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Hearing Research 132 (1999) 149–159

HEARING RESEARCH

Potentiation of octave-band noise induced auditory impairment by carbon monoxide

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Received 28 November 1998; received in revised form 18 February 1999; accepted 3 March 1999

Abstract

In previous studies from our lab, broadband noise induced hearing loss has been found to be potentiated by simultaneous carbon monoxide (CO) exposure. In the present study, octave-band noise induced auditory impairment was studied with the presence of CO at levels of 1500, 1200, 700, 500 and 300 ppm and zero (noise alone). Four octave-band noises (1.2–2.4, 2.4–4.8, 4.8–9.6 and 9.6–19.2 kHz) were used. Experimental subjects (rats) were grouped for the exposure (8 h) to each noise, CO and their combinations. The compound action potential (CAP) and cochlear microphonics (CM) were recorded 4 weeks after the exposure. The noise induced elevation of the CAP threshold and the CM iso-amplitude curve were potentiated by the simultaneous CO exposure when the CO level reached 500 ppm or higher. CO exposure alone had no effect on CAP or CM. The CO potentiation can occur in any frequency region depending on the noise band. The combined exposure can also induce threshold shifts in some cases in which both the noise and the CO alone did not cause threshold shifts. The size of the potentiation shown by CAP and CM was similar, indicating a possible origin of the CO potentiation from the damage to the outer hair cells. Interestingly, the hearing loss induced by noise alone gradually recovered (partially), but the hearing loss caused by the combined exposure did not. The potentiation may be due to the reduction of the cell's ability to repair noise induced damage by CO.

Key words: Hearing loss; Carbon monoxide; Ototoxicity; Octave-band noise; Compound action potential; Cochlear microphonics; Rat

1. Introduction

Intense noise is well known to cause hearing loss. Acoustic overstimulation may initially mechanically disrupt the stereocilia and cuticular plates, which affects the micromechanics of the transduction process (see review by Lim, 1986). The hair cell membrane has also been found to be damaged by the intense noise (Mulroy et al., 1998). Furthermore, following the noise exposure, many vacuoles are seen in the synaptic region of hair cells (Mulroy et al., 1990). Besides mechanical damage, intense noise may also interfere with the basic functions of the auditory cells. The increase of calcium concentration inside hair cell due to intense noise has been directly observed recently (Fridberger et al., 1998). Many functions including neurotransmitter release and outer hair cell length require calcium. The calcium increase is consistent with the decrease of the cochlear microphonic (CM) recorded at the same time. Other basic cellular functions (such as the protein, lipid, and glucose synthesis needed for cell repair and survival) may also be influenced by intense noise (Lim, 1986; Spoendlin, 1971).

Carbon monoxide (CO) has very high affinity to hemoglobin, and can reduce or block oxygen supply to tissues. Accidental exposure to high level CO in humans or pure CO injection to experimental animals causes severe hearing loss acutely, especially at high frequencies (Baker and Lilly, 1977; Fechter et al., 1987; Kowalska, 1980, 1981). Acute carbon monoxide toxicity is believed to be secondary to tissue hypoxia (Makishima et al., 1977). Recent studies indicate further that an excitotoxic mechanism may be involved in CO ototoxicity (CO inducing excessive glutamate release, Liu and

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0378-5955/99/$ – see front matter
PII: S0378-5955(99)00044-1

Fechter, 1995). Free radical generation seems also involved in CO ototoxicity (Fechter et al., 1997). However, CO exposure alone to experimental animals at 1200 ppm or 1500 ppm does not produce a permanent hearing loss (Fechter et al., 1988; Rao and Fechter, 1998). Interestingly, such CO exposure, which alone may not induce permanent hearing loss, can potentiate permanent noise induced auditory impairment (Fechter et al., 1988; Rao and Fechter, 1998; Young et al., 1987). The potentiation at high frequencies appears to be especially great (about 55 dB) following broadband noise exposure assessed by the reflex modification audiometry (Fechter et al., 1988). However, it is still unclear how CO interacts with intense noise. Is the potentiation frequency dependent? Is the noise induced permanent impairment necessary for the potentiation? What is the lowest CO level that shows an interaction with the noise? What is the mechanism of the CO potentiation?

In the present study four different octave-band noises at different intensities were used to induce different patterns of auditory impairments and to determine the frequency specificity of the CO potentiation. In the main experiment, a CO level of 1200 ppm was applied, which was similar to that used in previous reports (Fechter et al., 1988) to determine the CO potentiation in the auditory periphery. Gradually decreased CO levels were used to determine the lowest one which showed interaction with intense noises. The compound action potential (CAP) and CM were recorded in animals with different post-exposure recovery times to determine the development of the CO potentiation. The CO potentiations shown by CAP and CM were also compared, to help determine the origin of the potentiation. The mechanism of the CO potentiation is discussed in the present report.

2. Methods

Ninety-nine experimental animals (Long-Evans pigmented rats approximately 2 months of age) were acquired from Harlan Sprague-Dawley and housed in the University of Oklahoma Health Sciences Center animal facility. All animal facilities in OU are registered with the US Department of Agriculture and are inspected semiannually by the members of the Institutional Animal Care and Use Committee (IACUC). All procedures regarding the use and handling of animals were reviewed and approved by the IACUC serving the University of Oklahoma Health Sciences Center.

2.1. Noise and CO exposure

Broadband noise was generated by a synthesized function generator (Stanford Research System, model DS335). Octave-band noises were obtained by passing the broadband signal through a filter network (Frequency Devices, 9002) that provides a band-pass noise. The frequency band was set at 1.2–2.4 kHz, 2.4–4.8 kHz, 4.8–9.6 kHz and 9.6–19.2 kHz. The filtered signal (octave-band noise) was amplified with a power amplifier (SAE 2200) and delivered to two speakers (Vifa D25AG-05-06, 709) in the exposure chamber. The animal cages were located under the speakers with a vertical distance (to the cage floor) of about 7 inches. Noise intensity was measured with a Sound Level Meter (Quest Electronics) setting at linear weighting at the approximate level of the animals' ears. The noise level varied less than 2 dB within the space available to the animal. The intensity of the noises used was 100 dB (Ln) or 115 dB (Ln). The background noise level in the chambers with no added noise was about 40 dB (A). Air exchange rate in the exposure chamber was 8.5 m^3/min (providing for approximately 12 changes per hour), and airflow was monitored by a Top Trak 821-1-PS flowmeter. CO gas was delivered into the chamber and the CO level in the chamber was monitored by an Industrial Scientific 8000 Controller. The nominal CO level used in the main experiment was 1200 ppm. Nominal CO levels of 1500 ppm, 700 ppm, 500 ppm and 300 ppm were also used. The CO level in the exposure chamber reached its stable level within about 1 h, with about 95% of nominal value achieved within 40 min of exposure onset. Carboxyhemoglobin (HbCO) concentration in the blood was also measured in some animals. Blood was collected from the heart under anesthesia after exposure (within 5–15 min following exposure). When the noise was turned on (at 1.5 h after the onset of CO), the CO in the exposure chamber and the HbCO concentration in the blood had already reached their plateau levels. Noise exposure time was 8 h and the CO or air exposure time was 9.5 h (1.5+8 h). The rats were divided into groups randomly and exposed to each noise, CO, air and their combinations. The animals were not anesthetized and could move freely in separated cages. During exposure (daytime, inactive phase for rats) food and water were not available.

