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Full Length Article

Carbon disulfide potentiates the effects of impulse noise on the organ of Corti

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ABSTRACT

Occupational noise can damage workers' hearing, and the phenomenon is even more dangerous when noise is associated with an ototoxic solvent. Aromatic solvents are known to provoke chemical-induced hearing loss, but little is known about the effects on hearing of carbon disulfide (CS_2) when combined with noise. Co-exposure to CS_2 and noise may have a harmful effect on hearing, but the mechanisms involved are not well understood. For instance, CS_2 is not thought to have a cochleotoxic effect, but rather it is thought to cause retrocochlear hearing impairment. In other words, CS_2 could have a distal neuropathic effect on the auditory pathway. However, a possible pharmacological effect of CS_2 on the central nervous system (CNS) has never been mentioned in the literature. The aim of this study was to assess, in rats, the effects of a noise (continuous vs. impulse), associated with a low concentration of CS_2 [(short-term threshold limit value) x 10 as a safety factor] on the peripheral auditory receptor. The noise, whatever its nature, was an octave band noise centered at 8 kHz, and the 250-ppm CS_2 exposure lasted 15 min per hour, 6 h per day, for 5 consecutive days. The impact of the different experimental conditions on hearing loss was assessed using distortion product oto-acoustic emissions and histological analyses. Although the LEX,8 h (8-h time-weighted average exposure) for the impulse noise was lower (84 dB SPL) than that for the continuous noise (89 dB SPL), it appeared more damaging to the organ of Corti, in particular to the outer hair cells. CS_2 exposure alone did not have any effect on the organ of Corti, but co-exposure to continuous noise with CS_2 was less damaging than exposure to continuous noise alone. In contrast, the cochleo-traumatic effects of impulse noise were significantly enhanced by co-exposure to CS_2.

Therefore, CS_2 can clearly modulate the middle-ear reflex function. In fact, CS_2 may have two distinct effects: firstly, it has a pharmacological effect on the CNS, modifying the trigger of the acoustic reflex; and secondly, it can make the organ of Corti more susceptible to impulse noise. The pharmacological effects on the CNS and the effects of CS_2 on the organ of Corti are discussed to try to explain the overall effect of the solvent on hearing. Once again, the results reported in this article show that the temporal structure (continuous vs. impulse) of noise should be taken into consideration as a key parameter when establishing hearing conservation regulations.

1. Introduction

Despite preventive regulations, noise-induced hearing loss (NIHL) remains a major occupational health hazard. Noise exposure in workplaces can be either due to continuous noise or to impulse noises. Impulse noises are characterized by high intensities over a short duration, and the stereociliae at the top of the hair cells are known to be particularly vulnerable to acoustic

Abbreviations: CNS, central nervous system; dB (A), decibel weighted A; dB SPL, decibel sound pressure level; DPOAEs, distortion product oto-acoustic emissions; FFT, fast fourier transform; LEX,8 h, equivalent continuous noise level calculated over 8 h; MER, middle-ear reflex; NIHL, noise-induced hearing loss; IHC, inner hair cells; IS, internal standard; OHC, outer hair cells; SD, standard deviation; SDH, succinate dehydrogenase; SEM, scanning electron microscopy; STEL, short term exposure limit; TWA, time-weighted averages.

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injury from this type of noise (Hamernik et al., 1989). Impulse noise can provoke functional disruption of intercellular junctions in the sensory epithelium (Zheng and Hu, 2012). As far as the continuous noises are concerned, recent research has showed a possible disruption of the synaptic communication (Liberman and Kujawa, 2017; Kobel et al., 2017; Moser et al., 2013). However, the authorities do not distinguish between noise types when formulating recommendations to protect workers against NIHL. Both European and American frameworks and guidance documents for hearing conservation in workers require noise exposure to remain within certain limits: $L_{EX,8h}$ and peak values (Directive 2003/10/EC; http://www.worksafebc.com). The strategy used to assess noise-related danger relies on the equal energy principle over an 8-h workday ($L_{EX,8h}$), which presumes that hearing damage is mainly a function of the total acoustic energy received, not the pattern in which it arrives. Based on this assumption, the theory of conservation of acoustic energy (the 3-dB exchange rate in the European Union and the 5-dB exchange rate in the USA) should result in a constant hearing hazard whatever the type of noise. The maximum permissible noise exposure levels over an 8-h work shift are $L_{EX,8h}$ = 87 dB(A) in the European Union, and 90 dB(A) in the USA, and occupational exposure to noise must therefore be maintained below these limits. For impulse noise, peak value limits is 140 dB(C) in the European Union.

