Review Article

The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats

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Abstract

Human interaction and physical environmental factors are part of the stimuli presented to laboratory animals everyday, influencing their behaviour and physiology and contributing to their welfare. Certain environmental conditions and routine procedures in the animal facility might induce stress responses and when the animal is unable to maintain its homeostasis in the presence of a particular stressor, the animal's wellbeing is threatened. This review article summarizes several published studies on the impact of environmental factors such as light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. The behaviour and physiological responses of laboratory rats to different environmental housing conditions and routine procedures are reviewed. Recommendations on the welfare of laboratory rats and refinements in experimental design are discussed and how these can influence and improve the quality of scientific data.

Keywords: Environment, welfare, rats, light, noise, cage cleaning, transport

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With the development of Laboratory Animal Science and the application of the three Rs principles, proposed by William M S Russell and Rex L Burch in 1959 (replacement, reduction and refinement),[1] legislation and recommendations for the protection of animals used in experiments have been developed and applied. In Europe, guidelines for accommodation and care of laboratory animals are established in the *European convention for the protection of vertebrate animals used for experimental and other scientific purposes* (Council of Europe Convention ETS 123), with its Appendix A: *Guidelines for the accommodation and care of animals* (Council of Europe 1986 – revised in 2006, Strasbourg, http://conventions.coe.int/Treaty/EN/Treaties/PDF/123-Arev.pdf), which states that all experimental animals must be provided with proper housing, environment, at least a minimum degree of freedom of movement, food, water and care appropriate to their health and wellbeing. The animals' physiological and ethological needs should be satisfied as far as practicable and restrictions should be minimized (article 5).[2–4] Another European document containing specifications of housing laboratory animals is the 2007 European Commission recommendation on *Guidelines for the accommodation and care of animals used for experimental and other scientific purposes* (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:197:0001:0089:EN.PDF).[5]

Animal welfare can be related to the animal's emotional perception of its environment, to how the animals cope with their environment and how they have to compensate for aversive changes in their environment by behavioural and physiological adjustments.[3,6] Predictability and controllability are key concepts in this respect, but for optimal welfare some uncertainty (unpredictability and uncontrollability) is of great positive significance. Under natural conditions, animals are exposed to both negative and positive stimuli and 'living in harmony with its environment' probably implies that it must be possible to keep a (positive) balance between these stimuli.[7,8]

Laboratory animals are kept in confinement for their whole lifespan. In husbandry practices, the five freedoms (freedom from thirst, hunger and malnutrition, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behaviour and freedom from fear and distress), a concept first put forward in 1965 regarding farm animals,[9] should be applied to achieve animal's welfare, unless it interferes with the scientific objective. It has, however, been argued that the concept of the five freedoms is flawed in that it is not necessary to the welfare of an animal to have absolute freedom from hunger, cold, pain or fear; only that the animal should be able to cope with these problems by taking effective action to avoid suffering.[7]

Concerning animal welfare, environmental factors such as cage size and structure/enrichment, NH_3 and CO_2 levels, light (intensity, wavelength, photoperiod and flicker frequency), sounds, air/ventilation, temperature, relative humidity, odours, presence/absence of pathogens and human presence/interaction are as important as the presence or absence of conspecifics, their sex, and the predictability and controllability of the environment.[4,10,11] The capacity to adapt to new environments depends on the animal's phylogeny (species-specific morphology, physiology and behaviour), and on the individual's history (learning during ontogeny and adulthood). When environmental factors challenge the biological balance of the animal interfering with the animal's homeostasis (positive and negative experiences), and if the animal is unable to maintain this homeostasis by behavioural and physiological responses, stress will develop in the course of time.[4,7] Stress can be defined as the biological response elicited when an individual perceives a threat (stressor) to its homeostasis, but when the stress response truly threatens the animal's wellbeing, then the animal experiences distress ('bad stress').[12]

Presence or absence of certain natural behaviours (species-specific) is used as indicator of animal welfare. For instance, play behaviour is argued to be a reliable indicator of good welfare in mammals since one of the common characteristics of play behaviour is that it is absent under stressful conditions.[7] Motivated behaviour together with physiological data may provide a useful indicator of animal priorities and physical health, and of the effects of the environment, husbandry and experimental procedures performed on the animal.[3,7]

The environment of laboratory animals consists of a wide range of stimuli (physical and social environmental factors mentioned before) and should be adjusted to physiological and behavioural needs such as resting, nest building, hiding, exploring, foraging, gnawing and social contacts.[3] In practice, standardized environmental conditions are used to reduce variation between animals of the same experimental group and between studies, facilitating the detection of treatment effects and increasing reproducibility of results across laboratories.[13] However, this standardization developed to minimize uncontrolled environmental effects on the animals may be a primary source of pathological artefacts (stressor). Current thinking is that appropriate structuring of the cage/pen environment may be more beneficial than provision of a large floor area, although a certain area is necessary to provide a structured space. Except for locomotor activity (e.g. playing), animals do not actually use space, but instead use resources and structures within the area for specific behaviours.[3] Careful handling of laboratory rodents from a young age, together with conditioning to experimental and husbandry procedures, will probably reduce stress responses considerably.[14] A sense of security can be achieved by providing nestable and manipulable nesting material, hiding places and compatible cage mates.[3] For instance, rats are social animals and isolation can have permanent effects on their behaviour and physiology. When reared from weaning in an environment enriched with objects such as ladders, balls, tubes and boxes, rats are better in several learning tasks, are less defensive, show more exploratory behaviour, and have a thicker cerebral cortex and higher synaptic density than rats reared under standard conditions.[15,16] Environmental enrichment should comprise a well-designed and critically evaluated programme that benefits the animals as well as the experimental outcome, and it should be regarded as an essential component of the overall animal care programme, and just as important as nutrition and veterinary care.[3]

Regarding materials used as cage bedding, rats have been shown to prefer beddings that they can manipulate, consisting of large particles suitable for use as nesting material.[17] However, soft wood bedding materials (such as red cedar, white pine or ponderosa pine) have been shown to cause depression of sleeping time and induction of drug-metabolizing enzyme activity from both the supernatant and microsomal fraction of Sprague-Dawley rats' liver, whereas aspen does not.[18] Soft hydrothermal processing can remove many volatile components of the essential oil of red cedar decreasing the hepatic P450-inducing effect of this bedding material.[19] Appropriate knowledge about bedding material is therefore important.

As mentioned above, human interaction and physical environmental factors are part of the stimuli presented to laboratory animals everyday. The environmental factors light and noise, as well as cage cleaning and in-house transport were a source of discussion when starting a new facility where most of the rats are used for behavioural experiments and stress studies. Light and noise were important in discussions with engineers at the level of the physical structure of the animal facility and equipment installation. Animal facility routines, such as cage cleaning and transport of cages to an adjacent room, are procedures most of the times not taken into account for experimental design but also have an effect on laboratory animals. The present review will focus on the impact of light, noise, cage cleaning and in-house transport on welfare and stress of the laboratory rat, the second most commonly used vertebrate in research.

Light and vision

Light is an important abiotic environmental factor influencing laboratory animal behaviours and physiology. The effects of light can be related to aspects such as its intensity, wavelength or duration (photoperiod). Rats are nocturnal animals and are better adapted to dim light.[10,11] Initially, behaviour and electrophysiological experiments indicated that rats have no colour vision, but later it has been shown, by electroretinogram and behavioural discrimination tests, that rats may have dichromatic colour vision.[20] The rat's retina contains rods and cones, but they only have two classes of cones, one containing an ultraviolet (UV)-sensitive photopigment and the other containing a pigment maximally sensitive in the middle wavelengths of the visible spectrum.[20,21] One system has its maximal photopic sensitivity at a wavelength of 510 nm, which declines rapidly at wavelengths above 560 nm (red–infrared), and the other photopic mechanism has its maximal sensitivity at a wavelength of 360 nm, in the near UV range.[11,20,21]

Light intensity variations will be found inside transparent plastic cages on racks or shelves, depending on their

positioning relative to the light source, although variation is lowest in the cages farthest from the light source.[10] Rats are extremely sensitive to light. Retinal damage due to light exposure has frequently been reported. Albino rats have increased sensitivity to bright light since they lack the normal pigmentation of the eyes.[22–24]

Light and the rat's eye

Although several factors contribute in determining the extent of light-induced retinal damage (light intensity and duration of light exposure; wavelength of light; rat age, strain, and ocular pigmentation; light exposure history of the animal), retinal damage has been reported in albino rats at light intensities greater than 60 lx.[23,25,26] A preference test system has shown that, even though the effect was more pronounced in the albino rats, both albino and pigmented rats preferred cages with a low light intensity (< 100 lx) over those with higher light intensities (100–380 lx).[27] Using avoidance tests, Schlingmann *et al.*[28] have shown that albino rats avoid light intensities as low as 25 lx and pigmented rats from as low as 60 lx, but animal rooms need to have enough light for the technicians to perform animal care tasks. A minimum of 210 lx at working height as reported by the same authors has sufficient light in the room for the health and good performance of personnel.[29] Providing rats with a place to hide from too much light is therefore strongly advisable.

The cyclic (12 h light, 12 h dark; 12L:12D) light intensities under which the rats are reared have been shown to influence the rod outer segment length, photoreceptor cell density, whole retina visual pigment regeneration rates, the concentration of fatty acids and cholesterol in photoreceptor outer segment membranes and antioxidant capabilities of the rat retina. The retinas of rats reared under high-intensity cyclic light (i.e. 400 lx) acquired characteristics that made them less susceptible to light damage when compared with rats reared under low-intensity cyclic light (i.e. 6 lx). Such characteristics included reduced numbers of photoreceptor cells, reduced outer segment length and pigment concentration, decreased levels of polyunsaturated fatty acids and increased antioxidant capabilities.[23,30,31] Light-induced retinal damage is triggered by rhodopsin bleaching and albino rats have been shown to adapt to changes in environmental light intensities by adjusting the amount of rhodopsin per retina.[32,33] Case and Plummer[34] reported that the adult retina responds to a different lighting environment by a relatively rapid change in the size of photoreceptor segments, by a progressive and large change in number of ribbon synapses and by a slower progressive and large change in the size of photoreceptor nerve terminals.

Light history experienced by the animal also influences retinal response to light. Dim-light-reared rats have been shown to exhibit an age-related increase in retinal light damage susceptibility, whereas dark-reared rats were equally susceptible to damage at all ages.[26,33]

Retinal damage has been shown to be greater in rats exposed to a dose of intense light during their night period rather than in rats exposed to the same light treatment during their day period (circadian-dependent retinal light damage).[35] The mechanism by which susceptibility to retinal light damage in rats occurs seems to involve a light-induced apoptotic process of visual cell death.[26,33,35] A single, relatively short, intense light exposure can cause a circadian-dependent, oxidatively induced loss of photoreceptor cells.[35] Ideally, laboratory rats should be kept in the same light cycle and intensity for their life period in the laboratory in order to avoid physiological changes and damage as those mentioned above. Maintaining a standardized light environment of the animals would contribute to their welfare and to the reproducibility of experimental results.

Light, behaviour and physiology

Animal activity and behaviour are also influenced by environmental light intensity. Defecation rate in hooded rats (PVG/C) can be increased under bright lighting (given by a white 150 W bulb suspended centrally on top of a Y-maze placed in a grey-curtained enclosure of 1.37 × 1.37 × 1.22 m high).[10,36] Intense light conditions (572 lx) markedly suppressed social play behaviour of Wistar Han male juvenile rats accustomed to dim light conditions. In the intense light test conditions, pinning and boxing/wrestling was absent and following/chasing behaviour was markedly decreased.[37] These characteristic social play behaviours are important for the animals' (social) development, and the animals will learn a task to obtain the opportunity to play. Rats preferably play in sheltered places,[38] which are nearly absent under intense light conditions.[37] The presence of enrichment objects providing shelters can therefore be important, creating areas with reduced light intensity inside the cage and enabling rats to hide.