2.2. CAP and CM recording and analysis

Four weeks (1 or 2 weeks in some cases) after the exposure, the animals were anesthetized with xylazine (13 mg/kg, i.m.) and ketamine (87 mg/kg, i.m.). The round window was surgically exposed using a ventrolateral approach and a silver wire electrode was carefully placed on the round window under a surgery microscope for recording CAP and CM. A silver chloride electrode was placed in the neck muscle as the refer-

ence. The CAP and CM signals were amplified with a Grass AC preamplifier (Model P15). The gain was 1000. The band-pass frequency for CAP was 0.1–1.0 kHz and 0.1–50 kHz for CM. The CAP signals were observed using a digital oscilloscope (Nicolet Instrument Co., 2090-IIIA). The CM signals were sent to a SR530 Lock-in amplifier (Stanford Research Systems, Inc.) and then to a PC computer for automatic determination of the 1μV RMS amplitude.

Pure tones for eliciting CAP and CM were generated with the SR530 Lock-in amplifier (Stanford Research Systems, Inc.). The signals were attenuated by a programmable attenuator and then amplified by a high voltage amplifier and delivered to a high frequency phone (made from an ACO 1/2″ microphone, 7013) placed within a speculum that fit into the exposed external auditory meatus. The frequencies of the tones were 2, 4, 6, 8, 12, 16, 20, 24, 30, 35 and 40 kHz. Continuous tones were used for CM eliciting. Tone bursts were used for CAP eliciting. The duration of the tone bursts was 10 ms with a rise/fall time of 1.0 ms with a repetition rate of 9.7 times/s. Sound levels at all testing frequencies were calibrated with a probe microphone located near the eardrum.

The sound level of test frequencies that evoked a just detectable CAP was determined and used as the threshold at the frequency. The sound levels at all test frequencies that evoked 1 μV CM were determined by the computer program. The function relating the sound level eliciting the criterion amplitude response versus frequency was called the 1 μV CM iso-amplitude curve.

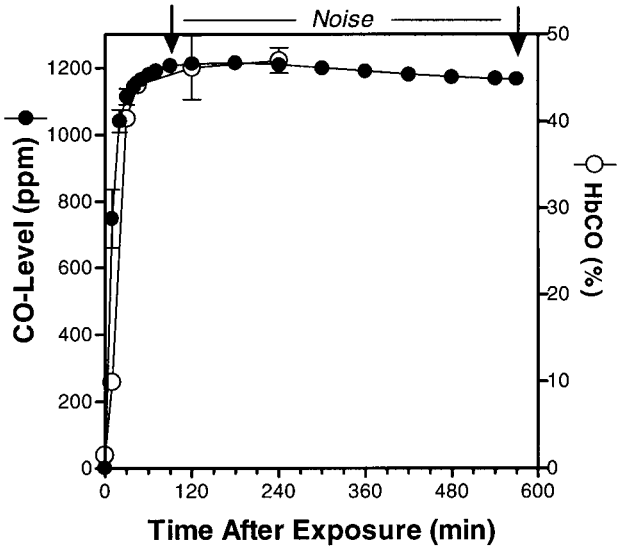

Fig. 1. Mean CO levels ($n=10$ exposures) in the exposure chamber (filled circles) and the HbCO concentration in the blood (open circles) to the exposure of a nominal 1200 ppm CO. Vertical bars are standard error (S.E.M.). Arrows indicate onset and offset of intense noise.

CAP threshold shifts were the difference between each CAP threshold and the mean control value (exposed to air alone) at the same frequency. CM iso-amplitude curve elevation was obtained in a similar way. The potentiation of noise induced hearing loss (NIHL) by CO was the difference between the CAP change or the CM change induced by the combined exposure and that by noise exposure alone.

A one-way analysis of variance (ANOVA, Newman-Keuls) was used to evaluate the variance among groups. Repeated measures analysis of variance (exposure condition and testing frequency) was also used to evaluate the overall significance of the main effects and their interactions.

3. Results

Chamber CO and blood HbCO concentrations reach their plateau within a short time of onset of exposure. Fig. 1 shows the averaged CO levels in the exposure chamber (filled circles) and the HbCO concentration in the blood (open circles) to the exposures of a nominal CO level of 1200 ppm. The pleateau blood HbCO concentration was $46.6 \pm 3.3\%$ ($\pm$S.D., $n=4$ rats). The noise exposure period is also indicated in Fig. 1.

The data presented here show impairment of CAPs and CMs caused by the exposure to intense octave-band noises and CO. The intense noises caused elevations of the CAP thresholds and the CM iso-amplitude curve. The noise induced auditory impairment was potentiated by the simultaneous CO exposure, while CO alone did not cause any CAP or CM change.

3.1. Carbon monoxide potentiating noise induced CAP threshold shift and the elevation of the CM iso-amplitude curve

In this experiment two octave-band noises (9.6–19.2 kHz and 2.4–4.8 kHz) were employed. The intensity of the high frequency noise was 100 dB (Ln) and for the low frequency noise it was 115 dB (Ln). The nominal CO level was 1200 ppm. The higher intensity of the low frequency noise was used because the low frequency noise did not induce hearing loss in rats at 100 dB (Ln) intensity (whether CO exposure was given or not). At a lower frequency band (1.2–2.4 kHz), even a 110 dB (Ln) intensity did not induce hearing loss.

