Specialist information

from the Committee for Humane Laboratory Animal Husbandry

Species-appropriate housing of laboratory rats

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1. Biology

Laboratory rats have been bred and kept for scientific purposes for more than 100 years. Today, a great many conventional inbred and outbred strains as well as an increasing number of genetically modified lines are available. The laboratory rat is descended from the brown rat (*Rattus norvegicus*), which spread as a synanthrope from Asia through the Balkans to Europe and ultimately worldwide, completely displacing the black rat or house rat (*Rattus rattus*) which was originally widespread here. Rats of both sexes are social animals. They live territorially in large groups with a promiscuous mating system, show shared rearing of young and marked intolerance towards members of other communities of the same animal species.

As wild animals, rats live in diverse natural and artificial habitats, establishing burrows that are complex in structure. Rats are very good climbers, swimmers and burrowers and have highly developed olfactory, acoustic and tactile senses. These sensory traits are characteristic of both wild and laboratory rats. In the case of laboratory rats, decades of breeding selection have led to a shift in morphological, physiological and ethological characteristics. The relative weights of liver, spleen, heart and brain are lower, while the weights of thymus and pituitary gland are higher than those in the wild rat. Females reach sexual maturity earlier and are more fertile than their wild conspecifics. Laboratory rats are less active and aggressive than wild rats. Their reduced neophobia and aggressiveness in particular makes it easier to keep them in cages under laboratory conditions and means they can be handled by humans without any problem.

Besides an appropriate spatial and physical housing environment, two aspects are considered especially important:

1. the development of species-appropriate social behaviour during the rearing phase and
2. housing in groups with stable social relations between group members.

2. Forms of housing and space requirements

2.1. Single or group housing

As also required in ETS 123, rats should in principle be housed in groups. This is especially important for young rats. Social isolation while growing up can result in lifelong behavioural deficits and a change in sensitivity to stress (e.g. Daskalakis 2012).

A stable group of 3-5 animals corresponding to the cage size is equally ideal for both sexes (Patterson-Kane 2004).

If animals have to be housed individually for experimental reasons, they should at least have acoustic, visual and olfactory contact with conspecifics. The individual housing of subadult animals (before day 52, see 2.1.2.) is to be avoided as a rule.

2.2. Effects of single housing of adult animals

Individually housed rats frequently show changes in behaviour patterns (Baenninger 1967, Hurst 1997, Krohn 2006) and changes in physiological values (Baer 1971), which can be interpreted as an expression of diminished wellbeing or stress due to isolation. Adrenal gland weights and plasma corticosterone concentrations are often increased (Dronjak 2004, Sánchez 1998, Serra 2005). Disturbances in the circadian secretion of hormones and in sleep patterns have also been recorded. Research results, for example, show significantly increased corticosterone, glucose or prolactin concentrations (Gambardella 1994, Baldwin 1995, Brown 1995), higher blood pressure and a higher heart rate (Sharp 2002) and less activity in individually housed rats than in rats living in a social environment (Lawson 2000). Patterson-Kane et al. (2002) also showed that female rats are much more motivated to “work” for social contacts than for other options (e.g. a bigger cage area or additional objects). Observations on the behaviour of rats housed individually and those housed in groups are inconsistent when they are studied in a test environment that is alien to the animal. Here, activity and timidity are described as both increasing and diminishing effects of individual housing.

2.3. Housing effects during the rearing phase

While rats are growing up, playful interactions with conspecifics are essential for the development of species-appropriate social and sexual behaviour. In the rough-and-tumble play commonly observed among young animals, they learn to apply these attacking and defensive behaviours according to the given situation (Wahlstrand 1983, Birke 1987, Akbari 2008). An important characteristic of play is that the interacting partners can switch roles at any time. Adult rats use the same behaviours later on as a means of regulating social relations. Rats that grow up without this experience may be socially less amenable later in life. Social isolation during the rearing phase leads to neurological and behavioural disorders (“isolation syndrome”) in later adulthood; male rats also become more aggressive. The period of social play is especially critical up to the age of 52 days (Wongwitcheda 1996, Zhao 2009, Hermes 2011). Generally, young rats should not be weaned before the age of 21 days; even short periods of separation should be avoided if possible, because the intact bond of the mother to her young is a basic prerequisite for normal development, and experimentally induced disturbances of this state (e.g. through the maternal separation paradigm or early weaning of young) can lead to long-term changes in relation to a wide variety of physiological and behaviour-relevant parameters (e.g. Meaney 1985, Kikusui 2008). Furthermore, epigenetic changes in offspring cannot be excluded if one has to assume that disturbing factors during relevant “sensitive phases” lead to changes in maternal care behaviour (Weaver 2004).

