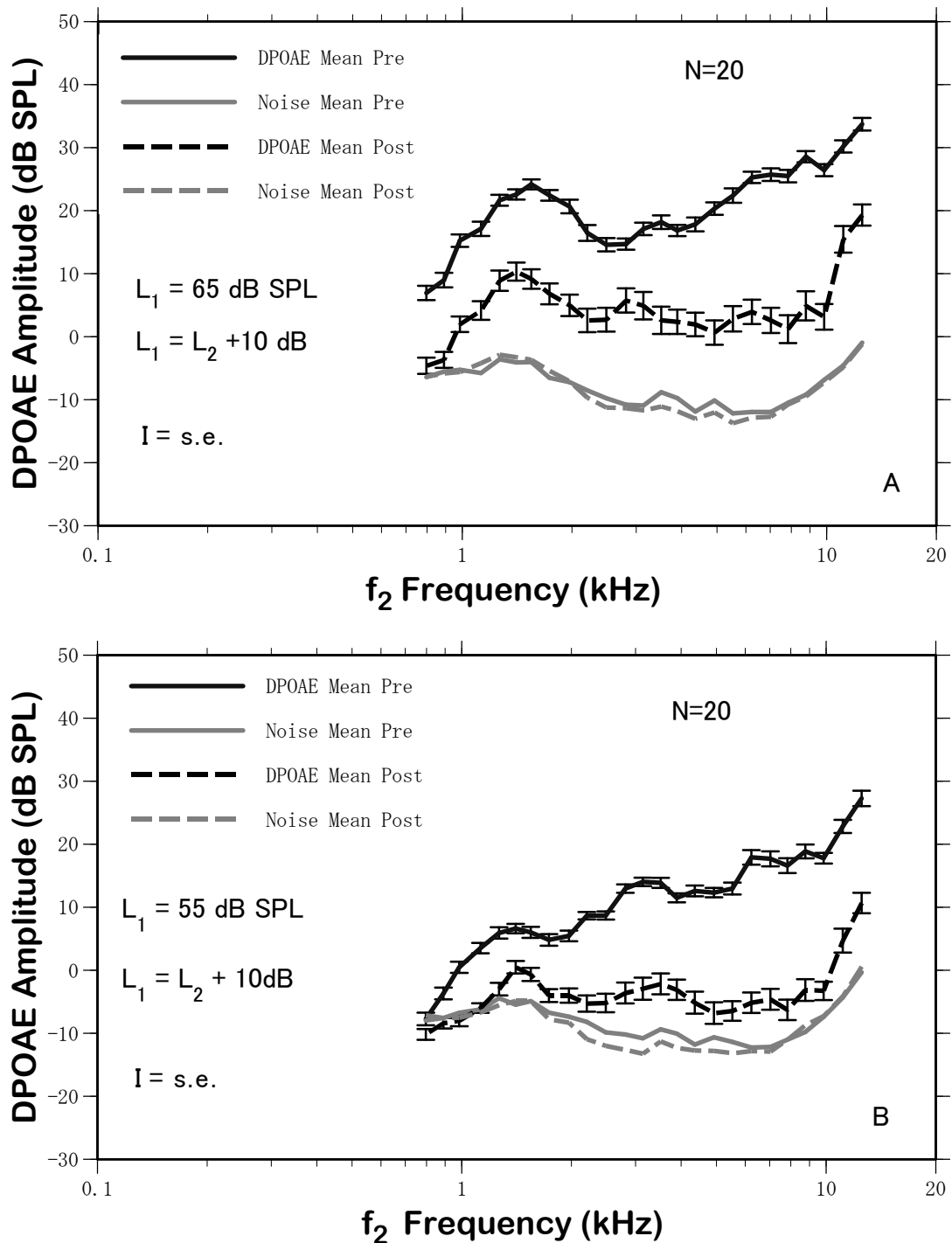


Figure 54. Phase III D-MET group mean ($N=20$) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) $L_1 = 65$ dB SPL and (B) $L_1 = 55$ dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 10, 158 dB peak SPL impulses (shock tube controls 10x @ 11 psi charge pressure) within less than 2 min. ($I = \text{s.e.}$)