The exposure to the high frequency noise alone (9.6–19.2 kHz, 100 dB (Ln), 8 h) induced obvious CAP threshold shifts (Fig. 2A, open circles, 4 weeks recovery time), especially at the frequencies at and higher than the noise band (>8 kHz). With combined exposure to noise and CO (1200 ppm), the CAP threshold shift at each frequency was obviously greater than that to the

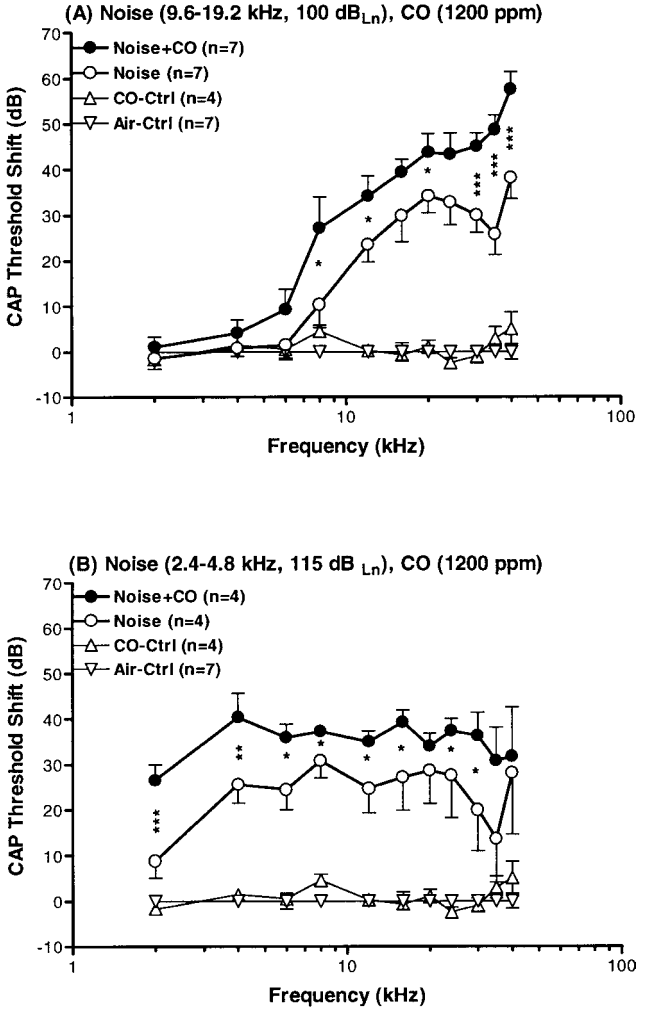

Fig. 2. CAP threshold shifts in animals to combined exposure of noise and CO (filled circles), noise alone (open circles), CO alone (open triangles) and air alone (open inverse triangles). Air exchange rate in the chamber: 8.5 m^3/min. CO nominal level 1200 ppm. CAP was recorded 4 weeks after exposure. Vertical bars: standard error (S.E.M.). The significance between the CAP threshold shift to the combined exposure and that to the noise alone is indicated with asterisks (*$P<0.05$, **$P<0.01$, ***$P<0.001$). A: Noise band: 9.6–19.2 kHz, 100 dB (Ln). B: Noise band: 2.4–4.8 kHz, 115 dB (Ln).

noise alone (filled circles). The significance of the difference between CAP threshold shift to the combined exposure and that to the noise alone is marked in Fig. 2A (***$P<0.001$; *$P<0.05$). The threshold shift potentiation becomes more obvious (or larger) as the frequency increases. At the frequencies lower than the noise frequency, however, the noise alone caused much smaller CAP threshold shifts or did not cause any threshold shift. At 8 kHz (close to the noise frequency), although a small averaged CAP threshold shift to the noise alone was observed, the difference between the threshold and the control value (open inverse triangle) was not significant ($P>0.05$). However, at the same frequency the combined exposure of noise and CO induced a greater CAP threshold shift (mean 27 dB). Compared to the control value, the effect of combined noise+CO exposure was very significant ($P<0.01$). The difference between the threshold shift to the combined exposure and that to the noise alone was also significant ($P<0.05$). At 6 kHz (a little lower than the noise frequency), the noise alone did not induce CAP threshold shift. The combined exposure induced about 9 dB threshold shift, though the threshold shift was not significant in comparison to the control data ($P>0.05$). At lower frequencies the noise alone did not induce a CAP threshold shift. Small but systematically greater threshold shifts to the combined exposure were still seen. CO exposure alone did not induce any CAP threshold shift (open triangles). Comparing the CO induced CAP threshold shifts to the control data, there was no difference across test frequencies ($F=0.16$, $P>0.05$). A significant loss of CAP threshold sensitivity was found between subjects receiving the different treatment conditions of combined exposure (filled circles), noise alone (open circles) and control subjects (open inverse triangles) ($F_{2,18}=50.8$, $P<0.01$). Additionally, both the effect of frequency ($F_{10,180}=54.2$, $P<0.01$) and the group-frequency interaction ($F_{20,180}=15.0$, $P<0.01$) were significant. This indicates that the CAP threshold shifts to different exposures are significantly different. The influence is frequency dependent. At high frequencies, noise caused obvious CAP threshold shifts. However, with the presence of CO, the noise exposure caused much greater CAP threshold shifts. At low frequencies, the exposure to noise alone did not induce CAP threshold shifts. However, the combined exposure tended to produce somewhat greater CAP threshold shifts.

The exposure to the low frequency noise alone (2.4–4.8 kHz, 115 dB (Ln), 8 h) also induced permanent CAP threshold impairment (up to about 31 dB, Fig. 2B, open circles). In distinction to the effect of high frequency noise (Fig. 2A), the low frequency noise induced similar CAP threshold shifts at all the testing frequencies. A relatively smaller threshold shift was observed at the 2 kHz tone (about 9 dB), a test frequency lower than the noise frequency. The combined exposure to the noise and the CO caused much greater CAP threshold impairment (filled circles). The significance of the difference between CAP threshold shift to the combined exposure and that to the noise alone is marked in Fig. 2B (***$P<0.001$; **$P<0.01$; *$P<0.05$). Although the threshold shift potentiation occurred at all the test frequencies, the potentiation is somewhat greater at low frequencies. A significant effect of group treatment ($F_{2,9}=16.1$, $P<0.01$) and frequency ($F_{10,90}=5.5$, $P<0.01$) was found. However, a significant group-frequency interaction was not seen ($F_{20,90}=1.3$, $P>0.1$). Thus, while the treatment effect

is statistically significant, the effect of treatment was independent of frequency.

