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Statement

of the Committee for Humane Laboratory Animal Housing

On Single Housing of Mice for Scientific Purposes

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It is not possible to answer in a single sentence the question before us as to whether a particular procedure (single housing of mice in customary mouse cages that are additionally placed in a containment for the measurement of respiratory gases) constitutes an animal experiment requiring approval.

We therefore refer to the legal framework conditions (EU and Germany) and differentiate between various groups of mice.

The requirements of EU law are clearly formulated:

ETS123 Appendix A (Council of Europe 2006): in principle, social animals should be housed in groups as long as these are “stable and harmonious”; the same convention acknowledges that this may be difficult “when housing male mice (…), as this can result in severe conspecific aggression. Animals may be housed individually if adverse effects or damage are likely to occur.”

Directive 2010/63/EU (Council of the Europe Union 2010): “Animals, except those which are naturally solitary, shall be socially housed in stable groups of compatible individuals. In cases where single housing is allowed in accordance with article 33(3) the duration shall be limited to the minimum period necessary and visual, auditory, olfactory and/or tactile contact shall be maintained.”

Not all mice are the same: relevant differences in social behaviour

Wild house mice live in polygamous family groups that coexist in larger populations, the population density depending on the supply of food and other resources (according to Olsson and Westlund 2007). Dominant males defend their home territory, in which several females and subadult young may live together, while communal use is made of food sources (according to Krohn et al. 2006). Males do not participate in the rearing of young mice.

On reaching sexual maturity, males either challenge the holder of the territory, which can result in fatal conflicts, or they move away to establish their own territories (according to Latham and Mason 2004). During the migration phase, the males live alone. Males that do not succeed in occupying territories of their own, lead permanently nomadic lives, unless they are tolerated by less aggressive dominant males in their colonies. The level of tolerance shown by dominant males towards subdominant animals depends primarily on the nature of the habitat and the population density (and also, at least in laboratory mice, on the mouse line, the degree of innate anxiety, age and experience). Tolerated subdominant males share the nest with other mice, especially as long as the dominant males sleep (according to Latham and Mason 2004). Preference tests in laboratory mice show that also males need active and passive social contacts that also include other male mice (van Loo 2004). The extent to which the emission of sounds in the ultrasonic range can serve as a criterion in this context is currently the subject of scientific investigations. For example, clear differences have been found here between males housed singly and those housed in groups, indicating individual emotional reactions in different contexts (Chabou et al. 2012, Levebvre et al. 2020). Non-territorial animals usually remain smaller than dominant males (according to Krohn et al. 2006).

Females show a markedly positive social behaviour; they prefer to raise their young in a nest together with other females, demonstrating clear preferences here. Females that rear their young together with a sister from the same litter are particularly successful in reproduction (number of young weaned), followed by females that share the nest with an unrelated female. Under natural conditions, 90% of females opt for the “communal nest” strategy. The effects of such a perinatal environment with communal rearing by several females has been shown to influence the development of the young and their behavioural phenotype in adulthood (Branchi et al. 2011; D'Andrea et al. 2010; Branchi et al. 2009). The least successful females are those that raise their young on their own (second-generation wild mice under laboratory conditions; König 1993).

For young females it is less dangerous to remain in the colony also after reaching sexual maturity. However, many females remain so subordinate that they never raise their own litter. These females, which do not reproduce themselves, help other females, in many cases closely related females, in rearing their young (according to Latham and Mason 2004).

The behavioural repertoire is basically very conservative and changed very little during domestication, especially as domestication history of mice is much shorter than that of other domestic animals. A major difference exists, however, in the quantity of the behaviours shown: laboratory mice, despite all the major differences between strains, are basically much calmer and less aggressive than wild mice.

Young mice, and adult females and males:

Young mice

Early social experiences influence the development of social skills and the ability to process stress adequately (according to Olsson and Westlund 2007). For example, mice that are separated from their mothers for 3 hours each day during the early phase of their life (days 1 to 14 after birth) show increased anxiety in adulthood, alongside with an elevated stress response to acute stressors, higher susceptibility to the negative consequences of chronic psychosocial stress, changes in aggressive behaviour (males: reduced territorial aggression; females: increase in maternal aggression), increased intestinal permeability and reduced exploratory behaviour (according to Veenema et al. 2009). On the other hand, a separation of the young for only 15 minutes each day has positive consequences on their sensitivity to stress in adulthood (Meaney and Aitken 1985, Meaney et al. 1988a, 1988b). While young in the wild migrate a few weeks after the end of the suckling period (Kikusui and Mory 2009, after Olsson and Westlund 2007), weaning of the young at this point is absolutely essential in the laboratory. However, single housing directly after weaning of the young has seems to have more serious negative consequences for mice than single housing in adulthood. It has been shown, for example, that isolation after weaning leads to an increase in stress reactivity, startle reaction, anxiety, aggressiveness, and alcohol consumption (according to Veenema et al. 2009).

Conclusion: Young mice should basically be reared communally and remain in a harmonious, stable group for as long as possible, initially in the litter and later with their siblings of the same sex.

Adult females

The females of most mouse strains are tolerant and can be housed in same-sex pairs or groups without any problem. Some studies have reported positive effects of communal housing (e.g. Meijer et al. 2006). The postoperative recovery phase of females housed in pairs is shorter than with singly housed females (van Loo et al. 2007, Jirkof et al. 2012). However, the groups should ideally grow up together, as socializing with older animals can partially lead to aggressive conflicts (cf. Jirkof et al. 2012). In general, it can be assumed that single housing is more stressful for female than for male mice (Blanchard et al. 2001).