It is difficult to separate the effects of light intensity from those caused by different light wavelengths and there are not many scientific data available on the effect of the colour of light on laboratory animals and even this is occasionally conflicting.[10,39] However, red fluorescent light has been shown to still serve as a synchronization pulse for rats kept in the dark,[40] even with light intensities below 1 lx.[39,40] Time of ovulation has been shown to be shifted by red light, which suggests that the circadian rhythm of luteinizing hormone (LH) secretion could be entrained by red light.[40] However, red light is still being used as dark (e.g. in behavioural studies). In mice, voluntary wheel-running activity has been shown to be influenced by differently coloured lights.[41] Red light might have an effect on the animals' behaviour and physiology, which made Wersinger and Martin[42] advise researchers performing studies on social behaviour not to assume that laboratory rodents are unable to detect dim red light and to minimize the exposure of subject animals to all sorts of light during the dark period.

During light periods, the fluorescent light that is commonly used in animal facilities provides a narrower range of wavelengths than sunlight; therefore, using full spectrum fluorescent bulbs could be considered a way of providing a more natural light environment in the laboratory.

Light is the most important environmental signal regulating the temporal pattern of animal behaviour and physiology, regulating circadian rhythms and stimulating and synchronizing breeding cycles.[10] The hypothalamic suprachiasmatic nucleus (SCN) contains the primary circadian oscillator in

mammals, and SCN neuronal activity is directly regulated by environmental light via the retinohypothalamic tract (pineal/pituitary/hypothalamic-neuroendocrine pathways, starting from retinal photoreceptors).[10,43,44]

Breeding cycles

The duration of the oestrus cycle in rats has been shown to be affected by changes in the proportions of the light:dark (L:D) regime used.[10] Time of ovulation and gestation length in rats are also influenced by the L:D cycle under which animals are reared.[10,45] Nocturnal exposure of female Sprague-Dawley rats to light of minimal intensity has been shown to produce a substantial incidence of ovarian changes, suggesting that the incidence of ovarian atrophy observed by the authors in a previous study may have been due to transient exposure to indirect nocturnal light of minimal intensity.[46]

Circadian rhythms

Continuous exposure to bright light has been shown to strongly suppress circadian rhythms of the sleep–wake cycle, drinking, locomotion, blood pressure, heart rate (HR) and body temperature (BT),[47–49] which are regulated by the SCN.[43,50] Constant light (LL) housing has been shown to increase corticosterone levels in both male and female rats, and is used as an experimental model of chronic stress.[51,52]

As mentioned above, in adult rats, after a long exposure to LL, the circadian periodicity disappears, as do rhythms of motor activity, BT,[47,53] plasma melatonin[54] and sexual hormones.[55] However, if previously subjected to LL during their lactation period, adult albino rats have been shown to exhibit a circadian rhythm of motor activity.[56,57] A critical period for sensitivity and ability to adapt to external factors seems to be located in the middle of the lactation stage of rats.[56] Light received during lactation affects the strength of the circadian pacemaker and its sensitivity to light.[58] Lighting conditions to which newborn animals are exposed are of great significance, as they will affect the circadian system and condition the further adaptation of the adult animal to the external conditions in which it lives.

The period length of the light/dark cycle under which rats are kept has also been shown to influence the body weight and food intake of young male Wistar rats.[59] A shift in the light cycle (reducing its photoperiod or changing the time at which lights are on and of) induces a shift of the circadian rhythms of blood pressure, HR and spontaneous locomotor activity in freely moving rats, which can take up to a week to fully synchronize with the new light cycle.[60,61]

Individually housed Sprague-Dawley and Spontaneous Hypertensive rats implanted with a radiotelemetry transmitter presented reduced basal HR when housed under 10 lx illumination or an 8:16 h L:D photocycle with 200 lx illumination, when compared with controls housed under a 12:12 h L:D photocycle with 200 lx illumination. Even though the pattern of effects varied between strains and between male and female rats, Azar *et al.*[62] concluded that housing rats under 12:12 h L:D, 200 lx ambient light conditions was potentially stressful. Rats' adaptation to a photoperiod reduction seems better when extending symmetrically the darkness into dawn and dusk.[60] The use of dimmers in rat rooms to create twilight periods between the light and dark cycles can be recommended[63] even though not much information is available comparing the physiological and behavioural changes in rats after sudden turn off of lights with gradual transition from light to dark and *vice versa*. Timers providing a gradual light transition would better mimic natural light at dawn and dusk, which have been suggested to be preferable in studies of social behaviour of crepuscular species.[42]

Effectiveness of light stimuli on daily circadian clock resetting can be modified by learning and events in the environment that reliably precede the onset of light.[64] Light stimuli can gain negative emotional significance through repeated pairing with aversive events, and consequently fail to provide the optimal stimulus for entrainment of circadian rhythms.[65]

In summary, environmental light is a fundamental factor with an important impact on the laboratory rat physiology and behaviour and should be a well-controlled factor in housing conditions. The housing photoperiod should be stable contributing to both animal wellbeing (i.e. avoiding distress, behavioural disturbances, retinal damage and circadian rhythm suppression) and good experimental results. Albino rats avoid areas with light levels over 25 lx, and as recommended in the European guidelines (*European convention for the protection of vertebrate animals used for experimental and other scientific purposes* – Appendix A, Council of Europe Convention ETS 123), the animal room should have a low light intensity (enough for the performance of husbandry procedures and inspection of animals,[2] e.g. around 210 lx at working height) and darker areas or hiding places should be provided inside the cages (e.g. tubes or shelters to provide rats with some control over their exposure to light). Cages on top shelves might be exposed to too much light and deserve special attention.

Sounds, audition and vocalization

The effects of sound on animal physiology and behaviour depend not only on its intensity (or loudness), which is measured in decibels (dB), its frequency, which is measured in hertz (Hz), and its duration and pattern (including vibration potential), but also on the hearing ability of the animal species and strain, the age and physiological state of the animal at the time of exposure, to what sounds the animal has been exposed to during its lifetime (noise exposure history of the animal) and to the predictability of the acoustic stimulus.[10,66–69] Meaningful sounds at relatively low-intensity levels can have a considerable impact on animal physiology and behaviour by engaging limbic structures and higher centres involved in determining context and meaning.[68]

Auditory sensitivity

Rodents have a different spectrum of audible sounds with maximum sensitivity at frequencies that are inaudible to

humans. Humans can perceive frequencies from 20 to 20,000 Hz, the frequencies from 400 to 4800 Hz being important for speech. Rodents not only produce sounds that we can hear, but also produce and hear frequencies that are inaudible to humans (above 20 kHz), perceiving sounds up to 80 kHz.[68,70–72] Different methods have been used for determining the auditory range of laboratory animals and the results presented in the literature are diverse.[66,71,73] Using a combination of an operant conditioning and the psychophysical method of constant stimuli, Gourevitch and Hack located the frequency region of greatest auditory sensitivity for the rat at approximately 1 octave wide in the vicinity of 40 kHz (frequency at which the rat is most sensitive). In this study, water-deprived Wistar rats were trained to respond to a tone by pressing a bar to obtain water as a reward (operant conditioning). The method of constant stimuli was applied by randomly varying the intensity of the signal from presentation to presentation among five intensities chosen from preliminary measurements so that the strongest signal was almost always audible to the rat and the weakest signal was only rarely audible.[74] Kelly and Masterton showed a range of hearing from 250 Hz to 80 kHz at 70 dB (SPL) for the (Sprague-Dawley strain) albino rat by using the conditioned suppression technique: first, the animals were water-deprived in their home cage and then trained to lick a spout for water reinforcement in the test apparatus; after this, animals were further trained to associate the offset of a pure 10 s tone with a foot shock until the tone elicited a freezing response incompatible with licking – this freezing or suppression of licking was then used as an indication of the animal's ability to hear a tone. The behavioural tests have shown that the absolute auditory sensitivity of the rats as less than 10 dB SPL at 38 kHz.[72] Using behavioural and electrophysiological techniques, Borg[75] showed the highest degree of normal auditory sensitivity of albino rats to be around 12–24 kHz, the upper hearing limit being 50 kHz. The behavioural audiogram of the hooded Norway rat was determined for frequencies from 250 Hz to 70 kHz. At a level of 60 dB SPL, the low-frequency limits are 530 Hz for the hooded rats and 400 Hz for the albinos. At the other end, the high-frequency limits are 68 kHz for the hooded rats with an estimated 76 kHz for the albino rats. No effect of albinism was detected by Heffner *et al.*[76] since there were no differences in the audiogram between albino and pigmented Norway rats (*Rattus norvegicus*). The audiogram of rats, compared with that of humans, is characterized by a lower capacity for detecting low frequencies (under 500 Hz) and a better capacity for detecting high frequencies (over 8000 Hz).[68]

There are differences in the structure of different cell types in the inner ear between albino and pigmented *R. norvegicus*, and differences in auditory sensitivity have been related to age, strain or stock differences.[67,68,71,76,77] For example, the rat strain Fisher 344 (F344) has a very different auditory sensitivity when compared with the F1 hybrid cross between the F344 and Brown Norway rat (FBN). F344 rats show approximately 20 dB better hearing at low frequencies (4 kHz), whereas FBN rats show approximately 20 dB better hearing at higher frequencies (32 kHz).[68] Age differences in hearing ability of rats have also been reported, the first auditory nerve–brainstem-evoked responses have been detected to begin in 7–8-day-old postnatal rat pups. The rat auditory sensitivity increases with the opening of the meatus of the ear at days 12–14 of age and reaches adult thresholds at about 20–22 days.[67,78] The inner ear of rat pups has been shown to have a maximum susceptibility to acoustic trauma at 22 days of life, after exposure to a continuous 120 dB SPL white noise for 30 min.[79,80] Auditory deprivation can also have profound effects on the hearing ability of the animal. Studies have suggested a sensitive period, from postnatal days 10 to 16 (including meatus opening), during which proper development of parts of the auditory system of the rat (namely, the dorsal and ventral cochlear nuclei) may require acoustic stimulation.[81] Poon and Chen[82] showed that exposure to trains of tones improved the ability of the exposed animals to process those same tones.

Vocalization/communication

Of course, rats not only react to but also transmit sounds. Ultrasound emission is an important communicating pathway used by rats in several situations, but they can also vocalize below 20 kHz. Ultrasounds were detected during aggressive interactions, sexual intercourse, mothering and during stressful situations (e.g. handling, pain or presence of a predator), and vocalization below 20 kHz were found in aggressive interactions, during the first days of life or while in pain.[66,67,69,83]

Most of the ultrasounds are emitted at a frequency of 21–32 kHz and are therefore called 22 kHz calls.[67,84] Sales[85] has described two major ultrasonic vocalizations during aggressive interactions between male rats: a short signal ranging from 40 to 70 kHz and a long one ranging from 23 to 30 kHz. Observing rats' behaviour, Sales proposed that long vocalizations were indicating submissive behaviour (by the intruder), while short pulses represented aggression from the resident male. Some studies report the emission of 22 kHz calls associated with a decrease in the aggressive behaviour response of the resident rat and in inhibition of attack, but in other studies the submissive rat was found to emit both long (22 kHz) and short (50 kHz) calls and some groups have been unable to find a correlation between the inhibition of aggressive behaviour and ultrasonic vocalization.[67,85] Female rats were also shown to emit both high- (32–60 kHz) and low-frequency (20–32 kHz) ultrasonic calls in agonistic encounters, with the rate of high-frequency calls enhanced during oestros. Low-frequency ultrasounds were shown to be shorter in duration and higher in frequency than those emitted by male rats in similar conditions.[86]

Groups of three male and two female Long-Evans rats, living in a visible burrow system, showed high levels of 18–24 kHz ultrasonic cries when presented to a cat (predator). These cries were emitted both during cat presentation and for 30 minutes following removal of the cat, with the rats preferentially staying in the tunnel/burrow system ('hiding'). In the same study, when single-housed rats were confronted with the cat, either with or without a place of concealment, almost no vocalizations were detected. Blanchard *et al.*[83] suggested that the production of

ultrasonic vocalizations during and after exposure to a predator is greatly facilitated by the presence of familiar conspecifics, and may serve as alarm cries.