2.4. Cage size

The height of the cage should allow the animals to adopt an upright position and hence engage in exploratory, playful and agonistic behaviour.

According to Annex III of Directive 2010/63/EU and Appendix A of ETS 123, the cage height should not be less than 18 cm. An optimized cage height that meets requirements can be achieved e.g. through the use of low cage walls or elevated cage covers to allow adult animals also to adopt an upright posture. Preference is to be given to the elevated cage cover or even a second level, because it facilitates better contact with the cage surroundings and with animals in neighbouring cages. The type IV cage has a height of 20 cm and can be raised to 27 cm by using an elevated cage cover.

According to Table A.2. of ETS 123, rat cages must have a floor area of at least 800 cm^2, with a minimum space allowance of 200 cm^2 per animal (up to 200 g BW.). The recommendation thus provides for the housing of 3-4 small rats or one mother with a litter in a type III cage (800 cm^2), but the required group housing and cage installation is hardly feasible in this size of cage. It is even more difficult to meet further requirements of Directive 2010/63/EU such as the need to provide “space of sufficient complexity to allow expression of a wide range of normal behaviour", e.g. through spaces for shelter or wood to gnaw on or possibilities for extending the range of species-specific manipulative and cognitive activities.

Type III cages should therefore only be used in limited cases for experimental purposes (experiment-related single housing).

The GV-SOLAS Committee for Humane Laboratory Animal Housing recommends as the standard for housing rats either a cage with a floor area of at least 1500 cm^2 or the cage widely used in Germany, which has a floor area of 1800 cm^2 (on one or two levels). Alternatively, cages with a floor area of 2000 cm^2 are also available, but the use of these cages entails commensurate human resources in view of their size and associated handling procedures.

The following factors are favour an increased housing area:

1. the possibility for a diversified use of space;
2. the need of growing rats for exercise after leaving the nest; energetic social play between littermates is important for the development of social characteristics in adult animals;
3. the need to keep rats in groups with a constant individual composition: in the type IV cage, groups of e.g. four rats weighing up to about 400 g can be kept together permanently without any necessity for reducing the size of the group; and
4. the fact that an area of less than 1800 cm^2 hardly allows any usable cage enrichment and group housing to be achieved.

Table 1: Minimum dimensions and space allowance for rat housing, breeding and experiments (extract from ETS 123, Table A.2. / Annex III of Directive 2010/63/EU, Table 1.2)

	Bodyweight (g)	Minimum cage area (cm^2)	Floor area/animal (cm^2)
Housing, experiment	≤ 200	800	200
	200-300	800	250
	300-400	800	350
	400-600	800	450
	≥ 600	1500	600
Breeding		800 mother & litter a further 400 cm^2 for each additional adult animal	

***** according to the requirements of ETS 123, priority should be given to maintaining the stability of the group over prolonged experimental periods regardless of bodyweight development.

2.5. Cage enrichment

ETS 123 stipulates nesting material and shelters. Shelters like nest boxes or tubes may only be dispensed with if sufficient materials are provided for a fully covered nest. Research results show that rats are prepared to work for access to a nest box, nesting material or a nest box + nesting material (Manser 1998a, b). In addition, for example, ladders or "roller balls" may be provided as motor enrichment as well as gnawing aids made of nylon or wood, although a careful benefit/risk assessment must always be undertaken in terms of any impact on the results of the experiment.

In a comparative study, (largely standardizable) environmental enrichment measures had a more positive impact on the wellbeing of male Wistar rats when they were used in the form of combined enrichment structures (multi-item cages) and not individually (Abu-Ismail 2011). Aside from nest boxes, tubes and/or additional levels, e.g. elevated boards for lying on, are also suitable as shelters (e.g. Connors 2014). Intensive use is made of several levels (Vachon 2014, Wheeler 2014, Makowska 2016).

