ARTICLE IN PRESS

Applied Animal Behaviour Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

The effects of mining machinery noise of different frequencies on the behaviour, faecal corticosterone and tissue morphology of wild mice (*Mus musculus*)

Karen F. Mancera[a,*], Allan Lisle[b], Rachel Allavena[c], Clive J.C. Phillips[a]

[a] Centre for Animal Welfare and Ethics, School of Veterinary Science, University of Queensland, 4343, Gatton, QLD, Australia
[b] School of Agriculture & Food Sciences, Faculty of Science, University of Queensland, P.C. 4343, Gatton, QLD, Australia
[c] School of Veterinary Science, University of Queensland, P.C. 4343, Gatton, QLD, Australia

ARTICLE INFO

Keywords:
Anthropogenic noise
Circling
Low frequency
High frequency
Mining
Stress

ABSTRACT

Mining noise has a wide variety of frequency spectra and is a potential source of stress for wildlife. We evaluated the effects of mining machinery noise on behaviour and associated physiological parameters at two isoenergetic frequency ranges: high (> 2 kHz) and low (≤ 2 kHz), the latter being less audible to mice, our model species. Effects of these two frequency spectra on behaviour, organ morphology and faecal corticosterone of wild mice were compared with a control treatment with no extra auditory stimuli. The mice exposed to high frequency noise spent less time in their nest than those exposed to low frequency noise or those in the control treatment, and they spent more time circling, especially anticlockwise, which in conjunction with elevated faecal corticosterone levels may reflect a greater right brain hemisphere stress-related response, particularly in females. Low frequency mining noise reduced grooming and circling, suggesting decreased physiological arousal due to mild stress. Low frequencies were also associated with increased faecal corticosterone in males compared to controls, which may be related to gender-based differences of the ear canal that affect frequency sensitivity. In conclusion, high frequency and low frequency mining machinery noise produced stress-related responses that may be important for the animals' welfare and survival.

1. Introduction

Anthropogenic noise is a source of stress for wildlife (Blickley and Patricelli, 2010; Wright et al., 2007). It can disturb acoustic communication, reproduction, community dynamics and behaviour (Blickley and Patricelli, 2010; Rabin et al., 2003; Wright et al., 2007). Chronic exposure to anthropogenic noise can decrease fitness by the repeated activation of the stress response (Romero and Butler, 2007). Noise exposure experiments on captive animals and humans have demonstrated negative effects on immunosuppression and reproductive function; these effects have been suggested as a possible outcome for animals living in the wild (Kight and Swaddle, 2011). Noise exposure also modifies captive animals' emotional state, generating anxiety and depression in rats (Naqvi et al., 2012) and increases in urinary corticoids, locomotion, distress vocalizations and escape attempts in pandas (Owen et al., 2004).

Stereotypic behaviour (i.e. repetitive behaviours induced by frustration, repeated attempts to cope, and/or central nervous system dysfunction, Mason and Rushen, 2008) has been related to noise exposure in primates (Patterson-Kane and Farnworth, 2006), rodents (Anthony et al., 1959) and pandas (Powell et al., 2006). Anthropogenic noise, especially from transportation, has been most studied in relation to its effects on birds and amphibians (Barber et al., 2010; Shannon et al., 2015). It typically has most of its energy output below 2 kHz (Barber et al., 2011; Roberts and Roberts 2009; Slabbekoorn and Peet, 2003). Other acoustic inputs with noxious potential, such as mining noise, have rarely been considered. However, open-cast mining machinery noise has been recognized as potentially dangerous for bats (Armstrong, 2010) and affecting birds' community dynamics (Read, 2000) in similar ways to related industries (rock crushing) (Saha and Padhy, 2011). Open-cast mining and rock crushing machinery produce predominantly low frequency sound waves (Barber et al., 2011; Roberts and Roberts 2009; Slabbekoorn and Peet, 2003). Most commonly used equipment also produces low frequency sound, e.g. dumper trucks and cooling fans from bulldozers whose output is 0.25–0.5 kHz and 0.3–3.5 kHz, respectively (Vardhan et al., 2004; Vardhan et al., 2005). However, rock cutting drills produce dominant frequencies between 2 and 4 kHz (Pal et al., 2006), resulting in a broad spectrum of

