Increased incidence of spontaneous seizures in laboratory mice in an IVC environment

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Abstract

Over the past decade, there has been an enormous shift away from the use of open top rodent cages to the use of individually ventilated cages (IVCs). This has brought with it many benefits, including better control of health status and minimisation of contamination of immunocompromised animals. At King's College London (KCL), the transfer of mice from open top cages to IVCs has coincided with an apparent increased incidence of spontaneous seizures in mice. The majority of seizures were observed when the cage was opened in a change station and it has therefore been proposed that a significant change in environmental stimuli may contribute to the incidence of seizures. This study aimed to record the incidence of seizures in mice following their transfer to IVCs and investigate the environmental differences between the inside and outside of IVCs, in particular, noise levels.

This study highlights the increase in spontaneous seizure incidence in mice in IVCs and the need to investigate the impact of environment on seizures amongst laboratory animals in order to ensure the highest level of animal welfare maintained.

Key words: mice, seizures, IVC

Introduction

A seizure is a sudden, uncontrolled electrical disturbance in the brain, which can be spontaneous, or disease related. Unexpected changes to electrical activity in the brain can induce jerks, shaking and unconsciousness due to messaging conductivity being disturbed resulting in an epileptic seizure. There are different types of seizure, for example: audiogenic which is caused by sound, photogenic caused by light, olfactory seizure caused by smell and many others.[1,2]

First recorded seizures in laboratory mice were reported in the 1930s–1940s. Audiogenic seizures were noted in rodents by Mirsky in 1943.[3] Furthermore around that time there were first reports of seizure-susceptible and seizure-resistant strains. Researchers also identified that not only sound but also frequency, modulation, rhythm and vibration might cause seizures in mice. Even at this early time, C57BL and DBA strains purchased from Jackson Laboratory and also some albino mice were being tested due to visible seizures.[4]

In laboratory mice, epilepsy can be experimentally induced for the purpose of scientific interest in order to provide cures for human diseases. It should be emphasised that in this study neither seizures nor epilepsy were induced and none of the mouse strains have a connection with epilepsy or seizure-related experimentation. This study is looking at increased number of spontaneous seizures in random mouse strains.

At the end of 2016 most of our mouse colonies were housed within the Biological Services Unit in the James Black Centre building in South London in either individually ventilated cages (IVC) systems or traditional open top cages. At that time, seizures were observed as very rare reoccurrences of single isolated cases, typically 4-6 months apart, particularly in two animal holding rooms. In September 2017 all our animals were moved temporarily to a new building next door (the Maurice Wohl Neuroscience building), while the James Black Centre building underwent major refurbishment and every mouse was then housed in IVC systems. The Maurice Wohl Neuroscience building's Biological Services Unit (Wohl BSU) is situated in the basement of the building with its cagewash which has a clean and a dirty side, one surgery room, two procedure rooms

and fifteen animal holding rooms, which house primarily breeding and stock mice. Breeding is not performed in four of the animal rooms as one holds rats in open top cages, immunocompromised mice, the third room acts as a quarantine room and holds all delivered mice (from external suppliers or animals moved from other King's College campuses) and the fourth room holds animals recovering after surgical procedures.

After relocation to the Wohl BSU with all the mice now being housed in IVCs, the number of seizures observed in mice increased. In November 2017 epileptic fits were mainly observed in mice in two of the animal holding rooms which corresponded to the animal holding rooms with observed seizures before relocation. Soon after, spontaneous seizures were noticed across the remainder of the Wohl BSU without discriminating between mouse strains. Seizures were recorded from January 2018 until the present time. The peak in recorded spontaneous seizure animals was in April 2018 when the present study began. To investigate these seizures in more detail, seizure report sheets were created and employed in every animal room. Additional investigations included monitoring genetic predisposition for the attacks and measuring noise levels in the animal rooms.