The data show that the intense octave-band noises can induce hearing loss at frequencies higher than the noise band, but little hearing loss at the lower frequencies. The simultaneous CO exposure may not only potentiate the hearing loss, but also expand the frequency region that shows NIHL.

The exposure to the high frequency noise alone (9.6–19.2 kHz, 100 dB (Ln)) induced an elevation of the CM iso-amplitude curve (Fig. 3A, open circles) not only in the high frequency region, but also in the low frequency region. The elevations of the CM iso-amplitude curve in the high frequency region (>10 kHz) were significant (except that at 12 kHz), but were relatively smaller than the corresponding CAP threshold shifts (see Fig. 2A). In the low frequency region the elevations were also obvious, though the CAP thresholds in this frequency region did not show significant change. This change in the low frequency region may reflect the recording bias to the high frequencies by the round window electrode. Similar to the CAP results, the CM was not affected by CO exposure alone (Fig. 3A, open triangles). The combined exposure of noise and CO, however, caused much greater elevations of the CM iso-amplitude curve (Fig. 3A, filled circles). There was no significant difference between the CM changes resulting from CO exposure and the control data (open inverse triangles). The significance of the difference between each CM change to the combined exposure and that to the noise alone is marked in Fig. 3A (***$P<0.001$; **$P<0.01$; *$P<0.05$). The potentiation of the CM change by the combined noise and CO was greater in the high frequency region than in the low frequency region. Significant effects of treatment ($F_{2,18}=34.4$, $P<0.01$), frequency ($F_{10,180}=11.7$, $P<0.01$) and the treatment-frequency interaction ($F_{20,180}=5.6$, $P<0.01$) were obtained.

The exposure to the low frequency noise (2.4–4.8 kHz, 115 dB (Ln)) also caused an elevation of the CM iso-amplitude curve (Fig. 3B, open circles). The elevations were much smaller than the corresponding CAP threshold shifts (see Fig. 2B) and at most of the frequencies (but 4, 6 and 8 kHz) were not significant. The combined exposure to the noise and CO caused much more remarkable elevation of the CM iso-amplitude curve (filled circles) than noise alone. The CM changes were all significant. Significant differences in CM between subjects receiving the combined exposure and those receiving noise alone are marked in Fig. 3B (*$P<0.05$). The significant difference only occurred at a few frequencies. Significant effects of treatment ($F_{2,9}=4.3$, $P<0.05$) and frequency ($F_{10,90}=2.1$, $P<0.05$) were obtained. However, a significant group-frequency interaction was not seen ($F_{20,90}=1.5$, $P>0.05$). Thus, while the treatment effect is statistically significant, the effect of treatment was independent of frequency.

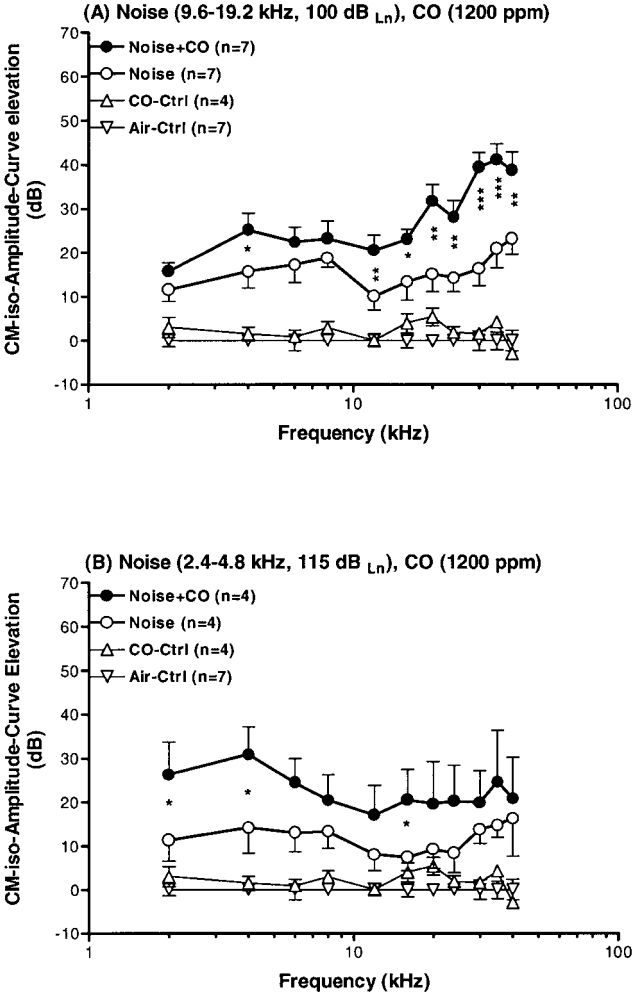

Fig. 3. Elevations of CM iso-amplitude curve to the combined exposure of noise and CO (filled circles), noise alone (open circles), CO alone (open triangles) and air alone (open inverse triangles). Air exchange rate in the chamber: 8.5 m^3/min. CO nominal level 1200 ppm. The CM was recorded 4 weeks after the exposure. Vertical bars: standard error (S.E.M.). The significance between CM changes to the combined exposure and that to the noise alone is indicated with asterisks (*$P<0.05$, **$P<0.01$, ***$P<0.001$). A: Noise band: 9.6–19.2 kHz, 100 dB (Ln). B: Noise band: 2.4–4.8 kHz, 115 dB (Ln).

3.2. Carbon monoxide increasing auditory system susceptibility to noise exposures

While a high frequency noise induces a hearing loss, a low frequency noise at the same intensity level may not. The rat auditory system is less susceptible to the low frequency noise exposure than to the high frequency noise exposure. However, in the presence of CO the vulnerability to the noise may increase. In this experiment, four octave-band noises were employed:

9.6–19.2 kHz, 4.8–9.6 kHz, 2.4–4.8 kHz and 1.2–2.4 kHz. The intensity of the noises was 100 dB (Ln) and the CO level 1200 ppm.