It is known that a highly structured housing environment ("super-enrichment"), especially when animals grow up in such an environment, has an impact on parameters of neurobiology, behavioural biology and stress physiology (Chapillon 2002, Simpson 2011, Wheeler 2014, Crofton 2015). These findings come almost exclusively from neurophysiology and stress physiology studies and were initiated to study environmental effects on neuronal plasticity and stress processing – not to arrive at conclusions concerning the wellbeing of the animals. However, the nature of the changes observed – namely, increased resistance to neurodegenerative processes, diminished timidity in corresponding behavioural tests and reduced stress reaction - can be interpreted as signs of improved wellbeing (e.g. Escorihuela 1994, Cui 2006). But it is unclear what effect these behavioural changes have in the practice of laboratory animal housing, e.g. if the animals have to be housed individually for experimental reasons (Wheeler 2014, Makowska 2016).

Still highly debated is the question as to the extent to which the enrichment impacts the variability of measurements. No enrichment measures should be implemented without prior evaluation. The available literature gives no evidence regarding the extent to which the experimental variance is affected by the housing environment. It appears to be very much dependent on the measurement parameters and applied enrichment measures (Eskola 1999, Mering 2001, Kemppinen 2010, Bardi 2016). It is therefore necessary, as mentioned earlier, to be familiar with the impact of housing enrichment and the modification of environmental factors in general and to take into consideration the potential impact of such factors before deploying them.

Directive 2010/63/EU does not require any concrete requirements for cage furnishing. Stocking densities and cage sizes have been adopted from ETS 123. However, the general refinement requirements formulated in ETS 123 probably go beyond a minimum degree of housing enrichment in many laboratory animal facilities.

2.6. Other forms of housing (containments)

There is little published experience concerning the housing of rats in special individually ventilated cages (IVCs). While experimental ethological and physiological parameters in IVC housing may change in comparison with open housing (Shan 2014, Krohn 2002), this form of housing does not seem to have any impact on the growth of rats (Kostomitsopoulos 2011).

The breeding and housing of germ-free rats in isolators is possible. To collect the relatively liquid faeces, the quantity of bedding material should be increased, where necessary, compared with normal housing. The transfer of germ-free rats into a conventional housing usually does not present any major problems, but adaptation processes associated with this, especially to the immune system, can take some weeks.

3. Feeding and drinking

Details on this are explained in the booklet *Fütterungskonzepte und -methoden in der Versuchstierhaltung und im Tierversuch – Ratte* (Feeding concepts and methods in laboratory animal housing and animal experiments – Rat) published by the GV-SOLAS Committee for Nutrition (http://www.gv-solas.de/index.php?id=40)

4. Physical environment

Climate data

Room temperature	20-24°C*)
Relative humidity	45-65%*)
Air change rate in room	15-20 times per h*)
Lighting	in cage optimal < 60 lux, resting place < 25 lux[1)] generally 130-325 lux[2)] at cage level of the animals
Light-dark cycle	as a rule 12:12, if necessary 14:10 no constant light
Noise:	< 60 dB, avoid noise and ultrasound!

*) Annex A of ETS 123

1) Schlingmann et al.1993b

2) Specialist information GV SOLAS – Planung und Organisation von Versuchstier-Haltungen und – Laboren (Stand 15.07.2015) Planning and organization of laboratory animal housing units and laboratories (15.07.2015)

4.1. Climate

Rats have a narrow thermoneutral range of 28-32°C (Refinetti 1989, Gordon 1990, 1993, Romanovsky 2002), which describes the temperature range within which the animals show neither sensitivity to heat nor sensitivity to cold. They can tolerate lower temperatures well, but higher temperatures hardly at all. Rapid and marked cooling leads to tremor. By means of nesting material and snuggling together, rats achieve a thermal comfort temperature that is well above room temperature values, which also provides a certain freedom of choice between temperature zones within the cage. All deviations of more than 2-4°C from the standard affect physiological parameters, such as blood flow, calorie consumption, feed intake and metabolic rate, as well as rest and sleep behaviour and the spontaneous motor activity of the animals. The housing of rats at 20-24°C under the conditions indicated in Table 2 is thus recommended.

Deviations in relative humidity beyond the range of 45-65% must be avoided, because morphological, physiological and behavioural abnormalities may occur when there are major fluctuations in temperature or humidity, which can have a negative impact on animal wellbeing and hence also affect the results of the experiment (Garard 1974, Gordon 1990).

