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Review

When Is Temporary Threshold Shift Injurious to Marine Mammals?

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Abstract: Evidence for synaptopathy, the acute loss of afferent auditory nerve terminals, and degeneration of spiral ganglion cells associated with temporary threshold shift (TTS) in traditional laboratory animal models (e.g., mice, guinea pigs) has brought into question whether TTS should be considered a non-injurious form of noise impact in marine mammals. Laboratory animal studies also demonstrate that both neuropathic and non-neuropathic forms of TTS exist, with synaptopathy and neural degeneration beginning over a narrow range of noise exposures differing by ~6–9 dB, all of which result in significant TTS. Most TTS studies in marine mammals characterize TTS within minutes of noise exposure cessation, and TTS generally does not achieve the levels measured in neuropathic laboratory animals, which have had initial TTS measurements made 6–24 h post-exposure. Given the recovery of the ear following the cessation of noise exposure, it seems unlikely that the magnitude of nearly all shifts studied in marine mammals to date would be sufficient to induce neuropathy. Although no empirical evidence in marine mammals exists to support this proposition, the regulatory application of impact thresholds based on the onset of TTS (6 dB) is certain to capture the onset of recoverable fatigue without tissue destruction.

Keywords: permanent threshold shift; synaptopathy; neuropathy; auditory brainstem response; behavioral thresholds

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1. Introduction

The last two decades have seen an explosion in the scientific literature related to the impact of anthropogenic noise on marine mammals. Arguably, the topic also dominates the distribution of research dollars related to marine mammal science. Research investment has sought to provide insight on the types of responses that marine mammals exhibit when exposed to anthropogenic noise, and more importantly, the short- and long-term consequences of such exposures. Relationships between animal responses and signal frequency, level, duration, and duty cycle, as well as the importance of novel to repeated exposures, have been the focus of many studies, the results of which have informed regulatory practices of countries actively engaged in the marine mammals and noise issue.

The avoidance of injury to marine mammals incidentally exposed to anthropogenic noise is a common goal of regulators. However, there is a lack of legal and regulatory consensus among countries with environmental management frameworks as to what defines an 'injury'. This contributes to differences in the noise exposure thresholds at which impacts are regulated, an issue that has particular relevance when considering direct, physiological impacts to the auditory system. For example, under the authority of the Marine Mammal Protection Act (MMPA), the National Marine Fisheries Service (NMFS) regulates noise producers in US territorial waters that have the potential to impact marine mammals. As part of its regulatory framework, NMFS adopted a definition of injury that involved the destruction of tissue [1,2]. This definition formed the basis for a legal distinction under the MMPA between two forms of noise-induced hearing loss (NIHL)–a temporary elevation of hearing threshold (temporary threshold shift, or TTS), which was believed to be a fully recoverable form of auditory fatigue, and permanent threshold shift

(PTS), which was a permanent loss of hearing sensitivity believed to arise from damage to the auditory system tissues (e.g., disarticulation of the middle ear bones, loss of hair cells). The occurrence of TTS was not considered injury under this regulatory interpretation, whereas PTS was. In contrast, all forms of hearing impairment caused by exposure to anthropogenic noise are considered injury under German regulation: "An injury within the meaning of the prohibition on taking under species protection law is an impairment of an animal's physical welfare or damage to its health. This encompasses any impairment of its physical integrity [3]." Thus, a TTS is considered an injury under German law once the threshold for TTS has been exceeded.

There has been a greater focus in recent years on whether TTS is truly non-injurious under an injury definition that incorporates the destruction of tissue [4]. Indeed, since TTS magnitude grows with the degree of noise exposure and eventually becomes PTS, there is obviously some threshold of noise exposure beyond which tissue damage occurs. What was historically less apparent was whether the onset of PTS and the onset of tissue damage were due to equivalent exposures. In 2009, Kujawa and Liberman [5] provided evidence that fully-recoverable threshold shifts in mice could be associated with the permanent loss of tissues within the auditory system. The question that followed for the marine mammal community was a natural extension of the findings–if TTS can be associated with the destruction of tissue, then at what magnitude of TTS can injury be present in marine mammals?

2. TTS and the Loss of Auditory System Tissues

There are relatively few studies demonstrating that TTS can be associated with the destruction of tissue. To date, relevant studies have only been performed in terrestrial laboratory animals. Kujawa and Liberman [5] exposed mice (*Mus musculus*) to octave-band noise (8–16 kHz) for two hours at a sound pressure level (SPL) of 100 dB re 20 µPa. Utilizing measurements of the auditory brainstem response (ABR), they measured a ~40 dB TTS in the mice 24 h after the noise exposure. (Note that a 40 dB shift reflects a several order of magnitude reduction in hearing sensitivity relative to the 6 dB of shift used to define the onset of TTS in some marine mammal regulations.) Kujawa and Liberman demonstrated that the mice suffered an acute loss of afferent nerve terminals, termed synaptopathy, while cochlear sensory (hair) cells remained intact. Degeneration of the cochlear nerve (loss of spiral ganglion cells) was also observed, although it occurred slowly over the course of one to two years. The magnitude and cochlear placement of the syanptopathy and nerve degeneration were spatially related to the corresponding frequency at which the greatest threshold shift was observed. Both phenomena were noted concomitant with hearing thresholds that returned to baseline days to weeks after the exposure, suggesting that conventional threshold testing alone was insufficient to determine pathologies associated with noise over-exposure.