Fig. 4 shows CAP threshold shifts (A) and the elevations of the CM iso-amplitude curves (B). The test frequencies were divided into low frequency group (2–8 kHz), mid frequency group (12–20 kHz) and high frequency group (24–40 kHz) for simplification. They are represented as three bars for each noise exposure group (low octave band at the left and higher octave bands at the right). Open bars represent changes of the CAP and the CM to noise alone, while the filled bars represent changes of the CAP or the CM to the combined exposure of noise and CO. With exposure to noise alone at

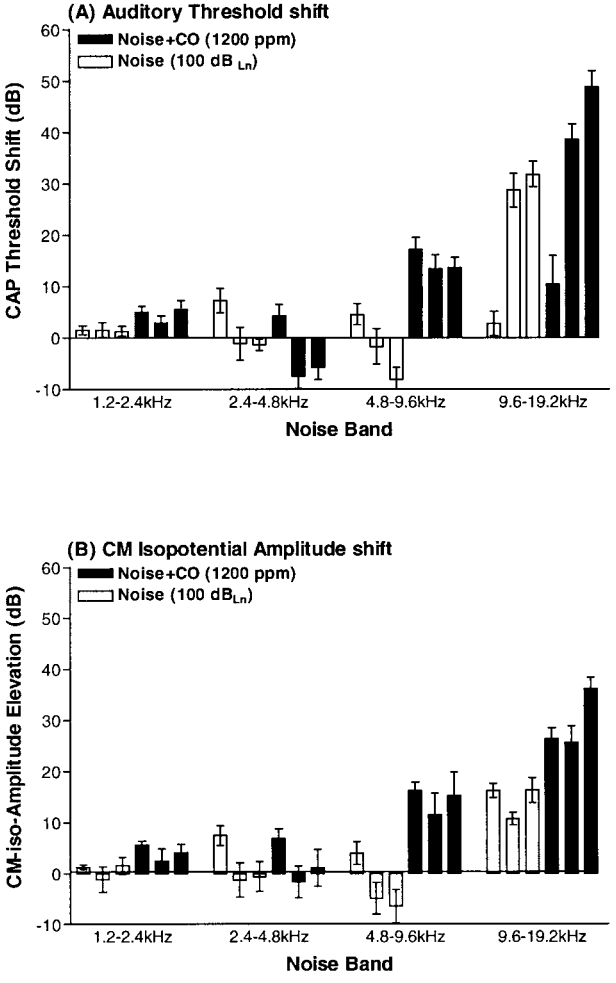

Fig. 4. CAP threshold shift and CM iso-amplitude curve elevation to noise in different frequency bands. Noise intensity was 100 dB (Ln). CO level was 1200 ppm. CAP and CM were recorded 4 weeks after the exposure. Filled bars represent the changes to the combined exposure of noise and CO and the open bars to the noise alone. The three bars in each group represent the changes of CAP or CM in the low (2, 4, 6, 8 kHz), mid (12, 16, 20 kHz) and high frequency regions (24, 30, 35, 40 kHz) respectively. Standard errors are indicated. A: CAP threshold shifts. B: CM iso-amplitude curve elevations.

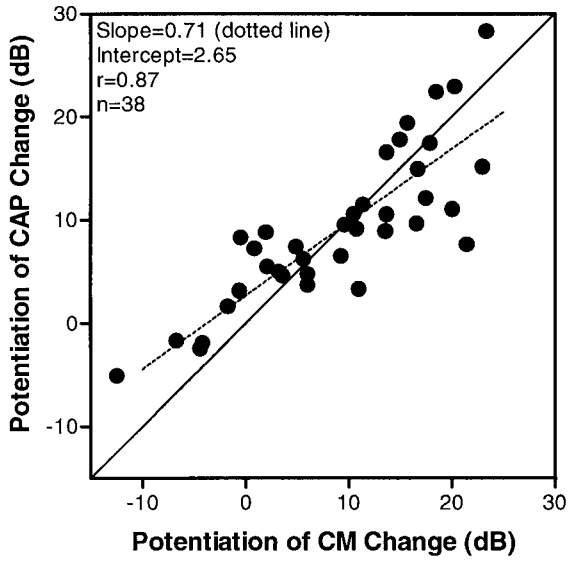

Fig. 5. Comparison between potentiations of CAP threshold shift and the elevation of CM iso-amplitude curve by CO. CO levels: 1200 ppm and 700 ppm; noise frequency bands: 9.6–19.2 kHz and 2.4–4.8 kHz. $CAP^P = 2.65 + 0.71 \times CM^P$, CAP^P is the potentiation of the noise induced CAP threshold shift. CM^P is the potentiation of the noise induced CM change. $r = 0.87$.

100 dB (Ln), only the highest frequency noise band (9.6–19.2 kHz) induced significant elevation of the CAP threshold and CM iso-amplitude curve (open bars). The change, especially of the CAP, occurred mainly in the high and mid frequency regions. The changes of CAP and CM to the other three noise bands were relatively smaller and distributed around the zero line. With combined exposure to noise and CO, the highest frequency noise band (9.6–19.2 kHz) induced much greater elevations, especially at the high and mid frequency regions, than that induced by the exposure to the noise alone. Importantly, in the presence of CO, the second highest frequency noise (4.8–9.6 kHz) also induced remarkable elevations of CAP threshold and CM iso-amplitude curve (see filled bars at noise band of 4.8–9.6 kHz). The noise (4.8–9.6 kHz, 100 dB (Ln)) did not cause hearing loss without the presence of CO. Two lower frequency noises (1.2–2.4 kHz and 2.4–4.8 kHz) did not induce any auditory impairment at 100 dB (Ln) intensity, with or without the CO exposure. In fact, the noise with the band of 1.2–2.4 kHz did not induce hearing loss even at 110 dB (Ln) intensity, with or without the presence of CO.

The data indicate that with frequency components lower than 10 kHz, the 100 dB noise did not induce auditory impairment in the rats. However, if CO was present in the environment (1200 ppm), all the octave-band noises (100 dB (Ln)) with frequency components higher than about 5 kHz disrupted the auditory system of the rats in the correspondent area.

3.3. Comparison between the potentiation of CAP and CM impairment by CO plus noise

Undoubtedly, noise induced CAP and CM change can be potentiated by the simultaneous CO exposure. To understand the mechanism of the CO potentiation it is helpful to know where and when the potentiation occurs. It is well known that the CM reflects electrical properties of outer hair cells (OHCs) mostly, but not entirely. The CAP, however, originates from the activities of auditory nerve fibers, which depend upon the normal functions of inner hair cells (IHCs), ganglion cells and also the OHCs (via the cochlear active process). The decline of the OHCs' function would cause not only the change of CM but also a corresponding decline of the output of the IHCs. However, the decline of the function of the IHCs may have limited influence on the CM. In this experiment the extent of CO potentiation of the effects of noise was compared between the CM and CAP.

