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Low welfare impact of noise: assessment in an experimental model of mice infected by *Herpes simplex*-1

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ABSTRACT

The breeding practices adopted and the equipment typically found in animal facilities produce sounds at frequencies within the auditory range of the mice (1 to 100 kHz), which can cause hearing and other non-hearing effects. Another aspect that could potentially affects the welfare of experimental animals would be their impaired health condition, since in addition to all the variables present in the environment, some of them are deliberately infected with pathogens. This study aimed to evaluate the possible effects of the chronic exposure of C57BL/6 and Tlr2/Tlr9$^{(-/-)}$ mice, uninfected and infected, with a low m.o.i. of *Herpes simplex*-1, at different noise levels present in the "Quiet" and "Noisy" rooms. Considering all procedures, a total of 51 male mice were used, 27 of the C57BL/6 and 24 of the Tlr2/Tlr9$^{(-/-)}$ strains. Physiological parameters such as weight gain, hemogram, cholesterol, glucose,corticosterone and cytokines involved in the immune response were evaluated together with the animals' behavioral responses in Open Field and Light/Dark tests. In relation to the physiological parameters in C57BL/6, there was infection x noise interaction ($P < 0.05$) with greater weight gain by the infected "Quiet" group ($P < 0.05$) when compared to the infected "Noisy" group. In the hemogram of C57BL/6 there was infection x noise interaction ($P < 0.01$) in the platelets, with increase by the infected "Quiet" group when compared to the control "Quiet" and infected "Noisy" group ($P < 0.05$). No significant differences were found between the groups in the cholesterol, glucose and corticosterone concentrations. Regarding the production of cytokines in C57BL/6 there was infection x noise interaction ($P < 0.05$), with a lower production of gamma interferon in control "Quiet" group when compared to the infected "Quiet" group and to the control "Noisy" group (all $P < 0.05$). In behavioral tests there was no difference between the groups. Our results demonstrated that although the noise and infection influence have caused changes in some phy-

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siological and immunological parameters, they were not sufficient to promote measurable change in the stress parameters evaluated, since that corticosterone concentration and the responses of the animals from different groups in the behavioral tests were not different. This study demonstrated that the noise level found in the animal facility caused a low impact on the welfare of experimental mice.

1. Introduction

Anthropogenic noise is currently recognized as a critical pollution problem (Popper and Hasting, 2009; Kight and Swaddle, 2011; Duarte et al., 2015). This kind of pollution, which usually increases with human occupation, presents many types of impacts on animals, from the masking of their communication (Duarte et al., 2018) to adverse effects on their welfare (Kight and Swaddle, 2011).

In an animal facility, animals are exposed to several environmental stressors in their micro and macro environments, such as odors, illumination, humidity, temperature and noise (Yamaguchi, 1995; Turner et al., 2005). Noise is rarely controlled as an environmental variable and is often monitored only in areas where some kind of damage to human hearing may occur (Lauer et al., 2009; Duarte et al., 2012). The sound pressure levels vary between the light and the dark periods and between weekdays and weekends and sounds with highs levels are produced during the work routine with the animals (Milligan et al., 1993; Voipio et al., 2006). Several routine procedures performed in animal facilities produce sounds with varying frequencies and sound pressure levels, yet within the auditory range of mice, 1–100 kHz (Turner et al., 2005).

With regard to rodents, the Guide for the Care and Use of Laboratory Animals (National Research Council, 2010) consider that exposure to noise above 85 dB can lead to the development of hearing and non-hearing effects. These changes are determined by characteristics inherent to the species and strain, by noise level intensity, duration, predictability, by the history of the animal and the context of exposure to noise. However, chronic exposure to moderate sound pressure levels (< 85 dB) (Tamura et al., 2012) can alter the way that sound is processed by modifying the perception of biologically relevant sounds and can trigger physiological, immunological and behavioral changes, thereby reducing animal welfare and, consequently, resistance to pathogens (Vazzana et al., 2017) and altering experimental results (Povroznik et al., 2017).