In summary, this study was carried out to address concerns in relation to spontaneous seizures in laboratory mice after transfer from traditional open top cages to IVCs in order to improve animal welfare by identifying the causes of spontaneous seizures in mice and reducing incidence of these attacks, which can cause brain disturbances, changes in behaviour and movement and alteration of consciousness. A further aim was to reduce the number of animals used for experimental procedures by eliminating false results or the need to increase sample size due to seizure activities. This study emphasises the importance of training for Animal Technologists and others in noticing and communicating spontaneous seizure activity in mice early, which is crucial for animal wellbeing and the successful completion of the experiment.

Materials and methods

Animal husbandry

In the Wohl BSU all mice are kept in individually ventilated cages which are GM500 Mouse IVC Green/SelfSeal Line supply by Tecniplast. Each room holds four racks of 60 IVC cages connected to either easy or smart flow air handling units set up at 25% air exhaust and 75% air supply. The individual cages are serviced within three different type of change station units: CS5 Evo Plus, CS5 Evo GP, ARIA CS48.

This study was performed primarily on animals under breeding and maintenance Project Licence (PPL) protocols held under the Animals (Scientific Procedures) Act 1986 (ASPA) and approximately 10% of animals under experimental procedures (data not published) licences also held under ASPA. Over 90% of the mice included in this study were on a C57BL background of which 45% were C57BL/6 (Charles River) and 45% were C57BL/6J (Envigo) with 10% of all studied mice were of unknown genetic background. There was no control group as each animal was studied on an individual basis. All age stages were taken into consideration and ranged from 3 weeks old to approximately 24 months of age (including ageing studies). Animals were only single housed for the welfare reasons. Primarily the study included different numbers of mice in one cage with 5 animals being the maximum. Mice had ad libitum access to LabDiet 5053 and reversed osmosis (RO) water throughout the study. Each cage contained Lignocel select bedding (aspen wood product) and environmental enrichment consisting of a mouse fun tunnel and a mouse chew stick with a disc of Bed-r'nest for nesting, along with cocoons as soft addition to supplement the nesting as required.

Recording of seizure and coat state

The study commenced in April 2018 due to a major concern about animal welfare related to spontaneous seizures. Mice seizure report sheets were prepared, discussed with BSU staff and placed in each mouse holding room. Each member of staff was educated on how to identify and record seizures. Animal Technologists responsible for their rooms recorded spontaneous seizures on a weekly basis as the attacks generally coincided with cages cleaning. Cage cleaning in BSU Wohl is performed every week or every second week depending on the number of animals in the cage. The IVC cage is taken into a working changing station where the cage lid is opened, and animals are transferred into an autoclaved cage base with sawdust, then sterilised environmental enrichment is added into the cage or for example when weaning animals are transferred into an autoclaved prepared complete cage with environmental enrichment already in place. Normally mouse cage cleaning takes less than one minute. However, in case of any suspected spontaneous seizure an Animal Technologist paused their work to observe the attack and allow full recovery from it. In the case of a severe seizure, the fit could last up to 1 minute. During the study only one animal died during a seizure – the remaining mice fully recovered from their seizures.

The incidence of seizures was recorded over an eight month period from April (when the seizures peaked) to November 2018. Recording included severity of seizure, number of incidences, age of mice, sex, genotype, coat state and cage location on IVC rack. Severity of seizures was differentiated as:

- Mild where stiffness and short jerks were observed.
- Moderate having multiple jerks and convulsions.

– Severe with violent convulsion that could cause an animal to dribble or lose consciousness for a time.

The coat dribble was added in order to compare the condition of animal. The sum of the different body parts score (head, neck, back and tail) determined the coat state. Smooth and shiny hair indicated a good coat condition and gave score equal 0 (0+0+0+0=0) and characterised the animal as well and in good health (as expected). Slightly fluffy coat with some spiky patches would be moderate coat state with score 0.5. Not groomed fluffy, jutting, greasy hair would be rated 1 point as bad coat (for example head-1, neck-1, back-1, tail-1) giving the highest possible score of 4 and signifying an unwell animal. Coat scoring was harmonised across all Animal Technologists with scores and their descriptions and recorded on mice seizure report sheets. Additional images were also attached showing a mouse with good coat (score 0) and other one with the highest coat score (4) is exemplified in Figure 1.