High air change rates are essential in order to remove noxious gases, especially ammonia. Draughts must be strictly avoided though. As a reference: the European occupational exposure limit for ammonia stands at 14 mg/m^3 or 20 ppm for an 8-hour exposure period and 36 mg/m^3 or 50 ppm for short-term exposure (IFA database 2011). Excessively high stocking densities or inadequate ventilation can lead to these limits being exceeded. If it is not possible to meet the required air change rate of 15-20 changes per hour in the room, the stocking density must be reduced accordingly. Alternatively, ventilated IVC cages may be used.

If animals are housed in such special containments (IVCs, isolator, filter-top cage, etc.), adequate ventilation within the cage must be ensured with long-term use.

4.2. Lighting

Ambient lighting up to 400 lux at working height is adequate for all necessary work in the animal room and at the same time meets the requirement to protect rats from intensive light and phototoxic retinopathy (Krinke, 2000, NIH 2011, Bellhorn, 1980). Albino rats usually prefer areas of low light intensity < 25 lux, pigmented strains < 60 lux (Schlingmann 1993a). Light intensity in the cage is very dependent on the position of the cage. The uppermost cage rows should be protected from direct light by an appropriate shield (CCAC 2003); ETS 123 already requires shelters, the preference of rats being for hiding places that are impervious to light rather than nesting materials, and they will “work” to achieve this (Manser 1998a,b). A place of retreat below 25 lux should be provided.

Retinal damage is due to many factors besides light intensity. Other factors that play a key role are wavelength, light cycle, period of exposure, origin, age, strain and sex. Light in the cyan range is more harmful than light in the red-yellow range; Wistar rats are more resistant than Lewis rats; and adult rats more sensitive than young rats (De Vera Mudry 2013, Faith 2009). In the case of retinal dystrophy, it has yet to be fully established under what housing conditions, especially under what lighting intensity and wavelengths, this can be prevented.

Adequate lighting of animal rooms is important for circadian rhythm, growth, behaviour and reproduction and is also essential for the care of the animals. Flickering light should be avoided (Voipio 2010, 2011).

During periods of darkness, even brief intervals of light, e.g. from an opened door, and light contamination (LAN, light at night), should be avoided e.g. by means of technical devices, because even minimal light exposure can affect the melatonin balance (≥ 0.1 lux) and the growth of tumours (≥ 0.21 lux) (Cos 2006, Faith 2009). Against this background, even the use of self-illuminating signs have to be viewed critically. If animal rooms need to be entered during a time of darkness, red light (≥ 650 nm) may be used, although it has to be taken into account that red light also increases brightness and can have lasting negative effects on circadian rhythm and characteristic physiological parameters (Dauchy 2015). Even minimal levels of light intensity in the animal room can lead to behavioural changes in mice and presumably also in rats (Bedrosian 2013).

Depending on the experimental design, it may also be appropriate, e.g. for behavioural studies in nocturnal animals, to work with a reverse light-dark rhythm. However, the animals should be acclimatized to this for 2 weeks beforehand.

4.3. Noise

In any animal housing facility, there are numerous sources of noise from technical installations, equipment and persons (Reynolds 2010). The noise caused by the rats themselves are largely negligible by comparison. Noise peaks of up to 100 dB have been reported during work in animal rooms (Pfaff 1976, Sales 1988, 1999, Voipio 2006). The level of unavoidable background noise (room and IVC ventilation system, animal noises, presence of people) was below 50 dB in all housing areas where noise was recorded. Peaks of just under 80 dB are generated when transferring animals in a transfer station or when changing bottles, but this only affects the animals in the immediate vicinity and then only for a short period of time. Taking into account also the fact that measurements were performed within the acoustic range of human hearing (31.5 Hz - 8 kHz), which is lower than that of rats (250 Hz - 70 or 80 kHz; Kelly 1977, Heffner 1994), it can be concluded that quiet handling is possible especially during the light and resting phase of the animals.

Numerous items of equipment (ventilation systems, monitors, washing machines) can also emit noise in the ultrasound frequency range. Ultrasound waves can be more easily blocked as a result of the short wavelength, but because they cannot be directly controlled, occasional measurements are of advantage.