In Fig. 5 the potentiation by CO of noise induced CM and CAP changes was obtained by subtracting the mean CM or CAP change caused by noise alone from that caused by the combined exposure to noise and CO. In this experiment five CO levels (300 ppm, 500 ppm, 700 ppm, 1200 ppm and 1500 ppm) were used. For exposure to high frequency noise (9.6–19.2 kHz) the CO potentiation was collected within the frequency region higher than 8 kHz. For lower frequency noise (2.4–4.8-kHz) frequencies from 2 to 6 kHz were studied. The potentiations of CAP threshold shift are plotted as a function of the potentiations of the CM iso-amplitude curve elevation. Obviously the CAP threshold shift potentiation increased with the potentiation of the CM iso-amplitude curve elevation. All the points distribute in the vicinity of the diagonal line (solid line). The slope of the regression line (dotted line) is 0.71 and the coefficient is 0.87. The equation of the regression line is:

$$CAP^P = 2.65 + 0.71 \times CM^P$$

Here CAP^P is the potentiation of the noise induced CAP threshold shift and CM^P is the potentiation of the noise induced CM change. These data indicate that the potentiations shown by CAP and CM are correlated and similar.

3.4. Recovery of auditory threshold over time in subjects exposed to noise alone and noise+CO

As described above, the hearing loss potentiation by 1200 ppm CO exposure was significant when it was measured 4 weeks after the exposure. However, it is not clear whether it shows a similar pattern of (partial) recovery as has been demonstrated in the case of noise exposure alone. In this experiment 9.6–19.2 kHz noise (100 dB (Ln)) and 1200 ppm CO were used. The CAP thresholds were measured at 1 week, 2 weeks and 4 weeks after the exposure in different group of animals. Fig. 6A presents the CAP threshold shifts obtained in different groups of animals at 1 and 4 weeks following exposure. Auditory thresholds in the high frequency region (>8 kHz) were significantly higher than the controls (open inverse triangles) in both subjects treated with noise alone and treated with combined exposure. Significant recovery of function, however, was observed among noise treated subjects between 1 week after the exposure (open squares) and 4 weeks after the exposure (open circles). At 12 and 16 kHz (within the

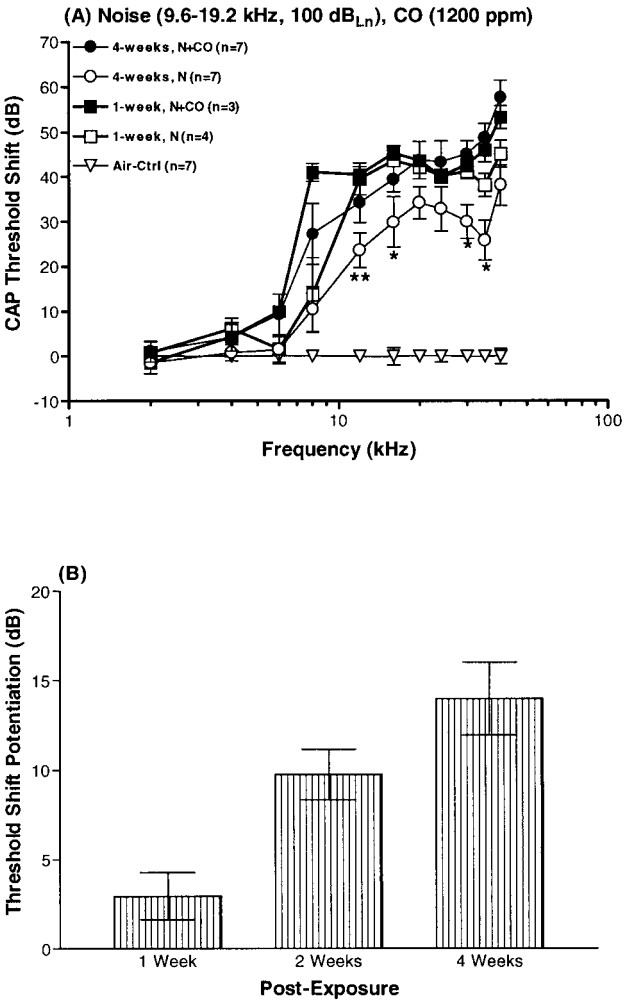

Fig. 6. CAP threshold shifts and the potentiation by CO measured at different post-exposure times. Open symbols: to noise alone (9.6–19.2 kHz, 100 dB (Ln)). Filled symbols: to combined exposure to the noise and CO (1200 ppm). Vertical bars: standard errors. A: CAP threshold shifts. The circles represent the CAP threshold shifts measured 4 weeks after the exposure, while the squares represent the CAP threshold shifts measured 1 week after the exposure. Open inverse triangles represent the data from control animals. B: Mean threshold shift potentiations within frequency region of 12–40 kHz.

noise frequency band) and at 30 and 35 kHz the difference was statistically significant. The significance is marked in Fig. 6A (**$P < 0.01$; *$P < 0.05$). By contrast, the subjects exposed to combined noise and CO showed a similar loss of threshold at both time intervals after the exposure (filled circles and filled squares). Even at 8 kHz, at which there is the largest difference between the means, the difference between the two groups is not significant ($P > 0.05$). The differences between the CAP threshold shifts to the combined exposure and that to noise alone within the frequency region of 12–40 kHz (within and higher than the noise band) were averaged to represent the CO potentiation. The mean CAP threshold shift potentiations of NIHL by CO at 1 week, 2 weeks and 4 weeks after the exposure are presented in Fig. 6B. The difference was very significant between the 1 week group and the 2 week group ($P < 0.01$) and between the 1 week group and the 4 week group ($P < 0.001$). However, between the 2 week group and the 4 week group the difference was not significant ($P > 0.05$) suggesting that asymptotic recovery levels were being reached at this time period.

3.5. Hearing loss potentiation and CO concentration

While potentiation of NIHL can be observed with exposures of 1200 ppm CO, it is essential to determine the lowest CO concentration that shows the noise-CO interaction.