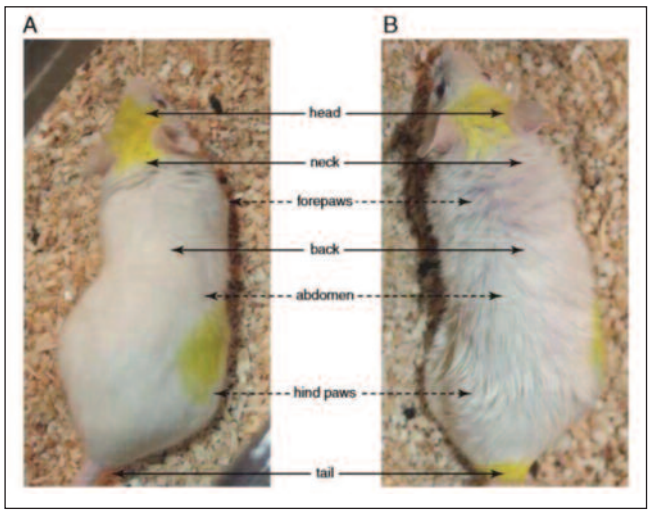

Image 1. Photograph of mice showing differences in coat condition. Current Protocols in Pharmacology 5.65.1-5.65.17, June 2013 Published online June 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/0471141755.ph0565s61 Copyright © 2013 John Wiley & Sons, Inc.

An example of coat scoring taking inspiration from above photos;

Body Part	Mouse A	Mouse B
Head	0	1
Neck	0	1
Back	0	1
Abdomen	0	1
Score	**0**	**4**

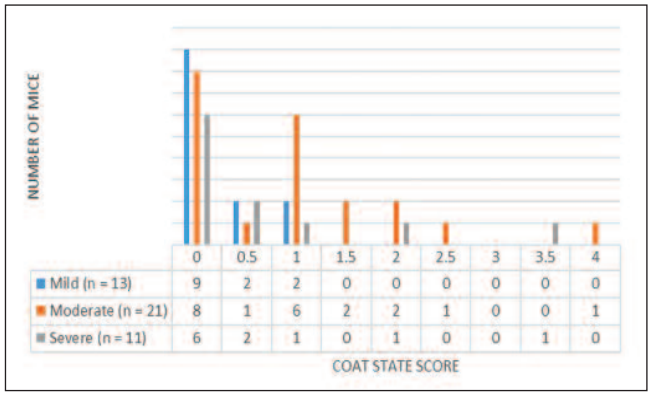

Figure 1. Coat score to seizure severity. Axis Y – Number of mice. Axis X – Coat state score

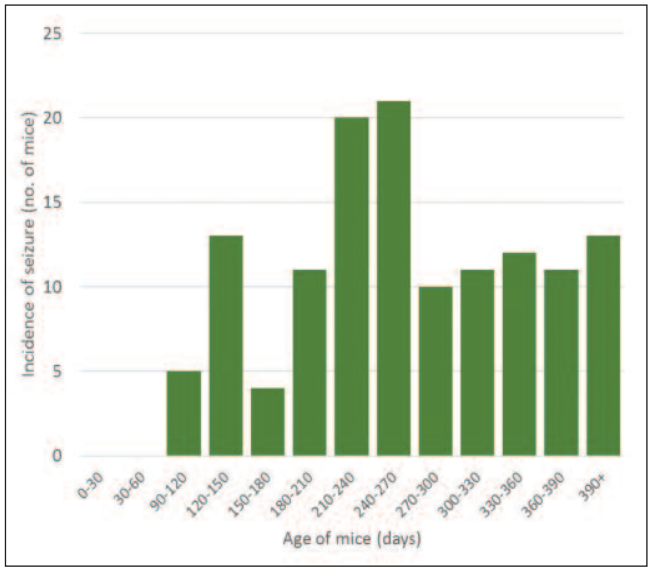

Figure 2. Age of mice with spontaneous seizure.