In various animal species, noise exposure leads to a decrease in activity and rate of reproduction, as well as to stress reactions (changes in blood glucose and corticoid concentrations, increase in anxious behaviour, reduced motor activity, lack of weight gain and sleep disturbances (e.g. Voipio 1997, Castelhano-Carlos 2009). Cardiovascular functions and circadian rhythm may also be affected (Turner 2007). Hearing damage occurs with prolonged sound pressure levels of 85 dB or more. By way of comparison: hearing protection must be provided for humans at work when exposed to 80 dB or more, and at 85 dB or more the wearing of hearing protection is compulsory (ordinance on the implementation of EC Directives 202/44/EC and 2003/10/EC on the protection of employees from the hazards of noise and vibrations). Sudden noises trigger fight-or-flight responses in rats; high frequencies are more stressful than low frequencies. Rats adapt to repeated noises, depending on the volume and duration of exposure, but the adaptation fades again very quickly (Castelhano-Carlos 2009).

Noise peaks in animal housing facilities should not exceed 85 dB and background noise levels should not be greater than 60 dB (Heine 1998). Specific attention should be paid to exposure in the ultrasound frequency range, especially in new buildings or after modifications of technical installations. Even quiet background music in the animal room during work time is a subject of controversy. Negative effects of moderate music are not known. Rats distinguish between different types of music and noise (Zhang 2009, Xing 2016, Lemmer 2009), but in selection experiments rats prefer silence (Krohn 2011).

5. Diseases associated with housing

Wild rats are distinguished by their remarkable adaptability. They live and reproduce in a very wide variety of climate zones and habitats. The breeding and housing of these animals under laboratory conditions is therefore usually unproblematic. Pathologies directly related to the housing system have not been described. However, there are environmental factors that can predispose an animal to disease.

Rats have very sensitive hearing and perceive frequencies in the ultrasound range (up to 80 kHz). Exposure to ultrasound-emitting equipment can damage the hearing and even cause deafness.

Albino rats are characterized by pigment deficiency in the iris and retina, making them extremely sensitive to light. With a light intensity of more than 60 lux in the cage, pathological changes to the retina have to be expected (see above).

High temperatures and relative humidity below 30% can lead to irritations of the eye and, in young rats up to weaning age, to ringtail formation (necrotic annular constrictions of the tail, often with loss of the distal part of the tail) (Percy 2001, Wijnbergen 2008). Exposure to air with ammonia irritates the airways and promotes infections with *M. pulmonis, P. pneumotropica, Streptococcus pneumoniae* and *C. kutscheri.* With *ad libitum* feeding, rats tend towards obesity, which leads to reduced life expectancy if they are not provided with sufficient space for physical activity.

6. Outlook

Along with mice, rats are the species most commonly used for animal experiments. In view of the fact that rats are increasingly used as a genetic model, it has to be assumed that the proportion of animals used for scientific studies has the potential to grow. There will thus be an increased demand in the future to look into the general conditions for housing this animal species in order to guarantee the best-possible animal welfare and scientifically valid conclusions. Aside from studies that demonstrate the impact of certain experimentally induced environmental conditions on behavioural physiology parameters, it is becoming increasingly important to consider also the variables that can be caused by the housing itself. Especially the use of new and little evaluated systems and enrichment options presents the scientific community with particular challenges, but also offers opportunities. It should be borne in mind that any kind of housing, standard or newly established, or enrichment has an impact on the experimental outcome. It is essential here not to adopt a one-sided focus that only takes account of hygiene, nutrition or the space provided, for example, but to see the housing of the animals in the overall context of the relevant scientific study concerned. Methodological investigations concerning the effects of environmental factors may also be included in the study when looking at specific parameters. Bearing in mind that housing-induced effects per se are valid parameters, the significance of animal experiments can thus be enhanced by precisely this knowledge.

The variables which are addressed here illustrate how demanding it is to provide species-adequate accommodation for any animal species under laboratory conditions.

Housing rats in line with animal welfare aspects requires proven enrichment which specifically takes into account their two and three-dimensional space requirements.

It is therefore essential that any change of housing conditions, e.g. modifications to enrichment during a study, has to be be avoided. Evaluating the effort involved, one should also bear in mind that sufficient data exist to show that environmental enrichment reduces sensitivity to stress (Segovia et al 2009, Ravenelle et al 2014). A reduction in the sensitivity to stress and a corresponding reduction in susceptibility to external parameters usually result in steady baseline values and minimal scatter in the experiments. If these species-specific factors are taken into account, laboratory rats can be usefully deployed in many scientific disciplines.

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