* Corresponding author.
E-mail address: dra.kelokumpu@gmail.com (K.F. Mancera).

http://dx.doi.org/10.1016/j.applanim.2017.08.008
Received 29 August 2016; Received in revised form 9 August 2017; Accepted 21 August 2017
0168-1591/

Please cite this article as: Mancera, K.F., Applied Animal Behaviour Science (2017), http://dx.doi.org/10.1016/j.applanim.2017.08.008

frequencies at the workface of coal mining sites (0.32 kHz to 8 kHz), but most in the mid to high range (Peng et al., 2010).

On site, mining noise can exceed amplitudes of 90 dB (A) and even reach 110 dB (A) (Ahmad et al., 2014; Utley, 1980). In surrounding areas the amplitude of noise from mining and related industries can exceed 80 dB (A), with small reductions in sound intensity as a result of limited acoustic input attenuating obstructions near the mining site (Mohapatra and Goswami, 2012; Saha and Padhy, 2011). Thus, mining machinery noise is likely to be perceived by a wide arrange of wildlife species, as a result of the broad frequency range and high energy intensity.

One of the animals commonly found in the vicinity of mining sites, due its opportunistic nature, is the mouse, (*Mus musculus*) (Fox and Fox, 2006; León et al., 2007). Its hearing range, from 2 to 92 kHz (measured at 60 dB Sound Pressure Level, SPL) (Heffner and Masterton, 1980; Heffner and Heffner, 2007), would allow it to perceive the mining machinery high frequency components as sound. Although the mouse is acoustically unresponsive to frequencies between 1–2 kHz (at 70–80 dB SPL, Heffner and Masterton, 1980), rodents can experience immunosuppression when exposed to inaudible low frequencies, as can humans in such situations (Aguas et al., 1999a, 1999b; Alves-Pereira and Castelo Branco, 2007).

Although no research exists that compares the effects of different frequencies of anthropogenic noise in mice, it is expected that both low and high frequency mining noise could potentially have negative effects on the behaviour and welfare of this species.

Stress is known to produce variations in rodent behaviours. Hiding, for instance, may increase as a response to threat (Hugie, 2003) and in females, nesting increases as it favors their security and that of their offspring (Taylor et al., 2000). Freezing is a behavioural response to fear and a reaction to perceived threats without a chance to escape (Blanchard et al., 1998; Blanchard et al., 2001). Likewise, maintenance behaviours such as eating and feeding can be effectively suppressed when rodents face environmental stressors (Morley and Levine, 1982; Aguilera et al., 1995).

One behaviour that has already been related to noise exposure in rodents is circling. Circling is an active motion of animals in a circular direction and is considered a stereotypy (Löscher 2010; Pycock 1980). Stress can increase circling behaviour due the actions of glucocorticoids on dopamine release, as glucocorticoids increase the secretion of enkephalines and tachykinines (Reiner and Anderson, 1990), which, in turn, increase nigrostriatal dopamine and locomotion (Biggio et al., 1978; Baruch et al., 1988). Furthermore, the direction of rotational behaviours is determined by hemispheric differences in dopaminergic activity, since animals will turn to the side opposite to the hemisphere with greater dopaminergic action (Carlson and Glick 1996; Ishiguro et al., 2007; Löscher 2010; Schirmer et al., 2007).

Circling behaviour has been observed before during exposure of rats to noise (Lukkes et al., 2009). As the right hemisphere of the brain is typically related to the activation of the stress response (a lateralized brain function, Rogers, 2010), exposure to mining noise could potentially increase this kind of stereotypy in mice. These behavioural responses can also be affected by the hearing sensitivity of animals at different frequencies.