Noise

Aware that noise is a big contributing factor for seizures in rodents, our study measured noise within the human hearing range (20 Hz – 20 kHz) and mouse hearing range (1 – 100 kHz) in IVCs. Noise levels were also measured with the change station turned on, representing the noise difference that animals experience when welfare checks/cleaning are being carried out. Noise in the presence of vacuuming was also determined. Noise was measure in 'A' weighting (dBA) which is standard measurement and covers frequency range from 20Hz up to 20kHz characteristic for human hearing and also in 'Z' weighting (dBZ) which is flat frequency between 10Hz and 20kHz ± 1.5dB and more closely resembles a mouse's hearing. While it does not represent mouse hearing per se, it lacks the 'adjustment' to human hearing that is present in 'A' weighting.

The noise was measured in several animal holding rooms that had one of three types of cage changing station. A smart phone application 'Decibel X pro' was used following advice from our Named Veterinary Surgeon (NVS). The measurements were performed

outside and inside an IVC cage (1) in the absence of any activity (no other people in the room, changing station switched off), (2) when the changing station was switched on, (3) when hoovering the floor, and (2) and (3) combined.

Measurement of noise was also performed inside the changing station type ARIA CS48. The measurements were carried out inside the complete IVC cage covered with the lid and without the lid with changing station on or off, trying to replicate the conditions during cage changing.

The IVC cage used for noise measurement had both clips, holding the lid in place, removed to avoid the 'clicking' noise interfering with measurement when the lid was being closed, and the same cage was used throughout the noise measurement. The cage was positioned on the IVC rack closest to the door and directly opposite to the changing station in column 4 and row C. Lignocel bedding, one fun tunnel, one chew stick and one disc of Bed-r'nest were present in the cage. No food or water was added to the IVC cage during the noise measurement. All sound recording was performed for 3 min.

Breeding

Breeding in our unit is run independently by several research groups. All groups manage their own breeding colonies according to their experimental requirements. To support the breeding performance a 12 hours light/dark cycle is maintained within the unit.

The majority of animals identified as having seizures during the study were kept on breeding and maintenance protocols. Following the advice from our NVS, any mice experiencing a seizure were not used for future breeding stock. As a consideration of animal welfare, mice which were notified with three or more seizure attacks were humanely killed.

Statistical analysis

A Kruskal-Wallis test was carried out to determine the relationship between seizure severity and coat state score. $P<0.05$ was considered to be significant.

Results

Spontaneous seizures were experienced by mice from a number of strains and ages leading to variation in the experimental group in this study. All studied mice were genetically altered and no spontaneous seizures were observed in any of the wild type mice within the unit.

General findings

In our study we observed a higher number of males were affected by spontaneous seizure attacks than females (Figure 3).

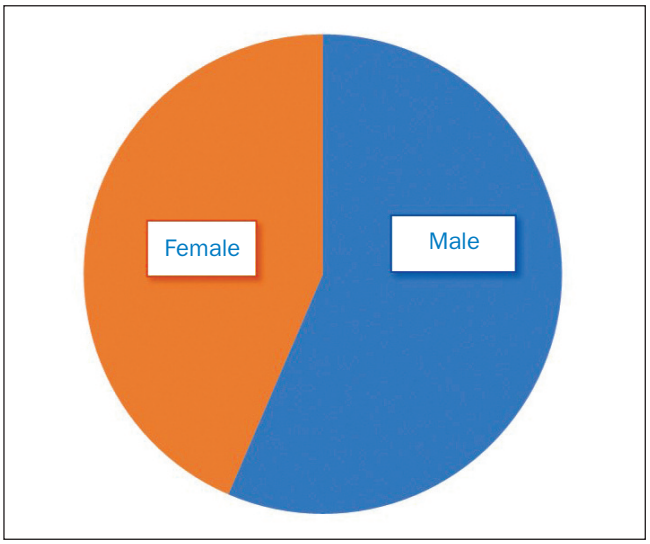

Figure 3. Percentage comparison of mice sex having a spontaneous seizure during the study.