In this experiment the CAP thresholds were measured 4 weeks after the combined exposure to 8 h band limited noise and different CO levels. The nominal CO level varied from 1500 ppm to 0 (noise alone). The potentiation of NIHL at each frequency and CO level was obtained by subtracting the threshold shift to noise alone from that to the combined exposure. In Fig. 7A the mean potentiation across frequencies higher than the noise band (24, 30, 35 and 40 kHz) is plotted as a function of CO level. At 300 ppm CO exposure, there was no NIHL potentiation observed. With an increase of CO level, potentiation also increased. The potentiation increased about 2 dB for each 100 ppm CO increment. Fig. 7B presents the potentiation across frequencies within the noise band (12, 16 and 20 kHz). Comparatively less potentiation was observed with the increase of CO concentration than that seen in Fig. 7A. Here each 100 ppm CO increase caused less than 1 dB potentiation increase. The CO level of 300 ppm caused a small potentiation of NIHL, though it was not statistically significant. Fig. 7C shows data across frequencies lower than the noise band (2, 4, 6 and 8 kHz). The potentiation was not seen until the CO level reached 1200 ppm. The data indicate that the lowest CO exposure level able to potentiate NIHL over an 8 h exposure lies between 300 ppm and 500 ppm.

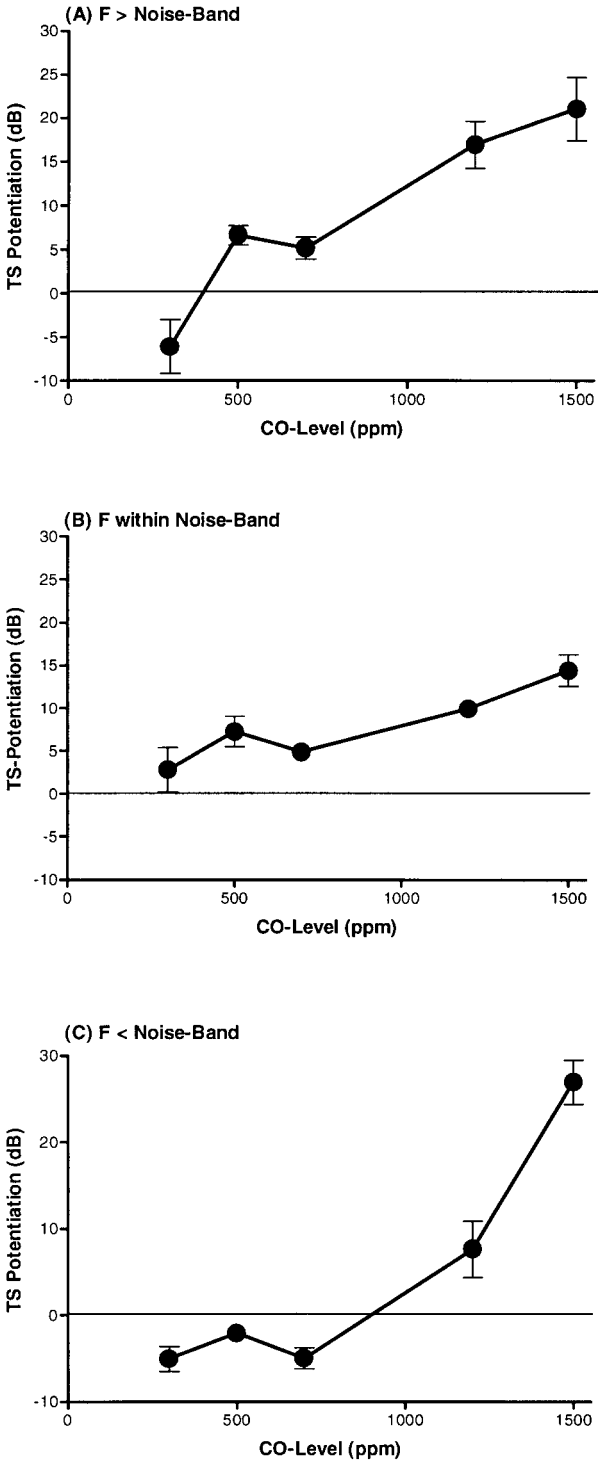

Fig. 7. NIHL potentiation as a function of the CO level in the exposure chamber. Noise: 9.6–19.2 kHz, 100 dB (Ln). CAP was recorded 4 weeks after the exposure. A: Data averaged across frequencies higher than the noise band (24, 30, 35 and 40 kHz). B: Data averaged across frequencies within the noise band (12, 16 and 20 kHz). C: Data averaged across frequencies lower than the noise band (2, 4, 6 and 8 kHz).

4. Discussion

In the present report the potentiation of noise induced auditory impairment by CO was studied. Noise induced elevation of CAP thresholds and the CM isoamplitude curve was potentiated significantly by simultaneous CO exposure. In other words, CO exposure may increase the vulnerability of the auditory system to noise damage. However, CO exposure alone did not cause any persistent change in the auditory responses. While previous studies using broadband noise suggested that potentiation of NIHL by CO was greatest for high frequency hearing, the current work demonstrates that the potentiation of NIHL can occur at any frequency, depending upon the nature of the noise band used. The potentiation is CO level dependent (above 500 ppm for 8 h) and each 100 ppm CO increase may cause about 1–2 dB extra threshold shift. The potentiations shown by CAP and CM are similar, indicating that the CO potentiation might originate from a functional change at the OHCs or some other impairment having common effects on both IHCs and OHCs. Such a finding is consistent with previous research showing a loss of OHCs with combined exposure to noise and CO (Fechter et al., 1988). The CO potentiation measured at a shorter recovery time was smaller than that observed at a longer post-exposure time, indicating that the CO potentiation results from a greater amount of permanent threshold shift.

The effect of CO exposure on NIHL has been studied previously (Fechter et al., 1988; Young et al., 1987). Fechter and colleagues (1988) reported that permanent threshold shifts and hair cell loss in rats, induced by broadband noise, were potentiated by the simultaneous CO exposure (1200 ppm). The potentiation was more prominent at the highest test frequency (40 kHz).

In the present study the CAP threshold shifts induced by octave-band noises were also found to be potentiated by CO at the same exposure level (1200 ppm). The potentiation may occur at high frequencies and may also occur at low frequencies, depending on the noise band. Potentiation was not specially related to the frequency dependent influence of CO on auditory functions (Fechter et al., 1987).

1200 ppm is a high level of CO that produces a concentration of HbCO in the blood of about 47%. Lower level CO exposure in the present study was also found to affect the noise induced CAP threshold shift, though the potentiation was smaller. The lowest CO concentration showing noise-CO interaction was 500 ppm. Burgess et al. (1977) measured CO concentrations found in a series of environments with automobile exhausts. They reported CO levels in excess of 500 ppm in roughly 30% of the samples they obtained. Treitwein et al. (1980) reported CO levels averaging 320 ppm and a range from 0 to nearly 5000 ppm in their studies of the work place of firefighters. The lungs of cigarette smokers may be exposed to 400–500 ppm CO at least during the period of smoking (US Environmental Protection Agency, 1991). Thus, while the exposure levels studied here were certainly very high compared to ambient air concentrations, they may be achieved in certain work environments and under special conditions.