Both male and female rats emit ultrasounds during copulation. The sexual ultrasonic calls were found to be divided into 22 and 50 kHz calls. The 22 kHz calls are emitted pre- and postejaculation.[87,88] Songs of male rats were described by Barfield and Geyer[87] as pulses of 1–3 s long and regular, 22–23 kHz in frequency and an intensity that can increase up to 80 dB. Female rats have been shown to produce short (10–200 ms) 40–70 kHz vocalizations during copulation.[67] Female rats emit different ultrasounds during the entire oestrus cycle. In response to a devocalized sexually experienced male, scent marking and 50 kHz ultrasonic mating vocalizations of Long-Evans female rats were shown to change in frequency across the cycle, both behaviours being highest at proestrus/early oestrus, which might indicate vocalization as an important factor coordinating reproduction.[89] Male pre-ejaculatory 22 kHz calls have also been suggested to be important for inhibition of female agonist behaviour and for stimulation of the female sexual responsiveness.[90]

Pregnant females emit more ultrasound than non-pregnant females and a diurnal variation has been shown for both, more sounds being emitted during the dark than during the light period.[91] Mother–pup relationship involves the emission of sounds, from the audible to the ultrasound range. Pups emit ultrasound distress calls in response to cold, isolation or unusual tactile stimulation.[67] Calls are emitted by rat pups when they are manipulated or moved by the mother. Unusual tactile stimulation can also induce vocalization. During handling, pups' calls are audible from day 2 onwards, later consisting of both audible sounds and ultrasounds, and at day 10 of only ultrasounds.[92]

When analysing the response of rats to two types of ultrasonic vocalizations (50 and 22 kHz) in an emergence test, Burman *et al.*[93] suggested that rats use different ultrasonic vocalizations to indicate either positive emotional state (50 kHz) or negative emotional state (22 kHz). Rats that received playback of the 22 kHz ultrasonic vocalizations showed an increased latency to emerge and spent less total time in the open arena than rats receiving playback of background noise, suggesting a state of increased anxiety; therefore, the authors suggested that 22 kHz vocalization could induce a negative emotional state in the rats hearing it and could therefore be useful as a welfare indicator for group-housed rats, including both callers and non-calling group mates.[93]

Because of its short wavelength, ultrasound fails to penetrate physical barriers well and do not pass through a standard plastic cage, which might protect rodents from ambient ultrasound (depending on the cage structure), except during cage changes or other procedures involving opening the cage.[42]

The emission of sounds by rats under stressful situations has also been reported. For example, ordinary handling of rats elicits both audible and ultrasonic calls.[67,94] Ultrasonic calls were detected when male rats were gently stroked on the head, neck, side of trunk or tail (with the most reactivity being shown when handling the dorsal neck and the least sensitive area being the tail).[67,94] The response to human handling consisted of multiple series of long 22 kHz calls. The number of ultrasound vocalizing (with the range from 21 to 32 kHz) Wistar Han rats in response to hand touch was shown to be significantly influenced by their housing condition – single versus community cages. Only 60% of the grouped-housed rats emitted ultrasonic calls during the first session of brief hand touch, compared with 100% of the single-housed rats responding to the same tactile stimuli. Rats became quickly habituated from session to session, until extinction of response. Brudzynski and Ociepa[94] suggested this quick habituation indicates that the distress response – ultrasonic vocalization of rats – could be caused by a potential danger or threat to the animal and it does not necessarily reflect physical discomfort or pain. Pain-induced vocalizations by laboratory rats are also reported in the literature. For example, electrical stimulation of tail or foot elicited both audible and ultrasonic vocalizations at 20–35 kHz.[95,96] Acoustic stimuli, which induce a startle response, were also shown to evoke ultrasonic vocalization in the rat. Startle-inducing acoustic stimuli evoked continuous ultrasonic calling, with 50–70% of male Wistar rats emitting long (0.5–1.2 s) ultrasonic vocalizations (22 kHz call). Comparing the development of vocalization behaviour of those rats with the development of freezing in experiments of fear conditioning, Kaltwasser[97] concluded that startle-eliciting stimuli may induce a state of fear in the rat.

Auditory effects of sound

Regarding noise influences on laboratory animals' physiology and behaviour, both auditory (hearing damage) and non-auditory effects have been reported and revised in the literature.[66,67,69,70,98,99] Intense noise exposure can damage the cochlea and inner ear and lead to a cascade of auditory effects along the entire central auditory cascade. Susceptibility to noise-induced hearing loss is species/strain dependent, and it has been shown to be genetically determined in inbred strains of mice.[68] Exposure to uniform stimulus patterns may lead more readily to hearing loss, whereas exposure to irregular patterns may be more likely to cause disorders due to repeated activation of the neuroendocrine system.[98] As previously mentioned, rat pups are most sensitive to auditory damage before 22 days of life, during the period of anatomical differentiation of the ear structures.[80] Age-related deterioration of hearing function has also been reported in rats, not only as a natural/physiological consequence of aging but also resulting from noise stimuli.[100,101] Hearing loss in aged rats has been reported in about 20–25-month-old rats, with the largest hearing loss reported between 24 and 40 kHz.[67,77,101] Constant white noise might also have negative consequences for the normal development of the auditory system of the animals by effectively masking out the normal input to the ear from vocalizations and other sources.[68]

Audiogenic seizures are another possible auditory effect of sound. An animal with these sound precipitated convulsions might crouch, shiver and indulge in substitute behaviour (e.g. grooming) after a sound stimulus. This stage is immediately followed by uncontrolled running and convulsion involving tonic and clonic episodes.[102] Audiogenic seizures can produce stressful effects in rats. D'Amour

et al. showed that the induction of multiple audiogenic seizures (15–20 in 1 day) had an effect on rats' physiology producing increases of adrenal weights measured as ratios of body weight. The same was not true with the induction of a single seizure.[103] In a study by Duncan, the adrenals of albino rats killed after single or repeated one-a-day audiogenic seizures were weighed and analysed for ascorbic acid, cholesterol and corticosteroids. Adrenal hypertrophy and an increase in corticosteroids were shown as response to repeated stress and decreases in cholesterol and ascorbic acid and increase in corticosteroids were demonstrated as acute response to audiogenic stress.[104] Rats' 'hyperbaric oxygen seizures' have been shown to be caused by the loud hissing of oxygen as it entered a chamber where the animals were placed, in combination with the rough handling of those animals, being in fact a consequence of noise and handling stress.[105] There are differences on susceptibility to audiogenic seizures among different strains of rodents (genetically-determined susceptibility), but non-susceptible strains can be made audiogenic seizure-susceptible by exposure to particular stimuli during a critical period of postnatal development. Acoustic priming of Long-Evans rats with intense sound during postnatal development subsequent to auditory function was shown to generate audiogenic seizure susceptibility. The rats were exposed to 125 dB SPL 10 kHz tone bursts at 14–36 days of age and tested with white noise at 14 or 19 days following sound exposure. All priming/testing combinations yielded audiogenic seizure susceptibility. All subjects displayed clonus at testing intensities of 120 dB, although some seizure behaviours could be elicited at 100 dB. Repeated testing at 120 dB increased latency to clonus and clonus duration, and total wild running activity.[106]

Non-auditory effects of sound

Several non-auditory effects of noise are described in the literature. The noise level in animal houses and laboratories can be sufficient to act as stressors. Noise activates the sympathetic division of the autonomic nervous system, producing a stress response with physiological characteristics similar to those triggered by other sensory stimuli.[68] Intense noise can cause alterations in gastrointestinal, immunological, reproductive, nervous, and cardiovascular systems, blood cell counts, as well as changes in development, hormone levels, adrenal structure, metabolism, organ weights, food intake, body weight and behaviour. These findings have been reported and revised by several authors.[10,66–68,70,98,99] Below are some examples of non-auditory effects of noise in laboratory rats.

A decreased body weight gain and food intake was shown in Wistar male rats submitted to noise stress (15 min daily exposure to an acoustic tone of 2640 Hz, 30 W).[107] Wistar rats exposed, in an acoustic chamber, to 1.5 h of white noise per day at intensities of 107–112 dB displayed increased adrenal weights when compared with the control condition of 60 dB (background noise level). High-intensity noise-exposed rats had increased total leukocyte counts and a relative eosinopaenia. During the six days of noise exposure, noise-exposed rats gained about 15% less weight than controls.[108] Increased vasoconstriction and increased HR have been reported as non-auditory effects of sound in rats (revised in Turner *et al.*[68]). Morseth *et al.*[109] showed an increase in blood pressure of normotensive female rats exposed daily to white noise (at 67–124 dB) for 8 h, during a two-week period. However, no change in blood pressure was noticed after lifelong (over 2 years) daily 10 h exposure to 85 or 105 dB broadband sound, which might be a consequence of habituation.[67,68,110]

Several studies have shown altered hormone levels in response to noise exposure. For example, increases in the levels of norepinephrine, cholesterol and corticosterone have been reported. The increase in stress hormone levels suggests a noise-induced activation of the hypothalamic–pituitary–adrenal axis (HPA axis), which might cause several problems related to abnormally elevated levels of circulating stress hormones.[68] Armario *et al.* studied the effects of acute and chronic noise on serum levels of pituitary hormones in male Wistar rats. They found that acute noise stimuli increased serum levels of corticosterone, prolactin, and LH and decreased serum growth hormone (GH), while follicle-stimulating hormone was unaffected by this stressor. Chronic noise did not modify basal levels of any hormone studied; however, responsiveness of some hormones to the same stimuli was altered: reduced corticosterone, prolactin and GH responses to noise were observed after previous chronic exposure to this stimuli.[111] Circulating testosterone levels were shown to be increased in Wistar rats that had been exposed to acute noise (1 h, 85 dB).[112] A rapid doubling of rat plasma corticosterone levels (lasting for 2–4 h) was also shown as a result of exposure to banging of metal cages in an animal room.[113] More recently, Burow *et al.* showed a noise-intensity-dependent increase in the plasma adrenocorticotrophine hormone and corticosterone (HPA axis products) in Sprague-Dawley rats in response to the perceived threat of loud noise, with levels beginning to rise at approximately 85 dB (A). In this study, the rats were exposed to noise for a period of 30 min, ranging from 80 dB (A) to 110 dB (A) (SPL) in increments of 5 dB (A). *c-fos* mRNA induction (a marker of regional brain activity) was very low in the brains of the control and 80 dB (A) groups, but several brain regions displayed a noise-intensity-related induction of this marker.[114]

The reproductive function of rats can also be affected by sounds. Zondek[115] showed that exposure of rats to ultrasounds of 50–80 kHz at 80–90 dB in the four days during the mating period reduced fertility by 73.2% and productivity by 84%. Exposure to 100 dB of 3–12 kHz for one minute during the four days of copulation reduced fertility by 70–80%.

Sleep disturbances induced by environmental noise have also been reported. Disturbances in sleep pattern were reported for pigmented Long-Evans rats after chronic exposure to environmental noise (similar to road traffic and railway noises: with a major octave band ranging from 20 to 3000 Hz) – composed of a background noise of 70 dB and several unpredictable noise events corresponding to a global intensity of 88 dB. A chronic exposure to this environmental noise (9 days) restricted continually amounts of slow wave sleep (SWS) and paradoxical sleep

(or rapid eye movement) and fragmented these two sleep stages with no habituation effect. Rabat *et al.*[116,117] also found evidence for the existence of subpopulations of rats that are either resistant or vulnerable to these deleterious effects of environmental noise on sleep and especially on SWS amounts, bouts number and bout duration.

An example of interference of sound with metabolism was reported by Friedman *et al.*,[118] who found that plasmid lipid (triglyceride) levels were doubled in rats that had been administered an oil-enriched meal during continuous 102 dB white noise with an intermittent one second burst of 114 dB of 200 Hz.

Loud noise exposure has also been reported to cause cellular effects. For example, ultrastructural alterations in Wistar rats' myocardium[119] and adrenal glands[120] have been shown in rats exposed to loud noise (100 dB) for 12 h. These ultrastructural changes were shown to involve mainly the mitochondria and endoplasmic reticulum. DNA damage in the above-mentioned organs was also found to be associated with this loud noise exposure.[119–121] Myocardium alterations were concomitant with increased *in situ* noradrenaline levels and utilization.[119]

Different levels of background noise were shown to influence learning and behaviour in rats.