Subsequent work has reinforced these findings. Lin and colleagues [6] performed a similar experiment to that of Kujawa and Liberman [5] but utilized guinea pigs (*Cavia porcellus*) in order to address concerns that neurons of the mouse ear might be particularly susceptible to noise over-exposure. Subjects were exposed to octave-band noise (4–8 kHz) for two hours at SPLs of 106 and 109 dB re 20 µPa. Again, utilizing ABR measurements, the magnitude of TTS 24 h after the noise exposure was found to be ~50 dB, but returned to normal by ten days after the exposure. Significant synaptopathy was noted at this time, although there was no loss of either inner or outer hair cells (IHCs and OHCs, respectively). Long-term monitoring subsequently showed the slow loss of spiral ganglion cells over the course of a two-year period, with losses closely associated with the sites of synaptopathy.

Wang and Ren [7] performed a noise exposure experiment in mice similar to that of Kujawa and Liberman [5] but utilized a repeated exposure paradigm in which a subset of mice were exposed to fatiguing stimuli, either two or three times following recovery of the initial ABR threshold shift. Mice were exposed to octave band noise centered at 12 kHz for two hours at ~100 dB re 20 µPa, and as in previous studies, ABR thresholds

were measured prior to and 24 h after the exposure. For mice receiving a single noise exposure, threshold shifts were initially found to be about the same as observed in previous experiments (30–40 dB), recovering to normal in approximately the same time. Similarly, the occurrence of synaptopathy without the loss of IHCs or OHCs was observed, although the loss of spiral ganglion cells was not investigated.

Collectively, these studies provide a small but sufficient amount of evidence to suggest that a fully recoverable TTS can occur despite permanent auditory system tissue damage. Further, progressive loss of auditory system tissues, specifically spiral ganglion cells, can occur long after recovery of hearing thresholds. How this applies to marine mammals warrants discussion, and the relevance of laboratory animal work conducted to date requires consideration in the context of marine mammal TTS studies.

3. Relevance of Laboratory Animal TTS Findings to Marine Mammals

To reconcile the findings of the TTS literature demonstrating tissue damage with the TTS work performed in marine mammals, there must first be an understanding of the magnitude of threshold shifts achieved in traditional laboratory animal models and the time courses at which shifts were measured. Threshold shifts for which tissue damage has been associated in laboratory animals range from ~30 to 50 dB of TTS [5–7]. Threshold shifts in these studies were measured 24 h post-exposure, and the measurements were made using ABR threshold procedures. The majority of marine mammal TTS studies have behaviorally measured smaller amounts of TTS (<20 dB) within minutes of noise exposure (for review, see [8]; for studies after 2015, see [9–14]), though initial threshold shifts measured behaviorally have occasionally been greater than 40 dB [15]. A smaller number of studies have measured ABR threshold shifts and found initial shifts as high as 63 dB when measured within a couple of minutes of the cessation of noise exposure [16]. Behavioral and ABR measurements are not equivalent, however, and reconciling threshold shifts measured with the two approaches requires an understanding of the differences between them [8].

Behavioral measurements of hearing sensitivity require an animal to act in response to hearing a sound (e.g., paddle push, produce a whistle), thus providing an integrated response that includes the animal's perception of the sound and its decision to respond. ABR measurements of hearing sensitivity do not reflect this integrated animal response but measure only voltages generated by portions of the ascending auditory system. Temporary threshold shifts determined from ABR measurements generally demonstrate an earlier onset of TTS, generally characterized as 6 dB of threshold shift, larger shifts than those observed with behavioral methods and longer recovery times than observed with behavioral methods. This suggests that some mechanism accommodates the restoration of the hearing threshold even though the auditory system has not fully recovered from the fatiguing noise exposure [8,17]. Finneran et al. [17] showed that TTS measured with ABRs could be 19–33 dB greater than those measured behaviorally and that ABR threshold shifts of ~10 dB could be found in the absence of a behavioral shift. The time courses of recovery measured with ABRs were always longer than those measured behaviorally. In a subsequent study of TTS induced by exposure to air gun impulses, no behavioral threshold shifts were observed, whereas a small amount of TTS was detected by measuring ABR thresholds [14]–in one dolphin, a 9-dB TTS was measured at a test frequency of 8 kHz. Thus, caution should be exercised in making comparisons between studies that used behavioral or ABR threshold measurement methods, and the synthesis of findings across studies should account for these differences.