The majority of reported seizures during the study were black mice, although we do not have comparable numbers of white/light colour mice within our unit (there is around one hundred white NSG mice stock and around fifty white BALB/c mice as sentinels within the unit).

Almost 50% of animals that entered our study because of spontaneous seizure attack were Cre transgenic mouse lines.

There was a significant spike in seizure attack in mice between 210 to 270 days old (Figure 2). Whether this is an effect of those mice being exposed to any stimuli in early stages in their lives or mice are more susceptible to seizure between 210 and 270 days of their lives is unclear.

Incidents of seizure and coat state scores

3 types of seizure attacks in mice were observed during our study;

(1) Mild with short jerks and sometimes body stiffness or freeze.
(2) Moderate with convulsions and often multiple jerks.
(3) Severe with violent convulsion when animal often would dribble or even be unconscious for a period of time.

The numbers of mild and moderate seizures were similar, while the number of severe seizures was much greater highlighting the concerns for animal welfare. Mice that suffered from seizures showed no erratic behaviour or abnormalities immediately before or after the attack, although the animals were not specifically monitored 24 hours a day. Therefore, seizures could have occurred at night when the mice are usually more active. It could be possible that an animal being disturbed during cage changing or health checks, could

trigger a seizure attack. Every spontaneous seizure was recorded for the study, consequently a range of different mice strains were observed without any preselection.

Some animals had only one or two notified attacks and lived for many months. Certain animals that showed one or two seizures were already under an experiment and lived to the end of the study.

The highest coat score in our study was 4, the lowest 0 with the score of 0.5 being most common. Some mice with coat score 0 were observed with severe seizure and some with score 4 were noticed with only moderate seizure. There were also animals notified with mild seizure when their coat score was 1 or 1.5. However, a Kruskal-Wallis statistical analysis showed that overall there was a significant relationship between coat condition score and seizure severity ($p=0.027$). However, the coat score was recorded after the seizure was observed, so there is a possibility that the attack could have contributed to the animal coat state, as opposed to mice in poor health having seizures (Figure 1).

Noise

The base line of noise measurement was within the complete IVC cage with lid on. The noise increased about 3% during measurement performed inside the cage with (1) a cage changing station switch on or (2) hoovering in the room (one activity at the time). During both activities 1 and 2 simultaneously noise was greater at approximately 6%. When measurement was carried out outside the IVC cage noise elevated about 12% with just the cage change station on and approximately 3% purely by hoovering. Both activities 1 and 2 simultaneously increased the noise in animal room roughly by 12% when measurement was performed outside of the cage.

Noise measurement inside the cage increased by 15% when the IVC cage was placed onto the working cage changing station and about 20% when the lid was removed from the IVC cage.

All sound frequencies measured during our study were too low for mice to hear.

Independent noise measurement was also undertaken within the Wohl BSU in March 2019 which also confirmed that the frequencies are too low for mice to be able to hear on the IVC rack or in the cage changing station.

Breeding

Breeding was stopped if one of the parents exhibited any seizures. Offspring with at least one parent identified with epilepsy were not used for future breeding stock. Observing seizures in first and second generation when at least one parent was affected could indicate that spontaneous seizure is in their genetic background (Figure 4). This is also consistent with a rapid decrease in the number of seizures observed from September 2018 after eliminating all affected animals from future breeding stock.

However, we still observe single cases of spontaneous seizure in mice in colonies where breeding could not be stopped on time for various reasons (data not published). This also suggests genetic implication to spontaneous seizure when dealing with subsequent generations.

Reduction of seizures by excluding animals with those exhibiting attacks from future breeding stock is one of the factors that instantly decreased spontaneous seizures in mice within our unit.