In a study using outbred Wistar rats and testing several different sounds, Voipio showed that the behavioural responses depend on the type of sounds. Noise type of sounds caused fear reactions, such as startle, flight and freezing, even if at a low sound pressure level. Wave-type sounds induced movement or no response at low SPL, but more clear effects at high SPL.[67]

A decrease in locomotor activity of rats was shown in response to either natural 22 kHz calls or artificial 38 kHz signals, during and after ultrasound exposure. These Wistar rats were exposed to 5 min of non-stimulus sounds (background noise correspondent to tape recorder and tape noise) followed by 5 min of stimulus signal (further noise, 38 kHz signals or 22 kHz calls) and finally a further 5 min of background noise. The SPL of each of the ultrasonic signals was set to about 65 dB, similar to levels of 22 kHz calls recorded from defeated rats. Natural calls also decreased loudspeaker sniffing compared with the response if the speaker only emitted the background noise of the tape. Sales suggested this was probably because the animals have already experienced these 22 kHz calls in their social context, in their home cages, and might have learnt to respond to them with reduced activity. Calls emitted by other rats in the colony room, during group transportation or in the experimental room could affect the behaviour of experimental rats that can hear them.[122]

In open field behaviour, continuous white noise of 85 dB was shown to increase defecation and reduce both social activities and non-social activities (e.g. sniffing, grooming or crawling) of male Sprague-Dawley rats when compared with 50, 65 or 75 dB. As suggested by Weyers *et al.*,[123] the changes in social activities and defecation may be interpreted as anxiety reactions.

In a recent study, genetically-defined rats (DA inbred strain) learned a complex maze exposed to noise of moderate intensity (70 dB) or to a quiet environment ($\leq$35 dB). Noise-exposed rats made fewer errors, explored less and finished their trials sooner. Previous studies have shown that noise of an intensity level as used in this study increased choline uptake in several brain regions including the prefrontal cortex and the hippocampus.[124] Therefore the facilitating effects of noise might have been due to increased cholinergic activity.[125] Thus, the acoustic environment is a factor that needs thorough control in studies with animal models of learning and memory.

Animals are able to adapt to sound. Outbred Wistar rats have been shown to adapt to sounds after repeated stimuli. During the exposure period to different sounds (16 days: 4 days with exposure to different sounds of 60 dB SPL; 2 days of pause; 4 days with exposure to different sounds of 80 dB SPL; 2 days of pause and 2 days with exposure to rat scream at 60 dB + 2 days with exposure to rat scream at 80 dB), the onset adaptation to less harmful sounds was short, but more harmful sounds caused intense response and habituation took longer. Furthermore, it was mentioned that the adaptation 'memory' was short and that similar type of sound caused similar response even on the next day.[67]

Noise in the animal facility

Several studies have been published showing the different sounds that can occur inside the animal facility where environmental noise is virtually unavoidable. The sources of sound can be technical devices (such as air-conditioners, air handlers, ventilated rack systems, electronics, video monitors, laboratory equipments and fire alarms); maintenance procedures done routinely (such as opening and closing doors, changing cages, cage washers, push carts, workers' speech and even rubbing cloth during movements and crinkling paper towels); and animals themselves by their movements (e.g. climbing and chewing on cages and accessories) and by their vocalizations (as mentioned previously).[42,68,98,99,126–130] For example, in the analysis of sounds associated with equipments used in an animal facility, Sales *et al.* recorded SPL of 56 dB (at 1 m distance) and 70 dB (at 0.1 m distance) in a high-frequency range for the two computer systems studied. The computer monitor appeared to be the source of most of the sound (10–100 kHz spectrum showing regular pronounced peaks at frequencies higher than 15 kHz).[128] The constant frequency and continuous ultrasound produced by oscilloscopes (28 kHz with overall SPL of 48 dB at 1 m) and visual display units (producing a harmonic series of sound frequencies from 16 kHz up to 160 kHz, with overall SPL around 60 dB at 0.5 m) have been shown to reduce total activity of male rats in an open field arena. In this study, the sound output of taps running into a sink was measured, being characterized as a complex sound of continuous noise with broadband short bursts of sound within a frequency range up to 160 kHz with an overall maximal SPL of 95 dB.[127] These are examples of (ultra)sounds that can be produced in the animal facility being apparently silent to humans but in the hearing range of laboratory animals, such as rats.

Sound levels are specially produced during animal care activities being variable between weekdays and weekends, and between day and night time.[98,126] Milligan *et al.* recorded

the acoustic environment of rooms housing rats over a 24 h period. In one of the facilities, there was a peak in sound levels about 90–100 dB in the lower frequency range during normal working hours. In the high-frequency range, the sound profiles were also increased during working hours with levels often reaching 70–85 dB.[126]

The sound exposure levels inside the rat cage and in the adjacent cage, while workers developed care procedures (e.g. pulling cage out of the rack, placing it onto a table and replacing the cage back into the rack, putting food into the food hopper), have been recently measured by Voipio *et al.* Hurried work with steel caused sound exposure levels exceeding 90 dB(R) when the cages were placed into the rack and about 80 dB(R) when pulling them out of the rack or placing them onto a table. With polycarbonate cages, the levels were 15 dB(R) lower. Unhurried calm working produced lower sound exposure levels than hurried working in many procedures (about 10–15 dB(R) differences in both cage materials). When the same procedures were performed in adjacent cages, the sound exposure levels were lower, but the differences were similar. When comparing rat and human hearing, H-weighted sound exposure levels (for human hearing sensitivity) were about 10–20 dB higher than R-weighted (for rat hearing sensitivity).[129]

The value of using masking noise, such as radios, to reduce adverse effects of impulsive noise has been discussed. Music treatment, produced by playing the Herbert Von Karajan Adagio compact disk through loudspeakers (less than 40 dB) from 09:00 h until 14:00 h daily during eight days, has been shown to decrease the metastaticity of WRC 256 cells in Sprague-Dawley male rats of both groups: submitted and non-submitted to auditory stress (exposing animals to the fire alarm bell (100 dB)).[131] Loudspeakers of most domestic radios have a limited sound output to below 16 kHz and, as shown by Sales *et al.*, this would not cover the full frequency range of environmental noise which is within the range of most laboratory animals. Using radios in the animal house might therefore be a benefit to animal house staff, being indirectly a benefit to animals taken care of by those workers,[128] but the effect of background noise, such as radio and different types of music, in masking the effects of ultrasound and loud noises needs further investigation.

Noise can be involved in several scientific studies, not as a controlled experimental variable but as an unintended environmental variable that can in some cases confound animal-based research results,[68] interfering with animal welfare and stress.

A 'silent fire alarm'[132] was developed taking into consideration the difference in hearing abilities between humans and rodents, in order to fulfil the need for an effective alarm signal in laboratory animal facilities that did not stress the animals every time it was tested. The device developed produced pure tones alternating between 430 and 470 Hz, giving a sound level of 97 dBC at 450 mm – the energy in the alarm signal was below the optimal hearing range for mice and rats. Producing a noise 'intensely irritating and disturbing' for humans, this alarm did not awake rats and mice from sleep, and if already awake, rodents did not show a startle response, ear twitching or other indication of auditory disturbance when this 'silent fire alarm' was switched on.[132]

In conclusion, both environmental and communication sounds are present in animal facilities having a wide variety of effects in animals' physiology and behaviour, and consequently influencing animal welfare and animal-based research results. Environmental noises differ among different animal facilities and a careful planning should be made before construction of the animal facility, with the help of acoustic engineers, taking into account the differences in the sound perception between rodents and humans,[69] in order to avoid stressful environmental sounds both for the animal and personnel. Procedures that may elicit both audible or ultrasound vocalizations should take place in a location that will not allow those sounds to reach other animals (unless that is part of the experiment).[42] Animal facility staff and researchers working at the animal facility should try to avoid uncontrolled ultrasounds (which might interfere with animals' communication sounds) and audible sound production. Measurements should be made in each animal facility in order to register existing environmental sounds and detect existing ultrasounds.

Cage cleaning and in-house transport

Laboratory rats are housed in standardized conditions and their maintenance requires handling by humans. Moving an animal from one cage to another by handling (cage cleaning) or transporting cages with animals inside a laboratory or between laboratories (in-house transport) are routine procedures in animal facilities. But how do animals react to these procedures? Does it interfere with animals' physiology and behaviour affecting their wellbeing by causing stress/distress, and if so, how long does it take for an animal to regain its normal physiological condition? This review addresses the impact of animal facility routines such as cage cleaning and in-house transport and its influences on laboratory rat physiology, behaviour and welfare.

Cage cleaning (and transport)

Several authors have shown that cage cleaning and in-house transport promote an acute stress response on laboratory animals. Armario *et al.* reported that cage cleaning of adult male Sprague-Dawley rats, housed in groups of three, elevated serum concentrations of corticosterone[133] and prolactin.[134] These endocrine stress responses were measured 15 min after the onset of acute stress (when rats were bled by decapitation) and were dependent on the intensity of the stimulus. When rats were submitted to cage cleaning alone, the increases in plasma corticosterone and prolactin were significant but lower than when rats were submitted to cage cleaning followed by transport to a new room. Cage cleaning and transport to a noisy room (alarm bell of 85 dB) were more stressful than cage cleaning and transport to a quiet room.[133,134] Barrett *et al.* reported a three-fold higher plasma corticosterone level in male Wistar rats bled after being transported to a laboratory compared with rats bled in their holding room.[113,135] Moving adult male Han:Sprague rat cages from the racks to the floor was shown to result in a significant increase in plasma

corticosterone levels within 5 min, reaching a peak at 15 min and returning back to baseline levels after 60 min.[136] In this experiment, Gärtner *et al.* also showed a significant increase of serum levels of prolactin, thyroid-stimulating hormone (TSH) and triiodothyronine (T3) within 15 min after moving cages, and after 60 min the levels of these hormones in the serum of transported rats were still elevated.[136] Regarding effects of moving cages on HR, Gärtner *et al.* reported an increase in HR from 340 to about 450 beats per minute (recorded telemetrically in unrestrained rats carrying a permanently implanted transmitter), tachycardia persisting for 10–15 min. Other effects on microcirculation, such as alterations of packed cell volume, haemoglobin and plasma protein, increased significantly within 2 min after moving cages and lasted about 10 min.[136] Disturbances in metabolism, which were suggested to be a consequence of endocrine and microcirculatory alterations and the activation of the sympathetic–adrenal system, were also found after moving cages: plasma glucose increased significantly 3–8 min after moving cage; plasma lactate and pyruvate increased 2–3-fold above control levels 1–3 min after cage moving. These effects of cage moving on metabolism lasted only for 10 min.[136]

Activation of the sympathetic nervous system has also been associated with routine housing procedures such as cage cleaning. Significant increases in plasma noradrenalin and adrenalin were reported by Boer *et al.* immediately after gently lifting catheterized male Wistar rats (individually housed) from their home cages and placing them in a new cage (identical to the first cage but without bedding, food and water) for a period of 15 min before returning the animals back to their original cage.[137] A significant increase in plasma corticosterone and glucose concentrations was also detected at 15 min after the placement of the rats into the new cage, returning to baseline levels at $t = 45$ min. The increased levels of catecholamines, corticosterone and glucose found in rats that have been handled and placed in a new cage were assumed to result from psychological activity in response to the change in environment (e.g. fear or emotional arousal), rather than being the result of physical activity or peripheral physiological changes induced by these environmental alteration.[137]

Even though using old anaesthetics that are not recommendable anymore,[138] Tabata *et al.* found a significant rise in plasma glucose levels in mice following handling and transport of the cage to an adjoining room, with plasma glucose levels appearing to return to normal levels after about one hour. The same set of procedures, when performed on F344 and Sprague-Dawley rats (males and females), seemed to have small or no observable effect on levels of plasma glucose.[139] The authors argue that rats might have acclimatized more easily to the handling procedure used than mice, which might have reduced the release of glucose into the bloodstream as a physiological stress response.[135,139]