With combined exposure to noise and CO, threshold shifts can also be observed even under noise conditions that are low and produce minimal effects by themselves. In some cases, noise alone did not induce a permanent threshold shift, but the combined exposure did. Possibly, the noise exposure alone only induced temporary auditory impairment. However, the combined exposure to noise and CO produced permanent damage.

Compared to the previous report (Fechter et al., 1988), the potentiation of NIHL by CO in the present study was relatively smaller. The maximal potentiation in the previous report was 55 dB (average 25 dB across test frequencies, Fechter et al., 1988). In the present study, the maximal potentiation by CO (1200 ppm) was only about 23 dB with exposure to 9.6–19.2 kHz octave-band noise (averaged 14 dB across the same test frequencies). Several possible explanations can be offered. First, the exposure period was different. In the previous study the noise exposure period was 2 h, compared to 8 h in the present study. Short-term noise exposure alone may cause temporary threshold shift. However, long-term noise exposure may cause permanent threshold shift. The CO influence is probably limited to cells not permanently damaged by noise. In fact, in the previous study the threshold shifts to the exposure of noise alone were very low (approximately 5 dB). Our preliminary data showed that a short-term exposure of the high frequency noise (2 h, 9.6–19.2 kHz, 100 dB (Ln)) alone only induced small threshold shifts. However, the short-term combined exposure to noise and CO induced as great threshold shifts as that to the long-term combined exposure presented in this report. As a result a greater potentiation was observed with short-term exposure than with long-term exposure. The influence of exposure time and exposure pattern on NIHL potentiation by CO will be reported separately. Secondly, the methods used to measure hearing loss were different. In the previous report, reflex modification audiometry was used, while CAP and CM recording were used in the present study. The CAP change reflects the auditory function change restricted to the auditory periphery while reflex modification also measures more central auditory system processes. It is possible that some of the effect of CO may be mediated more centrally than at the cochlea.

Similar to the CAP, CM amplitude was impaired following the exposure. CAP reflects the function

change of IHCs and OHCs and other cochlear functions including stria vascularis and cochlear fluid. The CM mainly reflects the function change of OHCs and other cochlear functions, but the influence of the damage in the IHCs is limited. The noise induced CM change was also potentiated by the simultaneous CO exposure. The potentiation shown by the CM was similar in size to that by the CAP. This suggests that the potentiation probably originated from damage to the outer hair cells or to other cochlear functions upon which the CM is dependent, such as changes in the stria vascularis or in the ion concentrations in perilymph or endolymph. This is consistent with the result of the previous report (Fechter et al., 1988). In the previous report the noise alone only caused limited hair cell loss restricted to the basal turn of cochlea. The combined exposure to noise and CO caused widespread hair cell loss especially outer hair cells.

The noise-CO interaction (potentiation) has been found to depend upon noise level (not noise frequency), CO level, and the recovery time after exposure. Low intensity noise did not induce hearing loss with or without the presence of CO. It seems that a certain noise level is needed for the noise-CO interaction. Probably noise intensity enough to induce temporary auditory impairment is required for the noise-CO interaction. CO exposure might make such a temporary change into permanent damage. High intensity noise and especially long-term exposure can cause permanent damage that may supersede the noise-CO interaction on normal auditory functions. The dead cell cannot show further damage as a result of combined exposure. Our preliminary histochemical data of cochlea hair cells showed the CAP and CM decrease being related to the decline of hair cell enzyme (dehydrogenase) activity. Combined exposure caused more decline of the enzyme activity than noise exposure alone. If CO mainly influences OHCs and causes the death of damaged OHCs, the NIHL potentiation is dependent upon the number of OHCs temporarily impaired by noise, not upon the unimpaired or dead OHCs caused by noise alone.

The potentiation increased with the CO concentration linearly when the level was higher than a threshold (500 ppm). Each 100 ppm CO increase may cause about 1–2 dB extra threshold shift. CO-exposure can reduce or block oxygen supply to tissues. Acute exposure to high levels of CO alone (HbCO in blood about 56%, Fechter et al., 1987) may cause a temporary threshold shift in the high frequency region (Baker and Lilly, 1977; Fechter et al., 1987; Kowalska, 1980, 1981). Recent studies further indicate that an excitotoxic mechanism is involved in this CO ototoxicity (CO inducing excessive glutamate release, Liu and Fechter, 1995). Free radical generation seems also to be involved in the CO ototoxicity as the acute effects are diminished or blocked by pretreatment with free radical scavengers (PBN) or with enzymatic inhibitors of free radical formation (allopurinol) (Fechter et al., 1997). However, in the present study the HbCO level in blood was relatively lower (about 47% for 1200 ppm CO inhalation). The CO exposure alone at 1200 ppm in the present study did not induce permanent CAP or CM change. Even when the CAP and CM were recorded immediately after the exposure, the CAP and CM changes seen with combined exposure of CO and noise were not observed with CO alone. Also the potentiation was not specially related to the high frequency region, in which the CO might potentially cause damage. Importantly, the potentiation may be observed at a much lower CO level (500 ppm). The noise-CO interaction might not be related to the special influence of the CO on the auditory cells. CO exposure causes reduction of oxygen supply to any kind of cells. When the CO treated cells are exposed to other toxic agents, like noise, the damage would be more severe than normal.

NIHL potentiation by CO emerged during the post-exposure time as NIHL showed recovery of temporary threshold shift. The potentiation increased with the post-exposure time non-linearly. From 1 week to 2 weeks post-exposure the potentiation increased more than from 2 weeks to 4 weeks post-exposure. The recovery of the noise induced damage slowing down might be the reason for the smaller increase of the potentiation. At 1 week post-exposure the potentiation was very small. Again, it indicates that the noise-CO interaction is not related to the special effect of the CO on auditory cells. The combined exposure produces sufficient damage to the outer hair cells that recovery of function is not possible. By contrast, subjects receiving noise alone are capable of recovery from a temporary threshold shift.

Acknowledgements

This work was supported by NIOSH Grant OH03481 to L.D. Fechter. Portions of the results have been reported in abstract form (Chen and Fechter, 1999; Chen et al., 1999). The authors thank Ms. M. McWilliams and Ms. D.B. Rao for assistance with the measurement of HbCO level in blood and Ms. P.A. Meder for editing of the manuscript.

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