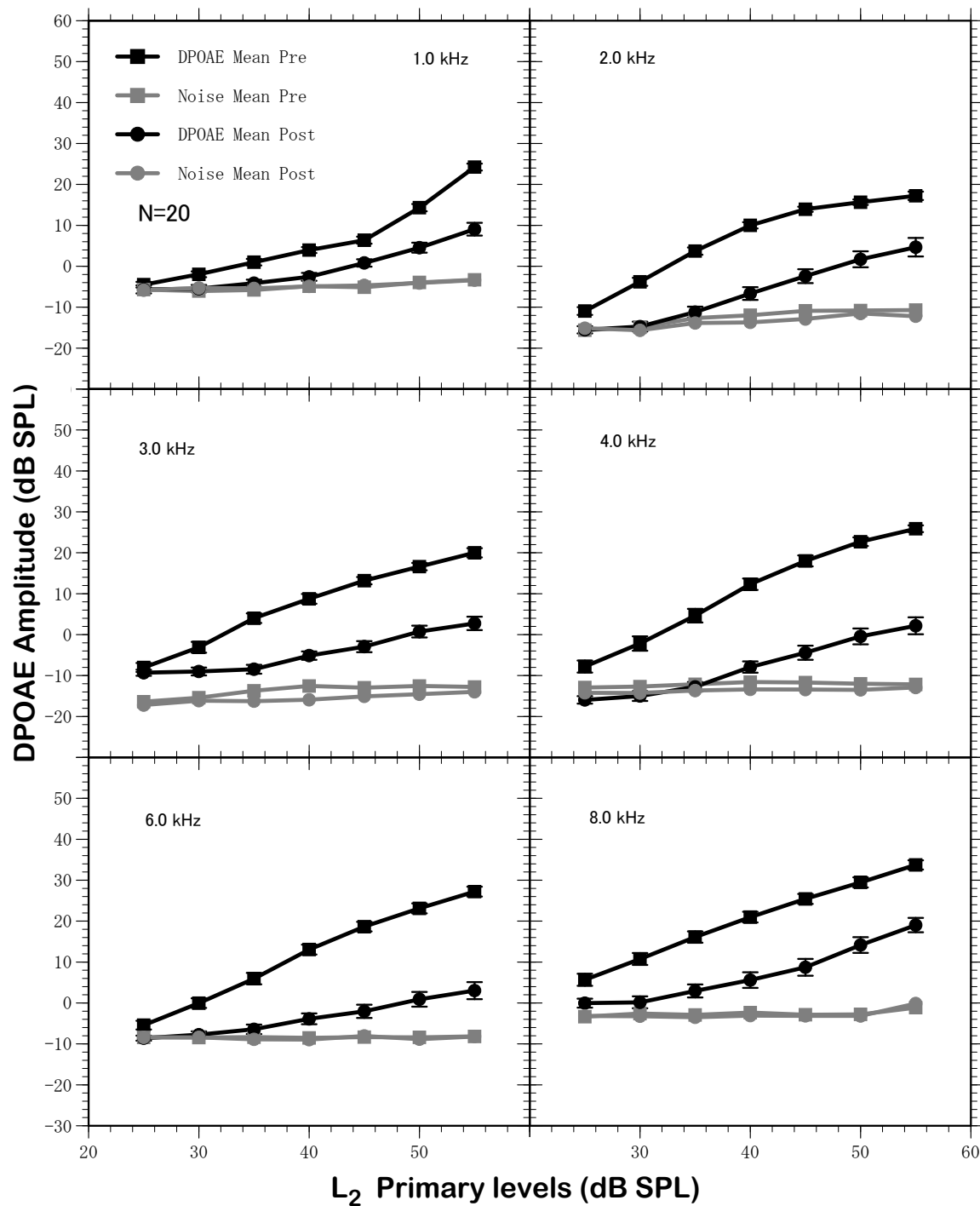


Figure 55. Phase III D-MET group mean (N=20) pre and post exposure cubic ($2f_1-f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 . Exposure: 10, 158 dB peak SPL impulses (shock tube controls 10x @ 11 psi charge pressure) within less than 2 min. (I = s.e.)