Laboratory rats' behavioural responses to cage cleaning have also been addressed. The behaviour of male Sprague-Dawley rats in their home cages have been shown to vary with time of day and cleaning regime, with rats showing more activity on cleaning days increasing behaviours such as locomotion, grooming, digging and climbing and reducing sitting.[140] Saibaba *et al.* interpreted this increased activity after cage changing as a sign of disturbance or a response to novelty, since the olfactory and possibly the visual and tactile environments were altered within the cleaned cage and could stimulate exploratory behaviours. Animals were also reported to tend to be more active in the morning periods than in the afternoon periods tested, which might be related to the arrival of staff and beginning of the working day with a general increase in noise levels.[140]

More recently, physiological responses to cage cleaning have been studied using radiotelemetry: increases in HR and blood pressure in both male and female rats have been reported in response to a variety of handling procedures.[141–146] For example, studying the effects of routine cage cleaning on cardiovascular and behavioural parameters, Duke *et al.* observed a prompt increase in systolic, diastolic and mean arterial blood pressure (MAP), HR, and cage behaviour (such as movement, rearing and grooming) in adult male Sprague-Dawley rats when placed in clean cages. Elevations in HR and MAP in response to cage change had a duration of approximately 45–60 min. Rats witnessing this procedure did not show significant changes in HR or MAP. Manipulated animals also became aroused, showing increased awake, moving, rearing and grooming behaviours for at least 45 min whereas witnessing animals showed much less activation, and most returned to sleep by 15–30 min after cage change occurred. Adding a small amount of soiled bedding from the previous home cage to the new one did not modify increases of HR, MAP or behaviour, indicating that familiar olfactory cues did not counteract the novelty of the new cage.[141] However, in male mice it made a remarkable difference in aggressive encounters whether soiled bedding or the old paper nest was transferred into the new clean cage.[147] Rats whose cage had not been changed for a period of two weeks presented an earlier onset of increased HR and MAP increased more rapidly in response to cage change, presenting a more prolonged cardiovascular response when compared with the response of rats whose cage has been changed weekly.[141] Grooming was less in the last 15 min of the observation period following cage change in the animals whose cage has been changed weekly compared with those whose cage had not been changed for a period of two weeks. Repeating these experiments in four consecutive weeks, researchers reported that cardiovascular and behavioural responses to the fourth change were not different from those observed in the first week, indicating the animals did not habituate to the procedure.[141]

When measuring the cardiovascular response of adult male Sprague-Dawley rats to several common procedures (cage change, restraint and subcutaneous injection, restraint and tail-vein injection, exposure to odour of urine and faeces from stressed rats, and exposure to the odour of dried rat blood), by radiotelemetry, Sharp *et al.*[145] also found significant increases in HR of rats housed individually, with one cage mate or with three cage mates, but HR in the four-to-a-cage rats decreased more rapidly, reaching baseline levels within 30 min. In response to routine cage change, rats housed individually or with one cage mate showed significant HR increases that were observed for 90–120 min after the movement to the

new cage. Changes in MAP after cage cleaning showed the same patterns as HR, except in the case of rats housed four per cage in which MAP decreased significantly below baseline values from 90 to 180 min after the cage change.[145] The decrease of HR and MAP below the baseline levels in the rats housed four per cage after routine cage change was suggested to result from the exposure to the odour of ammonia from soiled bedding that had accumulated in the cage during the four days since the cage was last changed. Manipulation of rats also affected their behaviour increasing the number of arousal behaviour such as moving, rearing and grooming, the increases being more prevalent in individually-housed than group-housed rats.[145] When testing individually or group-housed adult female Sprague-Dawley rats, the same authors also found significant increases in HR following routine cage changing and for durations of 30–90 min before HR returned to baseline, suggesting the rats were stressed.[143] In this study, transporting rats to another laboratory for subcutaneous injection was also shown to significantly increase HR (equivalently in all housing groups). Active behaviours of female rats in the home cage were increased for at least 30 min after the husbandry procedure.[143] Group-housing frequently reduced the stress-like response.[143,145] The physiological responses observed were similar during cage changing or simulated cage changing (where rats were returned to the original cage), suggesting the responses are caused by the physical manipulation rather than unfamiliar aspects of the new cage.[143] No noteworthy differences were registered by Sharp *et al.* in the cardiovascular (HR and MAP) and behavioural responses induced by cage changing or witnessing cage changing, between females in different stages of the oestrus cycle (proestrus–oestrus and metestrus–diestrus), but the authors noticed that female rats showed more cardiovascular (stress-like) and arousal responses than did male rats in the previous study, suggesting an influence of gonadal hormone status.[146] Female rats witnessing the cage change procedure also showed greater cardiovascular response than did male animals, and females showed noteworthy increases in response to room entry whereas males did not. Regarding home-cage behaviour, males showed two times more sleeping behaviour and much greater differences between manipulated and witnessing groups than females and no markedly effect of stage of the oestrus cycle was noticed in females.[146]

In another study by Sharp and collaborators, it was reported that witnessing routine procedures such as cage changing does not induce significant stress-like responses such as the ones observed in animals submitted to cage cleaning. Only Sprague-Dawley male rats housed alone or with only one cage mate had small increases in HR, MAP and home-cage behaviours while witnessing cage change.[142] In contrast, female Sprague-Dawley rats witnessing cage changes showed greater responses, but increases in HR and active home-cage behaviours were also reduced in females housed in groups than in single-housed females, suggesting that group-housing reduces stress or potential for stress.[144]

In 2006, a cross-laboratory study by Burn *et al.*[148] reported that cage cleaning frequency had no clear impact on rat welfare. Male Sprague-Dawley and Wistar rats, housed in groups of four, were kept in four different animal units and their cages were cleaned twice-weekly, weekly or every two weeks, and contained either aspen woodchips or absorbent paper bedding. Among other parameters, aggression, injuries and general health, weight gain, handleability and in-cage ammonia concentration were monitored. Frequent cleaning decreased ammonia concentrations and handleability, and non-aggressive skirmishing was highest in weekly cleaned rats. However, no clear welfare benefit or harm was associated with any of the cage cleaning frequencies of these socially-housed male rats, as no differences in growth rates or general health have been found when comparing animals from frequently cleaned with less frequently cleaned cages.[148] In agreement with previously mentioned studies (e.g. by Duke *et al.*[141] and by Sharp *et al.*[145]) when observing the behavioural activity of male Sprague-Dawley and Wistar rats immediately after cage cleaning, Burn *et al.* reported an increase in the general activity of rats, including walking, bedding manipulation and feeding, which were above baseline levels for the full 30 min observation period and during this period the number of rats resting did not return to precleaning levels. A marked increase in non-aggressive and play-like skirmishing to above precleaning levels was also noticed, but the effect was transient and after 15 min this skirmishing behaviour returned to below precleaning levels. Since cleaning frequency did not affect the magnitude of the postcleaning peak in skirmishing, Burn *et al.*[149] suggested that the peak is neither caused by any relative change in the olfactory environment nor influenced by how habituated rats are to disturbance. In this study, rats were also observed to move more frequently to sheltered areas after cage cleaning, which might indicate they are trying to avoid light while undisturbed rats (in the day before cleaning) remained in their resting position kept since the dark period or it can indicate an exploratory behaviour motivated by the environment of the new cage. No behavioural evidence was found suggesting that the increased postcleaning activity was an acute stress response corresponding to a negative effect of cage cleaning on rat welfare.[149] Chromodacryorrhoea (a dark red stress-related secretion produced by the Harderian gland in the orbit of the eye) was scored around the nose of rats after the behavioural observation period (i.e. 35–45 min after cage cleaning) and was found to be higher on the day before cage cleaning than after cleaning, suggesting that having soiled bedding was more stressful than the cleaning procedure itself, but since the scoring was made only after the observation period it might have been groomed away by rats before scoring (e.g. chromodacryorrhoea has been shown to be released between 16 and 30 min after acute stress induction by limb restraint).[149,150]

In-house transport effects

As previously mentioned, in-house transport of laboratory rats induces several physiological (endocrine, cardiovascular and metabolic) responses, such as increases in plasma corticosterone, prolactin, TSH and T3[113,133,134,136]; increase in HR; increases in haemoglobin

and plasma protein; and increase in plasma glucose.[136] Other reports can be found in the literature on behavioural and physiological changes resulting from in-house transport of laboratory rats, showing an acute stress response to the transportation procedure or to its witnessing. For example, the behaviour of non-transported Wistar Cpb:WU rats has been shown to be altered in the presence of rats that had been transported in their cages on top of a trolley pushed through the animal house for 2–3 min.[151] An open box with two adjacent small fields was used and each animal – one transported and one non-transported – was placed in each field and their behaviours were assessed: transported female rats showed significantly decreased sniffing and rearing and increased grooming (suggesting the induction of transportation stress), whereas non-transported rats displayed significantly increased sniffing and tended to urinate more frequently. Since there was no physical contact between transported and non-transported rats, de Laat *et al.*[151] suggested that communication might have occurred through sounds or odours from transportation-stressed rats affecting the behaviour of non-transported rats.

More recently, Dallmann *et al.*[152] have shown that moving the cage of group-housed male F344/Hw rats within the holding room or transferring the animals, in their cage, between holding and test rooms (through a noisy corridor with constant background noise caused by ventilation and pig holding rooms on the other side of the corridor) resulted in a significant increase in BT (T_b). When moving the cage inside the holding room, the stress-induced hyperthermia (SIH) corresponded to an elevation in rats' T_b of more than 0.5 °C for the following 120 min. Transportation between holding room and test room was shown to increase rats' T_b over time lasting for at least 60 min. The fact that rats' SIH lasted for 120 min when transporting the cage from the rack to a table inside the holding room made Dallmann *et al.* suggest that, before starting an experimental procedure, habituation periods after in-house transport should be up to 120 min or even longer.[152]

Cage cleaning and contact with odours

Odours presented to laboratory animals during cage cleaning might represent a stressor and have an impact on animal wellbeing. Personnel performing the routines of cage changing and cleaning can be a source of odours, personal odours or odours from other cages or animal rooms. Rats might fear humans carrying scents from pets[69] or from other animal rooms in the animal facility via fomites (clothing, hands), which is one important reason why all personnel working in the animal facility should wash their hands and change laboratory coats between rooms,[42] avoiding the transmission of odours (inter-species and between groups of animals of the same specie) and also avoiding contact of their clothes used outside the animal facility with the laboratory animals. Presenting predators' odours (e.g. cat fur/skin odours) to laboratory rats can elicit defensive behaviours, fear and anxiety (revised by Blanchard *et al.*[153]). Natural human odours or perfume and deodorants' odours associated to humans might also be stressful to the laboratory rats. For example, odorants and smells (volatile chemicals), often being present in perfumed products, can influence HPA activity and immune responsiveness[154] or be anxiolytic to rats.[155] Even though the effect of human odours (natural or artificial ones) in rats needs further investigation, humans should avoid bringing predators' smells, perfumes and deodorants' odours into the animal facility where they are in contact with laboratory animals, during routine husbandry procedures (such as cage cleaning) or experimental procedures.

Cage cleaning and ventilation

Another aspect that affects cage cleaning and animal welfare is ventilation. The air movement in the cage is related to other important environmental factors such as temperature (affecting the thermoregulatory capacity of animals) and relative humidity. Ventilation and air renewal at cage level affects its microenvironmental air quality in terms of microorganism concentrations, dust particles and noxious gases (such as ammonia and carbon dioxide).[10] To establish the frequency of cage cleaning in an animal facility, all these factors have to be considered because of their effects on the animals. For example, the prevalence of pneumonia was shown to increase with ammonia levels inside the cage (from 25 to 250 ppm) and pathological changes were found in the respiratory tract of rats exposed to soiled bedding with 100–200 ppm NH_3 levels, which can be found in rat cages (without filtered tops) after a week without cleaning.[156,157] Most rapid ammonia production occurs under conditions of high humidity[157] and the type of bedding also has an influence.[158] Ammonia and carbon dioxide levels in individually ventilated cages (IVC) were studied by Silverman *et al.* during a seven-day period without cage cleaning. In this study, ammonia levels reached values $\geq$ 500 ppm after three days and intracage carbon dioxide increased rapidly until values $\geq$ 10,000 ppm.[158] In another study analysing different ventilation rates and cage changing frequencies and their impact on C57BL/6J mice house in ventilated cages, Reeb-Whitaker *et al.*[159] concluded that cage changes once every 14 days and ventilation rates of 60 air changes per hour (at which ammonium levels were about 50 ppm in trio-mated mice) provide optimum conditions for animal health and practical husbandry and had no adverse health effects. Further studies are needed in order to have more information on the welfare consequences of increased ammonia concentrations and there are no guidelines for the maximum ammonia concentration to which rodents can be exposed to. Based on guidelines for human exposure and veterinary literature, Silverman *et al.* suggested an intracage concentration of 50 ppm should lead to cage cleaning of mice housed in IVC or disposable static cages.[158] Different IVC systems might also provide different cage microenvironments and each case has to be investigated.