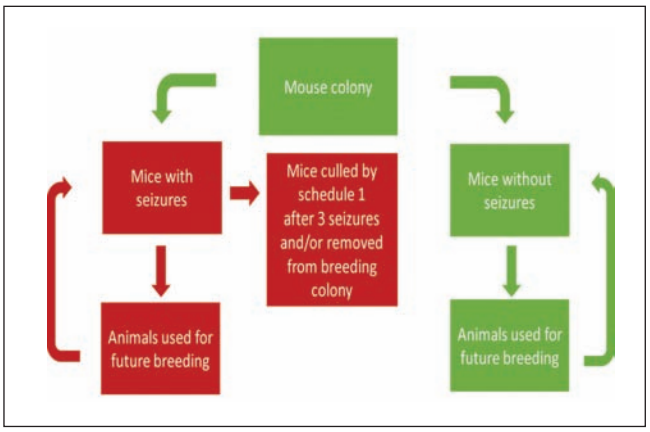

Figure 4. Breeding out of spontaneous seizures in mice.

Discussion

This study was carried out to address concerns in relation to spontaneous seizures in laboratory mice after transfer from traditional open top cages into IVCs which has been observed across the industry regardless of the IVC supplier. The primary concern is the welfare of animals; especially when multiple seizures were observed in individuals. Further concerns relate to the impact on experimental results, particularly for long-term studies where cohort numbers could be compromised, necessitating repeat experiments.

When the incidence of seizures in laboratory mice at King's College London was first identified, a policy was agreed in discussion with the Named Veterinary Surgeon (NVS) and consultation with the Personal Licence Holder (PIL) concerned, to humanely kill any mouse that had experienced 3 seizures or had welfare issues that may have arisen from a seizure. Records of the incidence of seizures in all mice held in IVCs in the Biological Services Unit in the Maurice Wohl Neuroscience Institute at King's College London were

subsequently maintained. Although seizures of varying degrees of severity were observed, mice generally returned to a state of 'normal' appearance within approximately 1 minute. Seizures in mice were mainly observed when IVC cages were opened in a cage change station. A small minority (<1%) were observed in mice that were resident in their cages while it was positioned on the holding rack but mice were generally not observed whilst on their racks and so this incidence could have been much higher.

Determining the cause of seizures

Mice held in individually ventilated cages experienced seizures regardless of age and sex in the present study and seizures were observed across range of colonies within the same facility. These results were compiled from one Biological Services Unit however seizures were also noted in mice in other KCL facilities upon transfer from open top cages to IVCs. A significant finding in the present study was that seizures were not only observed in the first generation of mice that were moved from open top cages to IVCs (and thus experienced both environments) but also in subsequent generations of offspring. This suggested that the incidence of seizures may have a genetic link rather than a purely environmental one. Related to this, most of the genetically altered mice held in facilities at King's College London are bred on a C57BL/6 background. Popularity of this strain can result in unidentified spontaneous mutations and genetic drift in C57BL/6 sub-strains can lead to unique known and unknown genomic sequences.

All mice that experienced a seizure were, where possible, excluded from any future breeding plans for their respective colonies in case of any genetic link to the incidence of spontaneous seizures. Seizures can have a genetic origin, as evidenced by various other publications. Steinmetz *et al*. (2017) concluded from their study of GCaMP6-expressing transgenic mouse lines that abnormal brain electrical activity may result from a combination of effects, including Cre toxicity (gemline Cre recombination with different Cre line), tTA (genotype) toxicity, and genetic background.[4] Considering all observations together, they also concluded that the broad expression of GCaMP (genetically encoded calcium sensor) itself, particularly during development, could be a major factor.[4,5]

It was not within the remit of this study to run a genetic analysis of the mice that experienced seizures but the incidence of seizures in strains where affected mice were excluded from breeding protocols were greatly reduced to the point where some strains no longer suffer seizures at all. Alternatively, seizures are still occasionally observed in strains where elimination of mice experiencing seizures from breeding regimes has not been possible. This is potentially an extremely important finding within this study, especially as elimination of a potential cause of seizures had a much greater potential welfare impact for laboratory mice being held in IVCs than simply eliminating potential environmental or procedural triggers. Training of animal care staff to identify seizure activity in mice at the earliest possible stage leads to a significant refinement of breeding protocols.

Determining environmental triggers of seizures

Key environmental variables within a Biological Services Unit are noise, temperature, humidity, air pressure, vibration and light. For the purpose of this study, we focussed on noise as a variable that may act as a trigger for spontaneous seizures. This does not eliminate other possible causes but these were beyond the scope of the present study. It is important to note that mice suffered seizures across a range of rooms within the same animal facility.