Therefore, in this experiment, the effects of mining machinery noise at two frequency ranges were compared with a control group in a laboratory colony of 'wild mice'. Wild mice in this instance were mice that had not intentionally been genetically modified for laboratory purposes, and which came in our case from the 10th generation of wild-caught animals bred in captivity. We hypothesized that mining noise would disrupt wild mice behaviours, specifically those which are known in rodents to be disturbed by environmental stressors, such as social play (Vanderschuren et al., 1995), grooming (Ducottet and Belzung, 2004) and relevant stereotypies, such as circling (Löscher, 2010; Pycock, 1980). As well as behaviour, we hypothesized that the mining noise would affect physiological parameters associated with stress, in particular faecal corticosterone and the size of immune organs (Zheng et al., 1997; Harper and Austad, 2000). We further hypothesized that the extent of these effects would vary with frequency of the sound.

2. Materials and methods

Procedures were approved by The University of Queensland's Animal Ethics Committee (UQAEC Research Approval Number CAWE/054/13; UQAEC colony approval number SAS/071/10/BREED (NF))

2.1. Study animals

Fifty-seven (34 females and 23 males) wild mice (*Mus musculus*) held at the University of Queensland (UQ) were utilized for the study. The UQ wild mice were originally captured in Darling Downs, Queensland, Australia during June 2004. The colony was originally composed of 16 males and 28 females in 7 litters, arranged on 12 triads (one male with two females). Females from the same litter were kept together to avoid aggression, which had been observed when animals from different litters were housed together. When animals were selected for this study, the colony was in its tenth generation. It had been kept as outbred as possible, making sure that closely related animals did not breed and that the animals still displayed the temperament and behaviour of wild mice. When animals were chosen for breeding there was no selection for any particular trait, to avoid inbreeding problems.

Sample size and sex ratios were established using a similar study previously performed by this group (Mancera, 2016) and was limited by the availability of individuals sourced from student handling practicals at our university. This methodology was chosen in order to follow the 3Rs principles for animal-based experimentation, and only animals that would have been otherwise euthanased were utilized in the study (Understanding Animal Welfare, 2014). All animals were born between 9 and 24 February 2013. Mice were weaned at 4 weeks and they were separated into single- and paired-housing at 4 months old, one week before the beginning of the experiment.

2.2. Diet and animal housing

Mice were fed Rat and Mouse Pellets (Specialty Feeds, Glen Forrest, Western Australia) *ad libitum*. Males were necessarily individually caged because of the risk of aggression, but females were able to be caged in pairs. Both were kept in conventional yellow plastic cages with metallic grid lids on top (females 40 × 24, x 14 cm high, males 31 × 14, x 12 cm high. A 12:12 light:dark cycle provided artificial lighting from 06:00 to 18:00 h, with a temperature range of 21–25 °C. Each cage was supplied with bedding (Sanichip, PJ Murphy Forest Products, USA), plastic tubes for hiding and nesting (2 cm diameter, 10 cm long; two for females and one for males), as well as shredded paper to provide enrichment and nesting material. Bedding and shredded paper were changed twice a week, during the faecal sampling to avoid disturbing the animals. Cages and enrichment tubes were changed for clean ones once weekly. Food and water were checked daily and more provided when needed.

2.3. Experimental treatments and generation of simulated mining noise

Based on the characteristics of mining noise and surface rock crushing (Pathak et al., 1999; Roy and Adhikari, 2007; Saha and Padhy, 2011; Scott et al., 2010) in a fly-in, fly-out (FIFO) mining system (typically operating 24 h, 7 days a week in Australia, (Perry and Rowe 2015) and in consultation with Dr. D. Bridgeman, Senior Director of Geological Services of Manning Mining, Australia, recordings of seven pieces of mining machinery were chosen to recreate the soundscape of open-cast mining facilities: coal truck, drill, bulldozer, shovel, dumper, rock crusher and dragline. A blast was added in order to recreate sound impact from the explosions that occur on mining sites. Specialized