As mentioned, constant air renewal is needed and adequate air changes in the rats' close environment are essential to control their microenvironmental temperature, humidity and air quality.[10] Animal facilities usually have between 15 and 20 air changes per hour in animal rooms, as indicated in Appendix A of Council of Europe Convention

ETS 123.[2] IVC systems enhance the ventilation inside the cages to higher rates of air changes per hour allowing facilities to reduce cage change frequency, but these systems constitute a relatively novel source of potential discomfort. For humans, air speed levels exceeding 0.2 m/s is considered draughty and this is also generally agreed to be an upper limit for rodents.[160] Higher intracage ventilation rate could induce chronic stress and heat loss due to the draught. It has been shown in preference tests that rats choose cages with lower than 80 air changes per hour.[161] In respect of mice, their physiology and behaviour were not affected when there were fewer than 80 air changes per hour, the air inlet came from the top and nesting material was provided. Thus, the location of the air supply in the cage (from the wall or from the top), the ventilation rate and the presence of nesting material are important when considering the impact of IVC housing on mice wellbeing.[162]

In conclusion, common husbandry procedures were indeed shown to induce stress-like responses in laboratory rodents. As mentioned above, cage cleaning and in-house transport have a considerable impact on physiological and behavioural responses in rats, characteristic of an acute stress response, but a clear impact on rats' welfare has not been proven yet. Researchers should be careful with the animal facility cage cleaning schedules when planning studies. Proper ventilation systems should provide a good ventilation control in the animal facility and consequently at the cage level. To establish the cage cleaning frequencies, animal facility personnel should have the possibility of making measurements of the intracage microenvironment (e.g. humidity, temperature, noxious gas levels) and these data should also be available for researchers. Care should be taken in dismissing husbandry procedures as non-stressful just because they are routine. These common procedures might influence experimental data if not taken into account or controlled for. For example, when measuring peripheral endocrine responses to stressors, researchers should perform blood sampling quickly (e.g. within 100 s of first touching the animals' cage) to avoid influences of the stress response to the animal/cage manipulation itself on experimental results[136]; in studies that require determination of basal cardiovascular parameters, Sharp *et al.*[143] recommended that data should not be obtained for at least 2 h after common husbandry procedures. By applying radiotelemetry technique in mice, Meijer showed that responses of HR and BT paralleled corticosterone responses to various routine procedures (e.g. different methods of restraint, injections by different routes, cage cleaning). Given the acute stress responses of laboratory animals to routine procedures, Meijer[163] stated that basal values of HR, BT or corticosterone should not be assessed directly after the animals have been subjected to such procedures – a recovery period of at least one or two hours was recommended in order to improve experimental procedures and to obtain reliable data for basal measurements.

Other stressors during cage cleaning might be the contact with humans and the odours they carry. When handling the animals for cage cleaning, all personnel should avoid the use of clothes that were in contact with rats' natural predators, the use of deodorants and perfumes, and use the appropriate protection clothing and procedures against odours spreading.

Conclusion

Human interaction and physical environmental factors are part of the stimuli presented to laboratory animals everyday, influencing their behaviour and physiology and contributing to their welfare. Certain environmental conditions might induce stress responses and when the animal is unable to maintain its homeostasis in the presence of that particular stressor, the animal's wellbeing is threatened and the animal might suffer from distress. Environmental factors such as cage size and structure/enrichment, NH_3 and CO_2 levels, light (intensity, wavelength, photoperiod and flicker frequency), sounds, air/ventilation, temperature, relative humidity, odours, presence/absence of pathogens and human presence/interaction are as important as the presence or absence of conspecifics, their sex, and the predictability and controllability of the environment in what refers to animal welfare implications.

Rats prefer low light intensity and a well-controlled photoperiod will certainly contribute to stable circadian rhythms. The position of the cage in a rack or room in relation to the light source, or the presence of enrichment objects allowing the animal to hide are determinant factors for the amount of light the animal is exposed to.

The auditory sensitivity of rodents is different from humans and special attention should be paid to the production of sounds and ultrasounds in the animal facility. Ultrasounds are more difficult to control because they are audible to rats but not to humans, so it might be advisable to measure the sources of these sounds in the animal facility. It is not completely clear if a background noise such as a radio could contribute to the laboratory animal's welfare, but it was also not shown to disturb them and it might be an 'enrichment' for animal care staff and consequently will benefit the animals. If considering the use of a radio in rat holding rooms, care should be taken to keep the volume low.

Routine procedures in laboratory animal facilities include moving animals from a dirty to a new clean cage – cage cleaning – and transporting cages with animals inside a room or between rooms – in-house transport. Even being usually simple and relatively quick procedures, several authors have shown that they induce acute stress responses in laboratory animals. Laboratory rats' behaviour and physiology can be altered as a result of these procedures for periods up to one or two hours. Even though it is not clear whether cage cleaning and in-house transport affect animal welfare, these procedures have been shown to alter physiological and behavioural parameters for a period of time. From the literature it is obvious that 'simple' routine procedures cannot be considered as non-stressful for the animals, even when best practices are adopted and the housing conditions are the most adequate for the animal species. Cleaning cages and simply moving a cage from the holding room to the experimental room interfere with the animal's behaviour and physiology and might interfere with experimental results if they are not controlled for.

Given the vast list of physiological and behavioural effects of light and acoustic environment and of husbandry reported in the literature and reviewed here, it is obvious that these factors are very important environmental factors to be controlled in the animal house in order to avoid causing distress to laboratory animals that might lead to poor animal welfare and consequently to poor experimental results. Animal facility routines such as cage cleaning and simple in-house procedures such as transport of cages to the experimental room should also be well established and accounted for when planning animal experiments, even though more research is needed to fully understand their impact on laboratory animals.

REFERENCES

1 Russell WMS, Burch RL. *The Principles of Humane Experimental Technique*. London: Methuen, 1959
2 Council of Europe Convention ETS 123. *European convention for the protection of vertebrate animals used for experimental and other scientific purposes*. 1986
3 Baumans V. Science-based assessment of animal welfare: laboratory animals. *Rev Sci Tech* 2005;**24**:503–13
4 Koolhaas JM, Baumans V, Blom HJM, von Holst D, Timmermans PJA, Wiepkema PR. Behaviour, stress and well-being. In: Van Zutphen LFM, Baumans V, Beynen AC, eds. *Principles of Laboratory Animal Science: A Contribution to the Humane Use and Care of Animals and to the Quality of Experimental Results*. 2nd edn. Amsterdam: Elsevier Science BV, 2001: 77–102
5 European Union. *Directive 86/609/EEC: Directive on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes*. 1986
6 Morton DB, Hau J. Welfare assessment and humane endpoints. In: Hau J, Van Hoosier GL Jr, eds. *Handbook of Laboratory Animal Science: Essential Principles and Practices*. Boca Raton: CRC Press LLC, 2003:457–86
7 Van der Harst J. *Tools to Measure and Improve Welfare of Laboratory Rats: Reward-related Behaviour and Environmental Enrichment* (PhD thesis). Faculty of Veterinary Medicine, Utrecht University, 2003
8 Wiepkema PR, Koolhaas JM. Stress and animal welfare. *Anim Welf* 1993;**2**:195–218
9 Brambell Committee. Report of the Technical Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems. London: Her Majesty's Stationary Office, 1965: Report No: Command paper 2836
10 Clough G. Environmental effects on animals used in biomedical research. *Biol Rev* 1982;**57**:487–523
11 Koolhaas JM. The laboratory rat. In: Poole T, ed. *The UFAW Handbook on the Care and Management of Laboratory Animals: Terrestrial Vertebrates*. 7th edn. Oxford: Blackwell Science, 1999:313–30
12 Moberg GP. Biological response to stress: implications for animal welfare. In: Moberg GP, Mench JA, eds. *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare* Wallingford, UK: CABI Publishing, 2000:1–21
13 Beynen AC, Gärtner K, Van Zutphen LFM. Standardization of animal experimentation. In: Van Zutphen LFM, Baumans V, Beynen AC, eds. *Principles of Laboratory Animal Science: A Contribution to the Humane Use and Care of Animals and to the Quality of Experimental Results*. 2nd edn. Amsterdam: Elsevier, 2001:103–10
14 Hurst JL. Introduction to rodents. In: Poole T, ed. *The UFAW Handbook on the Care and Management of Laboratory Animals: Terrestrial Vertebrates*. 7th edn. Oxford: Blackwell Science, 1999:262–73
15 Renner MJ, Rosenzweig MR. Social interactions among rats housed in grouped and enriched conditions. *Dev Psychobiol* 1986;**19**:303–13
16 Devenport L, Dallas S, Carpenter C, Renner MJ. The relationship between adrenal steroids and enrichment-induced brain growth. *Behav Neural Biol* 1992;**58**:45–50
17 Blom HJ, Van Tintelen G, Van Vorstenbosch CJ, Baumans V, Beynen AC. Preferences of mice and rats for types of bedding material. *Lab Anim* 1996;**30**:234–44
18 Vesell ES. Induction of drug-metabolizing enzymes in liver microsomes of mice and rats by softwood bedding. *Science* 1967;**157**:1057–8
19 Li Z, Okano S, Yoshinari K, *et al.* Soft-hydrothermal processing of red cedar bedding reduces its induction of cytochrome P450 in mouse liver. *Lab Anim* 2009;**43**:205–11
20 Jacobs GH, Fenwick JA, Williams GA. Cone-based vision of rats for ultraviolet and visible lights. *J Exp Biol* 2001;**204**:2439–46
21 Jacobs GH, Neitz J, Deegan JF. Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature* 1991;**353**:655–6
22 Perez J, Perentes E. Light-induced retinopathy in the albino rat in long-term studies. An immunohistochemical and quantitative approach. *Exp Toxicol Pathol* 1994;**46**:229–35
23 Semple-Rowland SL, Dawson WW. Retinal cyclic light damage threshold for albino rats. *Lab Anim Sci* 1987;**37**:289–98
24 Rao GN. Light intensity-associated eye lesions of Fischer 344 rats in long-term studies. *Toxicol Pathol* 1991;**19**:148–55
25 Penn JS, Baker BN, Howard AG, Williams TP. Retinal light-damage in albino rats: lysosomal enzymes, rhodopsin, and age. *Exp Eye Res* 1985;**41**:275–84
26 Organisciak DT, Darrow RM, Barsalou L, *et al.* Light history and age-related changes in retinal light damage. *Invest Ophthalmol Vis Sci* 1998;**39**:1107–16
27 Blom HJM, Van Tintelen G, Baumans V, Van den Broek J, Beynen AC. Development and application of a preference test system to evaluate housing conditions for laboratory rats. *Appl Anim Behav Sci* 1995;**43**:279–90
28 Schlingmann F, De Rijk H, Pereboom W, Remie R. Avoidance as a behavioural parameter in the determination of distress amongst albino and pigmented rats at various light intensities. *Anim Technol* 1993;**44**:87–96
29 Schlingmann F, De Rijk H, Pereboom W, Remie R. Light intensity in animal rooms and cages in relation to the care and management of albino rats. *Anim Technol* 1993;**44**:97–107
30 Penn JS, Naash MI, Anderson RE. Effect of light history on retinal antioxidants and light damage susceptibility in the rat. *Exp Eye Res* 1987;**44**:779–88
31 Penn JS, Anderson RE. Effect of light history on rod outer-segment membrane composition in the rat. *Exp Eye Res* 1987;**44**:767–78
32 Schremser JL, Williams TP. Rod outer segment (ROS) renewal as a mechanism for adaptation to a new intensity environment. I. Rhodopsin levels and ROS length. *Exp Eye Res* 1995;**61**:17–23
33 Organisciak DT, Darrow RM, Barsalou L, Kutty RK, Wiggert B. Susceptibility to retinal light damage in transgenic rats with rhodopsin mutations. *Invest Ophthalmol Vis Sci* 2003;**44**:486–92
34 Case CP, Plummer CJ. Changing the light intensity of the visual environment results in large differences in numbers of synapses and in photoreceptor size in the retina of the young adult rat. *Neuroscience* 1993;**55**:653–66
35 Organisciak DT, Darrow RM, Barsalou L, Kutty RK, Wiggert B. Circadian-dependent retinal light damage in rats. *Invest Ophthalmol Vis Sci* 2000;**41**:3694–701
36 Williams DI. Maze exploration in the rat under different levels of illumination. *Anim Behav* 1971;**19**:365–7
37 Vanderschuren LJ, Niesink RJ, Spruijt BM, Van Ree JM. Influence of environmental factors on social play behavior of juvenile rats. *Physiol Behav* 1995;**58**:119–23
38 Hole G. Proximity measures of social play in the laboratory rat. *Dev Psychobiol* 1991;**24**:117–33
39 Clough G. The animal house: design, equipment and environmental control. In: Poole T, ed. *The UFAW Handbook on the Care and Management of Laboratory Animals: Terrestrial Vertebrates*. 7th edn. Oxford: Blackwell Science, 1999:120–4
40 McCormack CE, Sontag CR. Entrainment by red light of running activity and ovulation rhythms of rats. *Am J Physiol* 1980;**239**:R450–3
41 Spalding JF, Holland LM, Tietjen GL. Influence of the visible color spectrum on activity in mice. II. Influence of sex, color, and age on activity. *Lab Anim Care* 1969;**19**:209–13
42 Wersinger SR, Martin LB. Optimization of laboratory conditions for the study of social behavior. *ILAR J* 2008;**50**:64–80