It is well known that exposure to noise may induce changes in the cardiovascular system, behavioural changes, hormone production and cause seizure susceptibility.[6] Guide for Care and Use of Laboratory Animals suggests that "changes in patterns of sound exposure have different effects on different animals" stating that "an excessive and unnecessary noise should be minimised by laboratory personnel wherever possible".[7] Noise and vibrations in the animal facilities should be controlled, however it is difficult to have control over building work around our facilities. We expect that the 1 noise in the Biological Service Unit with building, animal housing and the general laboratory activity to be 50-65 dBA[7]. We know hearing in mice is approximately 1 to 100kHz in ultrasonic frequencies compared to human hearing which ranges between 20Hz to 20kHz. Sound sensitivity is also different for mice at approximately 16kHz and for humans it is 1-4kHz which means that mice may hear a sound when humans cannot and vice versa. Other important factors include; age, noise exposure and disease which can all affect hearing of both mice and humans.[8] Several studies in the 1960s and 1970s tried to establish a difference in hearing in albino and pigmented mammals. It was discovered that acoustic deprivation induces audiogenic seizures in mice which would normally not result in this type of behaviour. Changes in hearing could be caused by conductive hearing loss, intense acoustic exposure and genetic manipulation especially in breeds showing hearing impairment[9].

Jeremy G. Turner found that mice hearing is greater than that in humans and can reach over 80,000Hz which is four times more than the highest range of human hearing.[6] In contrast, most laboratory inbred mice like DBA/2J, C57, BALB show progressive hearing

loss. The same strains also show tendency to noise-induced seizures. The author found that even strains resistant to seizures may develop tendency to those attacks if exposed to a few seconds of loud noise between 24-42 days of age. It is important to note that sound provides environmental information for nocturnal animals like mice. By stimulating central and peripheral systems sound controls autonomic motor responses, learning and memory, planning and executing and much more. According to Turner DBA/2 lose their hearing when they are around 2 weeks old and are deaf when they reach 3-4 months of age and their exposure to loud noise between 3-4 weeks of age may results in fatal seizures. Popular background strains as C57BL6 and BALB/c also show genetically progressive hearing lost which starts early in their lives but progresses slowly.[5]

Summary and conclusion

Mice have different hearing range to that of humans which is supported by published finding.[6,8-10] Unpleasant and noisy sounds for people may not be a disturbance to mice as the sound frequency is simply too low for the animals to hear.[8-10] Two separate noise measurement exercises confirmed that mice could not be able to hear the sound of technical equipment in the animal rooms. Our study has compared noise measurement for different activities in the animal room and in IVC caging however further study should be performed to find out if other factors other than noise may be a stimuli for spontaneous seizures in mice.

By selecting only future breeding stock that had not exhibited seizures in any consecutive generations, spontaneous seizures almost completely (99%) decreased in number of observed attacks throughout the unit. At that time no environmental changes or adjustments were undertaken.

The authors consider that the findings of our study of the incidence of spontaneous seizure in laboratory mice could impact on animal welfare. It is vital to identify spontaneous seizure activity in mice as early as possible to ensure that they are eliminated from future breeding stock. This requires animal care staff and researchers to be trained to identify all forms of seizures. The elimination of the cause of seizures will not only improve animal welfare but will also reduce the number of animals required to complete studies as seizures would result in the loss of an experimental cohort thus leading to a refinement in welfare and a reduction in the numbers of animals used.

Future work

Importance of genetic drift needs to be addressed as a main point of breeding and maintaining any animals' colonies. Sufficient training should be given to all those responsible for breeding including researchers and Animal Technologists to understand the strains they are working with and to differentiate unwanted side effects of genetic mutation in order to spot genetic drift as soon as possible and take proper action to improve animal welfare. Ideally, tissue samples should be obtained from any mouse that has a spontaneous seizure for polymerase chain reaction (PCR) analysis to detect a possible genetic cause of seizures.

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