If the difference between the magnitude of ABR and behavioral threshold shifts measured following noise exposure is consistent across mammals in general, then the modest behaviorally measured threshold shifts from marine mammal studies could appear similar to the ABR threshold shifts observed in laboratory animals that have been associated with tissue damage (e.g., a 20 dB behavioral TTS could potentially correspond to a 50 dB TTS measured with ABRs). However, the initial TTS measurements in marine mammal

studies are typically made within minutes of noise exposure, not 24 h after the exposure (as described for traditional laboratory animal models). Recovery from TTS induced by narrowband or tonal noise can crudely be described as a function of the logarithm of time with recovery rates increasing in variability as recovery time increases. In marine mammals, measured recovery rates range from ~4 to 23 dB/decade of time [12,17–27] and generally demonstrate a positive relationship with the magnitude of the initial threshold shift. Thus, TTS measured within minutes of the noise exposure would be much higher than that measured 24-h after the exposure. Comparisons between laboratory animal studies with 24-h post-exposure TTS measures and marine mammal studies made within minutes of exposure cessation must keep this difference in mind, particularly since marine mammal TTS studies often recover to baseline thresholds within 24 h of noise exposure, even when TTS measured behaviorally and immediately following the noise exposure was as high as ~30 dB. It is important to note that in the one marine mammal study in which PTS was observed, the behavioral threshold shift was ~30 dB 24 h after the exposure, which could equate to an ABR threshold shift as high as 60 dB [24].

Little information exists on the relationship between the growth of TTS and quantifiable tissue damage in terrestrial mammals, and none exists in marine mammals. However, some limited work in mice demonstrates that there exist both neuropathic and non-neuropathic levels of TTS. Mice exposed to octave-band (8–16 kHz) noise exposures ranging from 91 to 100 dB re 20 µPa for periods of two hours demonstrated significant synaptopathy at exposures >97 dB re 20 µPa, but not at exposures <94 dB re 20 µPa [28–30]. The degree of synaptopathy appeared progressive and frequency-dependent, i.e., the degree of synaptopathy varied as a function of the cochlear frequency-place map, as previously observed. The magnitude of TTS measured after noise exposure ranged from up to 55 dB measured 6 h after exposure to ~35–40 dB measured 24 h after exposure in non-neuropathic mice, showing substantial TTS could occur without the presence of synaptopapthy. However, the observance of synaptopathy onset at noise exposures that differed by as little to 6–9 dB from those that were non-neuropathic suggested a narrow range over which the onset and growth of synaptopathy occurs. Thus, the limited evidence that is available suggests that relatively large TTS (>30 dB, 24-h post-exposure) can occur without tissue damage, but that damage begins to occur along some noise exposure continuum as noise exposures (and TTS) increase.

4. Discussion

A limited amount of evidence from terrestrial laboratory animals suggests that both neuropathic and non-neuropathic TTS are feasible, with the onset of neuropathology occurring at noise exposures well exceeding those corresponding to the onset of TTS. Given this evidence, it is probable that threshold shifts in marine mammals can occur with noise exposures that also range in magnitude and effect from fully recoverable TTS without tissue damage, through fully recoverable TTS with tissue damage, to the destruction of tissue producing PTS. In other words, TTS is a graded phenomenon that is fully recoverable at low levels but can lead to tissue damage as it becomes more extreme–not all TTS results in the destruction of tissue. The threshold of exposure at which neuropathy would occur is unknown and likely varies between marine mammal species, as does the noise exposure required for the onset of TTS [8]. Based on laboratory animal studies, the onset of neuropathic TTS would appear to occur at only more extreme threshold shifts, exceeding the magnitude of TTS commonly induced in the marine mammal studies conducted thus far. Nevertheless, if a legal definition of injury includes the destruction of tissue, then synaptopathy qualifies as injury and must be considered in the framework of potential acoustic impacts to marine mammals.

Countries actively regulating the potential impact of ocean noise to marine mammals often employ thresholds for the onset of injury that would be conservative relative to the findings related to neuropathic TTS, regardless of whether following a broad definition of injury that encompasses impacts to behavior or one that more narrowly relies on a

definition involving the destruction of tissue. In the least conservative case, such as is employed by US regulators [31], the use of an initial (i.e., measured minutes after exposure) 40 dB of TTS as the onset of injury falls below the magnitude and time scale of TTS associated with neuropathic TTS (i.e., 30–50 dB of TTS measured 24 h after noise exposure) observed in conventional laboratory animal models. Therefore, even though it has been demonstrated that a fully-recoverable TTS of sufficient magnitude can result in underlying tissue damage [5], the implementation of regulatory thresholds based on TTS onset should encompass recoverable auditory fatigue without the occurrence of tissue damage [32].

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