While noise remains the predominant occupational hazard to hearing, there is growing evidence that a number of chemicals used in industry, such as organic solvents, may also affect hearing, or exacerbate the effects of occupational noise (Chen and Henderson, 2009; Campo et al., 2013). The toxicological effects of co-exposure to noise and chemicals are complex, but highly relevant when it comes to assessing worker risk. Co-exposures to noise and carbon disulfide (CS_2) are present in various industry sectors, but the most important use remains in the manufacturing of rayon and cellophane (Hodgkinson and Prasher, 2006). It can be use also as a solvent in chemical industry. Surprisingly, relatively little has been published on research into how CS_2 affects hearing in workers (Morata, 1989; Chang et al., 2003) or in animals (Clerici and Fechter, 1991). As CS_2 has neurotoxic effects, the threshold limit values – time-weighted averages (TWA) defined by Occupational Safety and Health Administration (OSHA) and the European agency for safety and health at work – are low: 20 and 5 ppm, respectively. However, the short-term exposure limits (STEL) are higher, 30 ppm is the ceiling value for 30 min exposure in the USA, whereas exposure is limited to a maximum of 25 ppm for 15 min in Europe. Based on these limits, the carbon disulfide concentration tested in the study presented in this paper (250 ppm) was chosen as it is 10 times (safety factor) higher than the European STEL value.

One concern of this investigation was to evaluate the relevance of the STEL values recommended in Europe and in the USA, taking a moderate safety factor into account. We also addressed the relevance of the TWA value in case of combined exposure to noise in factories. We continue to believe that it is important to take the type of noise into consideration when assessing risks to human hearing and that co-exposure to solvents should also be considered in this occupational context.

Several studies in rats have shown that solvents can have a pharmacological effect on olivocochlear nuclei. These effects explain, at least partially, the synergistic effects on hearing of combined exposure to noise and solvent (Campo et al., 2007; Wathier et al., 2016; Venet et al., 2011, 2015). For the study described in this paper, carried out in rats, two types of noise with similar spectra but different temporal structures (continuous *vs.* impulse) were tested to compare their impact on hearing when associated with CS_2. Impulse noise does not always trigger the middle-ear reflex (MER), and even when it does, the delay before its activation allows acoustic energy to penetrate into the cochlea. In contrast, the acoustic energy of continuous noise is significantly decreased by the protective effect of the MER (Venet et al., 2015). Therefore, the main goal of the current study was to test whether exposure to either type of noise in combination with CS_2 had different effects due to perturbation of the MER in a rat's model.

In summary, the goals of this study were first, to assess the relevance of the TWA values for CS_2 recommended in Europe and the USA, and second, to test whether CS_2 can modify the neuropharmacology of the MER and further weaken the resistance of the organ of Corti to noises.

2. Materials and methods

2.1. Animals

Adult female Long Evans (n = 78) rats weighing approximately 250 g were used in experiments. Animals were purchased from Janvier breeders (Le Genest St Isle, St Berthevin, 53941, France). Two rats were housed in each cage (1032 $cm^2 \times 20$ cm high) with irradiated cellulose BCell8 bedding (ANIBED, Pontvallain, France). All animals were 16-weeks-old before starting experiments. Food and tap water were available *ad libitum*, except during exposure periods. Animals were maintained on a 12 h/12 h day/night cycle during experiments. Room temperature and relative humidity in the animal facility were 22 ± 2 °C and $55 \pm 10\%$, respectively. The background noise level in the animal facilities was around 42 dB.

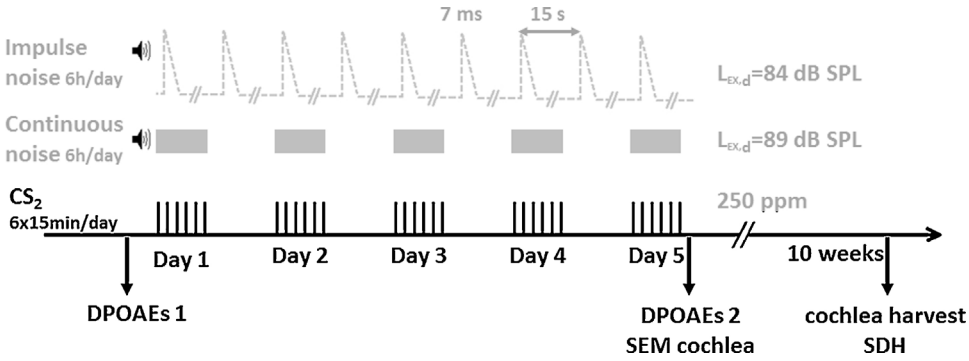

Fig. 1. Experimental protocol. The animals were exposed during 5 consecutive days for 6 h/day. Carbon disulfide (CS_2) exposure: each bar represents a period of 15 min exposure at 250 ppm. Continuous noise exposure: each gray rectangle represents a period of 6 h continuous noise with a $L_{EX,d}$ of 89 dB SPL. Impulse noise exposure: dotted line represents the temporal structure of the impulse noise emission. Each 7 ms pulse was separated by 15 s and this sequence was repeated during 6 h. Hearing loss was measured using the cubic distortion product oto-acoustic emissions (DPOAEs) before (DPOAEs1) and after the 5 days of exposure (DPOAEs2). Scanning electron micrographs (SEM): rats were sacrificed at the end of the exposures. Succinate dehydrogenase (SDH): animals were sacrificed 10 weeks after the end of the exposures.