Conclusion: Female mice should basically be housed in groups or at least in pairs, bearing in mind also that the other cage occupants should ideally be litter siblings or that they have at least grown up together.

Adult males

Group housing of males serves as a model for chronic psychosocial stress under certain conditions. For example, it has been shown that social instability within a male mouse colony (the composition of the group in each case was changed twice a week over seven weeks) leads to an increase in behaviour resembling anxiety and depression, reduced exploratory behaviour, adrenal hypertrophy, a disturbed circadian rhythm of corticosterone, a change in the expression of neuropeptides in the brain and a reduced negative feedback effect on the hypothalamic-pituitary-adrenal (HPA) axis (Schmidt et al. 2007, Sterlemann et al. 2009). But aside from social instability within a mouse colony, lasting subordination within a group of male mice that is induced experimentally (by co-housing four relatively light laboratory mice with a heavier and more aggressive resident = chronic subordinate colony housing (CSC)) leads to characteristic signs of chronic stress, such as reduced weight gain, adrenal hypertrophy, increased anxiousness, increased alcohol consumption, spontaneous enterocolitis and loss of social preferences (Reber et al. 2007). This experimental design can easily emerge by chance und unintentionally by group housing of male mice. Indeed, such hierarchical relationships also form within a group of male mice that had the same age and the same weight at the time when the group was formed. The effects are somewhat less prominent than in subordinate CSC mice, but control animals in group housing likewise show increased anxiousness, reduced readiness to take risks and explore, as well as reduced weight gain (Singewald et al. 2009) in contrast to singly housed controls (Singewald et al. 2009). Combined studies on group vs single housing and an impoverished vs an enriched environment in B6 males show that signs of learned helplessness in males grouped together in an impoverished environment (cage with bedding) are more pronounced than in males grouped together in an enriched environment (bedding, nesting material and tunnel) and in singly housed males (both forms of housing). The group housing of males thus acts as a stress factor, which is reinforced by an impoverished environment (no possibility for hiding). By contrast, single housing does not appear to have any negative effects on sensitivity to stress (Chourbaji et al. 2005) and does not in itself induce any immunological or endocrine stress symptoms (Gasparotto et al. 2005). Even in male rats where social isolation was described in some studies as a chronic stressor (Wallace et al. 2009), no immunological, ethological or endocrine differences were observed between controls housed singly and those housed in groups in other studies (Nyuyki et al. 2012). As regards the enrichment of the cage environment, however, studies must also be mentioned - for the sake of completeness - which clearly show that group housing of males

can result in increased aggression between the occupants of the cage and thus leads to a marked increase in stress caused by the formation of hierarchies (Marashi et al. 2003). This is also utilized in the so-called visible burrow system, which is deployed throughout the world in male rats and mice as a model for chronic psychosocial stress (Blanchard et al. 1995; Arakawa et al. 2007). Through the creation of an extremely enriched habitat (several boxes connected with each other by tunnel systems) territorial aggression between males is markedly increased here, which results in a fiercely contested hierarchical structure and enormous ethological, physiological and neuronal changes. Partial cage dividers, on the other hand, significantly reduced aggressive-like behaviour in group-housed male mice (Tallent et al. 2018).

Co-housing of a single male with 1-3 females probably approximates most closely to the natural way of life of adult, dominant males and may also represent the least stressful mode of husbandry for males: experimental co-housing of males with ovariectomised females resulted in lower heart rates and longer uninterrupted periods of sleep than in singly housed males (Späni et al. 2003).

Conclusion: After reaching a certain age (depending on the strain), single housing of male mice is preferable to group housing with other males. The occurrence of bite wounds must not be the sole criterion here, because housing-related stress and anxiety states without any physical injury must also be avoided as far as possible. In addition, from a scientific standpoint it seems questionable to include animals in the evaluation that come from different housing conditions without taking any account of this factor and thus usefully to aim at a feasible form of housing as a parameter that can be standardized.

It is emphasized here that single housing does not mean isolation. Visual, audible and olfactory contact remain intact.

Conclusion: Does the single housing of mice for scientific purposes in principle require approval?

The decision as to whether an individual procedure requires approval or not is a matter for the authorities. The Committee for Humane Laboratory Animal Housing cannot answer legal queries, but can only contribute to solutions in terms of content.

Clearly, the authorities can only make a decision on a possible approval if they have knowledge of a planned procedure. Therefore, when the scientists and animal welfare officers concerned have the slightest doubt, planned procedures must be submitted to the authorities so that they can be assessed.

In the current German animal welfare act (TierSchG), “animal experiment” is defined as follows: “Animal experiments within the meaning of the law are procedures or treatments for scientific purposes (…) if they may be associated with pain, suffering or harm”, which also includes changes in the genotype (TierSchG §7 Para. (1)). This definition is retained in the draft amendment and simply extended (to include animals prior to birth and newborns and also a part of the previous notification procedures, e.g., teaching purposes; see homepage of Germany’s Federal Ministry of Food and Agriculture [BMELV]).

Conversely, when there is no possibility of pain, suffering or harm occurring, it is not deemed to be an animal experiment, even if the work involved has a scientific purpose within a given research project. The occurrence of pain, suffering or harm as a result of single housing depends on the sex, age and strain of the mice and also the duration and housing conditions during single housing. While there is no scientific evidence that the welfare of adult animals is compromised by a short period of single housing, the prolonged single housing of females or early single housing of young animals can be associated with suffering on the part of the animals. In the case of temporary single housing, however, it must be borne in mind that the return to a group can prove extremely problematic, especially if the animals have reached the age of sexual maturity and such changes in the constellation can result in dominance reversal, which is associated with stress (Mondragon et al. 1987). Single housing of adult male mice is an acceptable form of housing.

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