43 Ikeda M, Sagara M, Inoue S. Continuous exposure to dim illumination uncouples temporal patterns of sleep, body temperature, locomotion and drinking behavior in the rat. *Neurosci Lett* 2000;**279**:185–9

44 Inouye ST, Kawamura H. Persistence of circadian rhythmicity in a mammalian hypothalamic 'island' containing the suprachiasmatic nucleus. *Proc Natl Acad Sci USA* 1979;**76**:5962–6

45 Mitchell JA, Yochim JM. Influence of environmental lighting on duration of pregnancy in the rat. *Endocrinology* 1970;**87**:472–80

46 Beys E, Hodge T, Nohynek GJ. Ovarian changes in Sprague-Dawley rats produced by nocturnal exposure to low intensity light. *Lab Anim* 1995;**29**:335–8

47 Deprés-Brummer P, Levi F, Metzger G, Touitou Y. Light-induced suppression of the rat circadian system. *Am J Physiol* 1995;**268**:R1111–16

48 Albers HE, Gerall AA, Axelson JF. Circadian rhythm dissociation in the rat: effects of long-term constant illumination. *Neurosci Lett* 1981;**25**:89–94

49 Briaud SA, Zhang BL, Sannajust F. Continuous light exposure and sympathectomy suppress circadian rhythm of blood pressure in rats. *J Cardiovasc Pharmacol Ther* 2004;**9**:97–105

50 Scheer FA, Pirovano C, Van Someren EJ, Buijs RM. Environmental light and suprachiasmatic nucleus interact in the regulation of body temperature. *Neuroscience* 2005;**132**:465–77

51 Welberg L, Thrivikraman KV, Plotsky PM. Combined pre- and postnatal environmental enrichment programs the HPA axis differentially in male and female rats. *Psychoneuroendocrinology* 2006;**31**:553–64

52 Morimoto Y, Oishi T, Arisue K, Ogawa Z, Tanaka F. Circadian rhythm of plasma corticosteroid in adult female rats: chronological shifts in abnormal lighting regimens and connection with oestrous cycle. *Acta Endocrinol (Copenhagen)* 1975;**80**:527–41

53 Eastman C, Rechtschaffen A. Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiol Behav* 1983;**31**:417–27

54 Honma S, Kanematsu N, Katsuno Y, Honma K. Persistence of circadian oscillation while locomotor activity and plasma melatonin levels became aperiodic under prolonged continuous light in the rat. *Neurosci Lett* 1996;**216**:49–52

55 Takeo Y. Influence of continuous illumination on estrous cycle of rats: time course of changes in levels of gonadotropins and ovarian steroids until occurrence of persistent estrus. *Neuroendocrinology* 1984;**39**:97–104

56 Canal-Corretger MM, Cambras T, Vilaplana J, ez-Noguera A. Bright light during lactation alters the functioning of the circadian system of adult rats. *Am J Physiol Regul Integr Comp Physiol* 2000;**278**:R201–8

57 Cambras T, Vilaplana J, Torres A, *et al.* Constant bright light (LL) during lactation in rats prevents arrhythmicity due to LL. *Physiol Behav* 1998;**63**:875–82

58 Canal-Corretger MM, Cambras T, ez-Noguera A. Effect of light during lactation on the phasic and tonic responses of the rat pacemaker. *Chronobiol Int* 2003;**20**:21–35

59 Vilaplana J, Madrid JA, Sanchez-Vazquez J, Campuzano A, Cambras T, ez-Noguera A. Influence of period length of light/dark cycles on the body weight and food intake of young rats. *Physiol Behav* 1995;**58**:9–13

60 Zhang BL, Zannou E, Sannajust F. Effects of photoperiod reduction on rat circadian rhythms of BP, heart rate, and locomotor activity. *Am J Physiol Regul Integr Comp Physiol* 2000;**279**:R169–78

61 van den Buuse M. Circadian rhythms of blood pressure and heart rate in conscious rats: effects of light cycle shift and timed feeding. *Physiol Behav* 1999;**68**:9–15

62 Azar TA, Sharp JL, Lawson DM. Effect of housing rats in dim light or long nights on heart rate. *J Am Assoc Lab Anim Sci* 2008;**47**:25–34

63 ARRP Guideline 20. Guideline for housing rats in scientific institutions (http://www.animalethics.org.au/policies-and-guidelines/animal-care)

64 Amir S, Stewart J. Conditioning in the circadian system. *Chronobiol Int* 1998;**15**:447–56

65 Amir S, Stewart J. The effectiveness of light on the circadian clock is linked to its emotional value. *Neuroscience* 1999;**88**:339–45

66 Gamble MR. Sound and its significance for laboratory animals. *Biol Rev Camb Philos Soc* 1982;**57**:395–421

67 Voipio HM. How do rats react to sound? *Scand J Lab Anim Sci* 1997;**24**(Suppl. 1):1–80

68 Turner JG, Parrish JL, Hughes LF, Toth LA, Caspary DM. Hearing in laboratory animals: strain differences and nonauditory effects of noise. *Comp Med* 2005;**55**:12–23

69 Burn CC. What is it like to be a rat? Rat sensory perception and its implications for experimental design and rat welfare. *Appl Anim Behav Sci* 2008;**112**:1–32

70 Pfaff J. Noise as an environmental problem in the animal house. *Lab Anim* 1974;**8**:347–54

71 Bjork E, Nevalainen T, Hakumaki M, Voipio HM. R-weighting provides better estimation for rat hearing sensitivity. *Lab Anim* 2000;**34**:136–44

72 Kelly JB, Masterton B. Auditory sensitivity of the albino rat. *J Comp Physiol Psychol* 1977;**91**:930–6

73 Brown AM, Pye JD. Auditory sensitivity at high frequencies in mammals. *Adv Comp Physiol Biochem* 1975;**6**:1–73

74 Gourevitch G, Hack MH. Audibility in the rat. *J Comp Physiol Psychol* 1966;**62**:289–91

75 Borg E. Auditory thresholds in rats of different age and strain. A behavioral and electrophysiological study. *Hear Res* 1982;**8**:101–15

76 Heffner HE, Heffner RS, Contos C, Ott T. Audiogram of the hooded Norway rat. *Hear Res* 1994;**73**:244–7

77 Backoff PM, Caspary DM. Age-related changes in auditory brainstem responses in Fischer 344 rats: effects of rate and intensity. *Hear Res* 1994;**73**:163–72

78 Geal-Dor M, Freeman S, Li G, Sohmer H. Development of hearing in neonatal rats: air and bone conducted ABR thresholds. *Hear Res* 1993;**69**:236–42

79 Lenoir M, Pujol R. Sensitive period to acoustic trauma in the rat pup cochlea. Histological findings. *Acta Otolaryngol* 1980;**89**:317–22

80 Lenoir M, Bock GR, Pujol R. Supra-normal susceptibility to acoustic trauma of the rat pup cochlea. *J Physiol* (Paris) 1979;**75**:521–4

81 Coleman J, Blatchley BJ, Williams JE. Development of the dorsal and ventral cochlear nuclei in rat and effects of acoustic deprivation. *Brain Res* 1982;**256**:119–23

82 Poon PW, Chen X. Postnatal exposure to tones alters the tuning characteristics of inferior collicular neurons in the rat. *Brain Res* 1992;**585**:391–4

83 Blanchard RJ, Blanchard DC, Agullana R, Weiss SM. Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. *Physiol Behav* 1991;**50**:967–72

84 Anisko JJ, Suer SF, McClintock MK, Adler NT. Relation between 22-kHz ultrasonic signals and sociosexual behavior in rats. *J Comp Physiol Psychol* 1978;**92**:821–9

85 Sales GD. Ultrasound and aggressive behaviour in rats and other small mammals. *Anim Behav* 1972;**20**:88–100

86 Haney M, Miczek KA. Ultrasounds during agonistic interactions between female rats (*Rattus norvegicus*). *J Comp Psychol* 1993;**107**:373–9

87 Barfield RJ, Geyer LA. Sexual behavior: ultrasonic postejaculatory song of the male rat. *Science* 1972;**176**:1349–50

88 White NR, Barfield RJ. Effects of male pre-ejaculatory vocalizations on female receptive behavior in the rat (*Rattus norvegicus*). *J Comp Psychol* 1990;**104**:140–6

89 Matochik JA, White NR, Barfield RJ. Variations in scent marking and ultrasonic vocalizations by Long-Evans rats across the estrous cycle. *Physiol Behav* 1992;**51**:783–6

90 Brown RE. The 22-kHz pre-ejaculatory vocalizations of the male rat. *Physiol Behav* 1979;**22**:483–9

91 Lewis PR, Schriefer JA. Ultrasound production by pregnant rats. *Behav Neural Biol* 1982;**35**:422–5

92 Ihnat R, White NR, Barfield RJ. Pup's broadband vocalizations and maternal behavior in the rat. *Behav Processes* 1995;**33**:257–71

93 Burman OHP, Ilyat A, Jones G, Mendl M. Ultrasonic vocalizations as indicators of welfare for laboratory rats (*Rattus norvegicus*). *Appl Anim Behav Sci* 2007;**104**:116–29

94 Brudzynski SM, Ociepa D. Ultrasonic vocalization of laboratory rats in response to handling and touch. *Physiol Behav* 1992;**52**:655–60

95 Jourdan D, Ardid D, Chapuy E, Eschalier A, Le BD. Audible and ultrasonic vocalization elicited by single electrical nociceptive stimuli to the tail in the rat. *Pain* 1995;**63**:237–49

96 Levine JD, Feldmesser M, Tecott L, Gordon NC, Izdebski K. Pain-induced vocalization in the rat and its modification by pharmacological agents. *Brain Res* 1984;**296**:121–7

97 Kaltwasser MT. Startle-inducing acoustic stimuli evoke ultrasonic vocalization in the rat. *Physiol Behav* 1990;**48**:13–17

98 Peterson EA. Noise and laboratory animals. *Lab Anim Sci* 1980;**30**:422–39

99 Fletcher JL. Influence of noise on animals. In: McSheehy T, ed. *Control of the Animal House Environment*. Laboratory Animal Handbook 7. Huntingdon, UK: Laboratory Animals Ltd, 1976:51–62