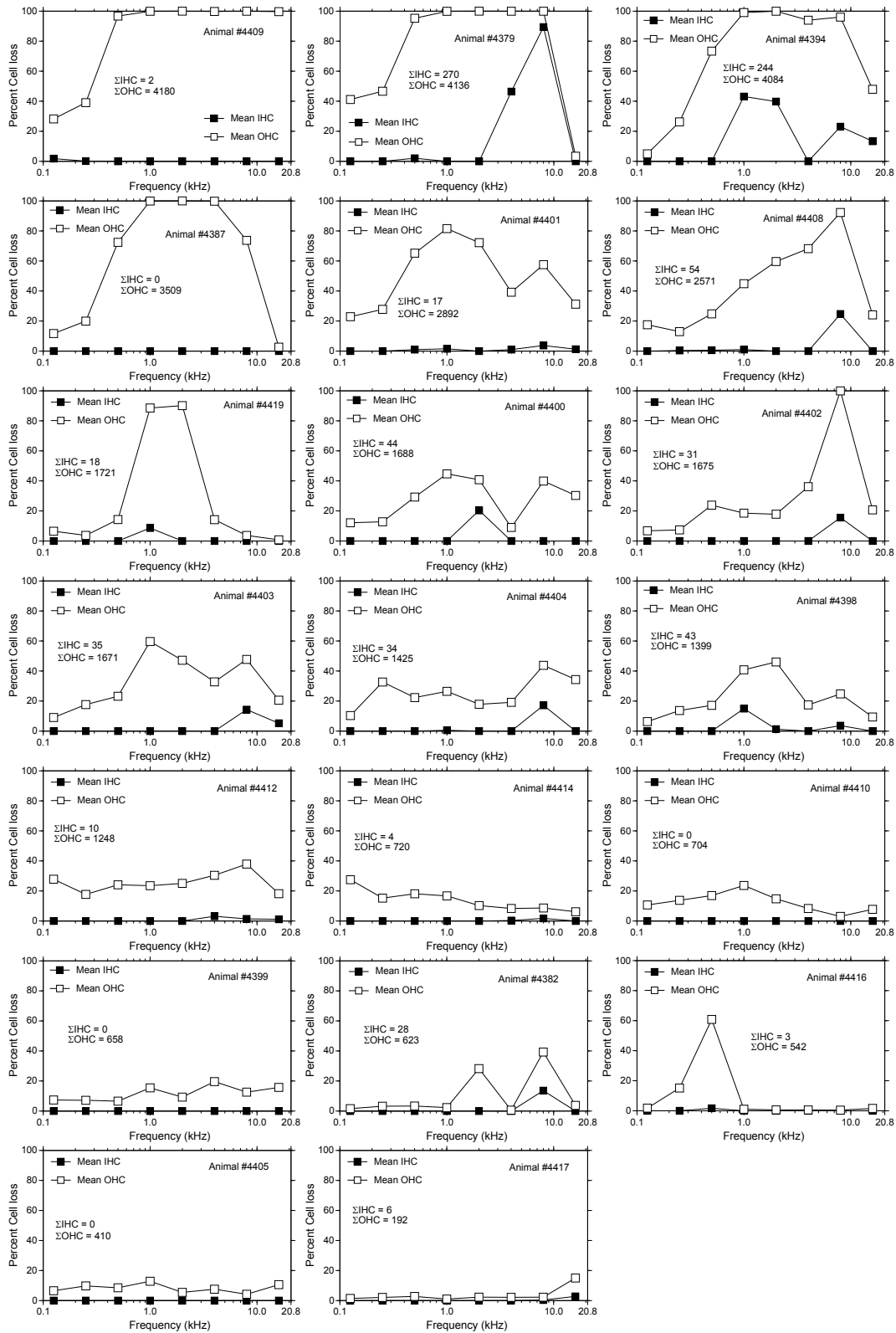


Figure 56. Phase III D-MET individual cochleograms for the 20 animals exposed to the 158 dB peak SPL shock wave (11 psi charge pressure) that were subjected to pre and post exposure tympanometry.