Furthermore, the early exposure to certain levels of noise can alter the structural and functional development of the central nervous system, leading to greater susceptibility to environmental changes (Cheng et al., 2011). In experimental animals, another aspect that potentially affects the welfare of experimental animals is their impaired health condition, since in addition to all the variables present in the environment, some of them are deliberately infected with pathogens of interest. In this context, the welfare of a sick animal may be compromised, however, the magnitude of disease effects on welfare still needs to be studied, since little is known about the degree of suffering associated with many diseases (Broom and Molento, 2004).

Herpes simplex-1 (HSV-1) is an infectious agent with a worldwide distribution, high prevalence among the human population and has been the focus of many studies in murine models. Toll-like receptors (TLRs) are responsible for recognizing molecules associated with pathogens, and Tlr2 and Tlr9 together are responsible for the defense of vertebrates against HSV-1 (Lima et al., 2010; Zolini et al., 2014; Lucinda et al., 2017). C57BL/6 mice produce cytokines against HSV-1 and are resistant to this virus, surviving the infection. However, Tlr2/Tlr9 knockout mice produce a smaller amount of proinflammatory cytokines and are susceptible to the infection with this virus in a dose dependent manner.

The purpose of this study was to evaluate whether the chronic exposure of C57BL/6 and Tlr2/Tlr9$^{(-/-)}$ mice, uninfected (control) and infected by HSV-1, to different noise levels in experimental animal facility, promotes impacts on their physiological, immune and behavioral responses. Here, we tested the hypothesis that noise pollution in an experimental animal facility promotes different effects on the physiology, immune response and behavior of both infected and uninfected mice. Our predictions were that different noise in the experimental animal facility could alter the evaluated parameters in immune competent mice (C57BL/6) and/or immune deficient mice (Tlr2/Tlr9$^{(-/-)}$), because the noise could be an additional stressor component of the experiment.

2. Material and methods

2.1. Ethical considerations

This project was approved by the Ethical Committee of Animal Handling (CEUA) from FIOCRUZ, Brazil (License number: LW-20/15) and carried out in accordance with the recommendations of the Brazilian regulations for the scientific use of laboratory animals (Lei Arouca 11.794/2008), as well as the CONCEA/Brazil.

2.2. Survey of the noise levels in the animal facility

Initially, a survey of the noise levels usually found in four different animal rooms (anthropophony and technophony) was carried in the Instituto René Rachou - Experimental Animal Facility, with the purpose of selecting the rooms which presents the lowest and highest noise level. The internal composition of each room is variable containing: only open iron shelves and/or individually ventilated cage (IVC) racks and/or ventilated cabinet and/or mice change station. The number of researchers and technicians varies in each room. The frequency range selected for the noise levels measurements was from 1 kHz to 100 kHz, since this is the auditory spectrum of mice. However, data on frequency from 20 Hz to 20 kHz in the L_{Aeq} (human - A) and L_{Zeq} (linear - Z) were also evaluated for comparative purposes, since in many studies the results are expressed in the A or Z curves. Noise was measured daily at different times throughout the day for four weeks, during and outside the work day. The morning period included the time from 6:00 to 12:00 h, the afternoon from 12:00 to 18:00 h and the night from 18:00 to 6:00 h of the following day. During day work, two types of records were made according to the traffic of people: (1) only with the presence of the technicians and (2) with the presence of researchers and technicians. Each measurement had a duration of 10 min, with one register of sound level per second. The sound pressure meters were placed at six different points in each room and the mean of these points was used for the calculation of the noise levels per room and the definition of the rooms with the lowest ("Quiet") and the highest noise levels ("Noisy").

2.3. Recording of noise levels and configuration of sound level meters

The noise level measurements were done using two sound level meters: B&K2270 (type 2270-S, Bruel & Kjaer, Denmark) and AR125BAT (AR125BAT, Binary Technology Acoustic, USA). The B&K2270 sound level meter is capable of measuring the equivalent continuous level during the time interval determined by the operator in frequencies from 20 Hz to 20 kHz on curves A, C and Z that can exceed 100 dB. It is able to record up to twelve different descriptors measured simultaneously, making possible the post-treatment of data of measurements and analysis of noise levels over time. This instrument has serial analyzers in frequency bands and it is equipped with a 1/2″