100 Popelar J, Groh D, Pelanova J, Canlon B, Syka J. Age-related changes in cochlear and brainstem auditory functions in Fischer 344 rats. *Neurobiol Aging* 2006;**27**:490–500

101 Keithley EM, Lo J, Ryan AF. 2-Deoxyglucose uptake patterns in response to pure tone stimuli in the aged rat inferior colliculus. *Hear Res* 1994;**80**:79–85

102 Bevan W. Sound-precipitated convulsions 1947–1954. *Psychol Bull* 1955;**52**:473–504

103 D'Amour FE, Shaklee AB. Effect of audiogenic seizures on adrenal weight. *Am J Physiol* 1955;**183**:269–71

104 Duncan IW. The effect of audiogenic seizures in rats on the adrenal weight, ascorbic acid, cholesterol, and corticosteroids. *J Biol Chem* 1957;**229**:563–8

105 Boyle E, Villanueva PA. Hyperbaric oxygen seizures in rats: effects of handling and chamber noise. *Lab Anim Sci* 1976;**26**:100–1

106 Ross KC, Coleman JR. Audiogenic seizures in the developmentally primed Long-Evans rat. *Dev Psychobiol* 1999;**34**:303–13

107 Alario P, Gamallo A, Beato MJ, Trancho G. Body weight gain, food intake and adrenal development in chronic noise stressed rats. *Physiol Behav* 1987;**40**:29–32

108 Nayfield KC, Besch EL. Comparative responses of rabbits and rats to elevated noise. *Lab Anim Sci* 1981;**31**:386–90

109 Morseth SL, Dengerink HA, Wright JW. Effect of impulse noise on water consumption and blood pressure in the female rat. *Physiol Behav* 1985;**34**:1013–16

110 Borg E, Moller AR. Noise and blood pressure: effect of lifelong exposure in the rat. *Acta Physiol Scand* 1978;**103**:340–2

111 Armario A, Castellanos JM, Balasch J. Adaptation of anterior pituitary hormones to chronic noise stress in male rats. *Behav Neural Biol* 1984;**41**:71–6

112 Armario A, Castellanos JM. Effect of acute and chronic stress on testosterone secretion in male rats. *J Endocrinol Invest* 1984;**7**:659–61

113 Barrett AM, Stockham MA. The effect of housing conditions and simple experimental procedures upon the corticosterone level in the plasma of rats. *J Endocrinol* 1963;**26**:97–105

114 Burow A, Day HE, Campeau S. A detailed characterization of loud noise stress: intensity analysis of hypothalamo-pituitary-adrenocortical axis and brain activation. *Brain Res* 2005;**1062**:63–73

115 Zondek B. Effect of auditory stimuli on female reproductive organs. *Trans N Engl Obstet Gynecol Soc* 1964;**18**:177–85

116 Rabat A, Bouyer JJ, Aran JM, Courtiere A, Mayo W, Le MM. Deleterious effects of an environmental noise on sleep and contribution of its physical components in a rat model. *Brain Res* 2004;**1009**:88–97

117 Rabat A, Bouyer JJ, Aran JM, Le MM, Mayo W. Chronic exposure to an environmental noise permanently disturbs sleep in rats: inter-individual vulnerability. *Brain Res* 2005;**1059**:72–82

118 Friedman M, Byers SO, Brown AE. Plasma lipid responses of rats and rabbits to an auditory stimulus. *Am J Physiol* 1967;**212**:1174–8

119 Lenzi P, Frenzilli G, Gesi M, *et al.* DNA damage associated with ultrastructural alterations in rat myocardium after loud noise exposure. *Environ Health Perspect* 2003;**111**:467–71

120 Pellegrini A, Soldani P, Gesi M, Lenzi P, Natale G, Paparelli A. Effect of varying noise stress duration on rat adrenal gland: an ultrastructural study. *Tissue Cell* 1997;**29**:597–602

121 Frenzilli G, Lenzi P, Scarcelli V, *et al.* Effects of loud noise exposure on DNA integrity in rat adrenal gland. *Environ Health Perspect* 2004;**112**:1671–2

122 Sales GD. The effect of 22 kHz calls and artificial 38 kHz signals on activity in rats. *Behav Processes* 1991;**24**:83–93

123 Weyers P, Janke W, Macht M, Weijers H-G. Social and non-social open field behaviour of rats under light and noise stimulation. *Behav Processes* 1994;**31**:257–67

124 Lai H. Acute exposure to noise affects sodium-dependent high-affinity choline uptake in the central nervous system of the rat. *Pharmacol Biochem Behav* 1987;**28**:147–51

125 Prior H. Effects of the acoustic environment on learning in rats. *Physiol Behav* 2006;**87**:162–5

126 Milligan SR, Sales GD, Khirnykh K. Sound levels in rooms housing laboratory animals: an uncontrolled daily variable. *Physiol Behav* 1993;**53**:1067–76

127 Sales GD, Wilson KJ, Spencer KE, Milligan SR. Environmental ultrasound in laboratories and animal houses: a possible cause for concern in the welfare and use of laboratory animals. *Lab Anim* 1988;**22**:369–75

128 Sales GD, Milligan SR, Khirnykh K. Sources of sound in the laboratory animal environment: a survey of the sounds produced by procedures and equipment. *Anim Welf* 1999;**8**:97–115

129 Voipio HM, Nevalainen T, Halonen P, Hakumaki M, Bjork E. Role of cage material, working style and hearing sensitivity in perception of animal care noise. *Lab Anim* 2006;**40**:400–9

130 Pfaff J, Stecker M. Loudness level and frequency content of noise in the animal house. *Lab Anim* 1976;**10**:111–17

131 Nunez MJ, Mana P, Linares D, *et al.* Music, immunity and cancer. *Life Sci* 2002;**71**:1047–57

132 Clough G, Fasham JA. A 'silent' fire alarm. *Lab Anim* 1975;**9**:193–6

133 Armario A, Montero JL, Balasch J. Sensitivity of corticosterone and some metabolic variables to graded levels of low intensity stresses in adult male rats. *Physiol Behav* 1986;**37**:559–61

134 Armario A, Lopez-Calderon A, Jolin T, Castellanos JM. Sensitivity of anterior pituitary hormones to graded levels of psychological stress. *Life Sci* 1986;**39**:471–5

135 Balcombe JP, Barnard ND, Sandusky C. Laboratory routines cause animal stress. *Contemp Top Lab Anim Sci* 2004;**43**:42–51

136 Gartner K, Buttner D, Dohler K, Friedel R, Lindena J, Trautschold I. Stress response of rats to handling and experimental procedures. *Lab Anim* 1980;**14**:267–74

137 De Boer SF, Koopmans SJ, Slangen JL, Van der Gugten J. Plasma catecholamine, corticosterone and glucose responses to repeated stress in rats: effect of interstressor interval length. *Physiol Behav* 1990;**47**:1117–24

138 Flecknell PA. *Laboratory Animal Anesthesia: A Practical Introduction for Research Workers and Technicians*. 2nd edn. Newcastle-upon-Tyne: Academic Press, 1996

139 Tabata H, Kitamura T, Nagamatsu N. Comparison of effects of restraint, cage transportation, anaesthesia and repeated bleeding on plasma glucose levels between mice and rats. *Lab Anim* 1998;**32**:143–8

140 Saibaba P, Sales GD, Stodulski G, Hau J. Behaviour of rats in their home cages: daytime variations and effects of routine husbandry procedures analysed by time sampling techniques. *Lab Anim* 1996;**30**:13–21

141 Duke JL, Zammit TG, Lawson DM. The effects of routine cage-changing on cardiovascular and behavioral parameters in male Sprague-Dawley rats. *Contemp Top Lab Anim Sci* 2001;**40**:17–20

142 Sharp J, Zammit T, Azar T, Lawson D. Does witnessing experimental procedures produce stress in male rats? *Contemp Top Lab Anim Sci* 2002;**41**:8–12

143 Sharp J, Zammit T, Azar T, Lawson D. Stress-like responses to common procedures in individually and group-housed female rats. *Contemp Top Lab Anim Sci* 2003;**42**:9–18

144 Sharp J, Zammit T, Azar T, Lawson D. Are 'by-stander' female Sprague-Dawley rats affected by experimental procedures? *Contemp Top Lab Anim Sci* 2003;**42**:19–27

145 Sharp JL, Zammit TG, Azar TA, Lawson DM. Stress-like responses to common procedures in male rats housed alone or with other rats. *Contemp Top Lab Anim Sci* 2002;**41**:8–14

146 Sharp JL, Zammit TG, Lawson DM. Stress-like responses to common procedures in rats: effect of the estrous cycle. *Contemp Top Lab Anim Sci* 2002;**41**:15–22

147 Van Loo PLP, Kruitwagen CLJJ, Van Zutphen LFM, Koolhaas JM, Baumans V. Modulation of aggression in male mice: influence of cage cleaning regime and scent marks. *Anim Welf* 2000;**9**:281–95

148 Burn CC, Peters A, Day MJ, Mason GJ. Long-term effects of cage-cleaning frequency and bedding type on laboratory rat health, welfare, and handleability: a cross-laboratory study. *Lab Anim* 2006;**40**:353–70

149 Burn CC, Peters A, Mason GJ. Acute effects of cage cleaning at different frequencies on laboratory rat behaviour and welfare. *Anim Welf* 2006;**15**:161–71

150 Harkness JE, Ridgway MD. Chromodacryorrhea in laboratory rats (*Rattus norvegicus*): etiologic considerations. *Lab Anim Sci* 1980;**30**: 841–4

151 de Laat JM, Van Tintelen G, Beynen AC. Transportation of rats affects behaviour of non-transported rats in the absence of physical contact (preliminary communication). *Z Versuchstierkd* 1989;**32**:235–7

152 Dallmann R, Steinlechner S, von Hörsten S, Karl T. Stress-induced hyperthermia in the rat: comparison of classical and novel recording methods. *Lab Anim* 2006;**40**:186–93
153 Blanchard DC, Griebel G, Blanchard RJ. Conditioning and residual emotionality effects of predator stimuli: some reflections on stress and emotion. *Prog Neuropsychopharmacol Biol Psychiatry* 2003;**27**: 1177–85
154 Komori T, Miyahara S, Yamamoto M, *et al.* Effects of odorants on the hypothalamic-pituitary-adrenal axis and interleukin-6 (IL-6) and IL-6 receptor mRNA expression in rat hypothalamus after restraint stress. *Chem Senses* 2003;**28**:767–71
155 Nakashima T, Akamatsu M, Hatanaka A, Kiyohara T. Attenuation of stress-induced elevations in plasma ACTH level and body temperature in rats by green odor. *Physiol Behav* 2004;**80**:481–8
156 Broderson JR, Lindsey JR, Crawford JE. The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am J Pathol* 1976;**85**:115–30
157 Gamble MR, Clough G. Ammonia build-up in animal boxes and its effect on rat tracheal epithelium. *Lab Anim* 1976;**10**:93–104
158 Silverman J, Bays DW, Baker SP. Ammonia and carbon dioxide concentrations in disposable and reusable static mouse cages. *Lab Anim (NY)* 2009;**38**:16–23
159 Reeb-Whitaker CK, Paigen B, Beamer WG, *et al.* The impact of reduced frequency of cage changes on the health of mice housed in ventilated cages. *Lab Anim* 2001;**35**:58–73
160 Lipman NS. Isolator rodent caging systems (state of the art): a critical view. *Contemp Top Lab Anim Sci* 1999;**38**:9–17
161 Krohn TC, Hansen AK, Dragsted N. The impact of cage ventilation on rats housed in IVC systems. *Lab Anim* 2003;**37**:85–93
162 Baumans V, Schlingmann F, Vonck M, van Lith HA. Individually ventilated cages: beneficial for mice and men? *Contemp Top Lab Anim Sci* 2002;**41**:13–19
163 Meijer MK. *Neglected Impact of Routine: Refinement of Experimental Procedures in Laboratory Mice* (PhD thesis). Faculty of Veterinary Medicine, Utrecht University, 2006

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