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Module 2, Video 7: Housing considerations when using male and female animals

Concept introduction: Recent pushes to improve preclinical reproducibility have emphasized that all experimental conditions be transparently reported, including those related to rearing and testing. The home cage environment is often an overlooked source of experimental variability that can have profound effects on how animals behave outside of the cage [1]. Some of these home cage conditions have different effects in males and females. In this video, we will discuss examples of home cage housing conditions that demonstrate how the context can matter by introducing phenotypic variation in males and females both within and across cages.

Deciding whether to house your animals individually or in same-sex pairs or small groups may be the most important of all the housing variables to consider. This decision is often made based on the experimental aims. As social animals, most rodents are well adapted to living in a group and social contact with conspecifics is important for their overall well-being [1]. However, sometimes single housing is used as an experimental design modification to, for example, obtain accurate behavioral measurements during home cage monitoring or ensure the safety of animals following a surgical procedure. If you choose to house your animals individually, it is important to consider that physical and social isolation can produce stress, especially when done chronically. These effects are widely documented in rodents and other social animals and can be expressed as variations in physiology [5-8]. Stress can also be manifested via changes in anxiety and depression phenotypes as well as altered learning and memory.

The effects of isolation are different between males and females and may depend upon the developmental time period, with effects in adulthood skewed towards females and effects post-weaning and adolescence skewed towards males [8-24].

When social isolation is necessary, the different effects on males and females should be accounted for as a potential confounder, particular during development. Alternatively, some mitigation strategies have been proposed including allowing for olfactory, visual and auditory contact with conspecifics [25] or providing environmental enrichment [26].

On the other end of the spectrum, group housing may also lead to unintended phenotypic variation in males and females. There are two primary concerns of group housing. The first is unintentional crowding through either a decrease in spatial density or an increase in social density. In rat studies where the available space was decreased and the number of rats per group was increased, males showed greater HPA responsivity; in contrast, females subjected to the same changes showed either no effects or even positive effects of crowding [27-29]. High housing density has also been suggested to either cause or exacerbate the prevalence of barbering, a repetitive abnormal behavior indicating social stress, especially among female mice [30, 31].

The second concern of group housing is aggressive behavior associated with the establishment and maintenance of social hierarchies. Across species, social hierarchies and aggression are typically studied in the context of male inter-sexual competition, with greater competition leading to higher aggression in males. This aggression can lead to large inter-cage variations in phenotypic expression, with greater effects in male subordinates [32]. Fewer studies have examined the role of hierarchies on phenotypic variation in females, outside of the specific case of the maternal defense of offspring.

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But other group-housed females also form hierarchies, which should be accounted for in design and analysis.

Another important point to consider is whether or not males and females should be housed in separate rooms in the animal care facility. Generally, there are two reasons to argue against this strategy. First, cross-room comparisons are limited when males and females in the same experiment are kept in separate rooms. This is because subtle room-to-room environmental variations cannot be accounted for statistically. However, if the experimental conditions dictate that males and females be housed in separate rooms, replicate batches must be used where the rooms for males and females are switched between the batches to control for between room variables.

Second, the presence of male pheromones is needed for proper female hormone cycling and reproductive viability [35-44]. For example, females housed in groups and isolated from male odors exhibit a prolongation or suppression of the estrous cycle [33, 34] and delayed puberty in juvenile animals.

The presence of cycling females is also needed for proper androgen and sperm production in male mice [45]. In addition, ovarian synchrony in females can be induced by either housing female rats together with males in the same cage or in the same room with airborne olfactory cue exposure [46, 47]; similar findings have been observed in other species [48, 49].

Breeders, however, should always be housed separately from experimental animals for two main reasons. First, female mice exposed to urine from pregnant or lactating females have longer periods of estrus than mice exposed to the urine of single housed females [50]. Second, smelling the urine of an unfamiliar male can block embryo implantation early in pregnancy [51-52], resulting in a return to ovulation.

As demonstrated in this video, the context in which an animal is housed can have wide-reaching implications on experimental outcomes. Males and females can have different behavioral and neurochemical changes in response to the same housing conditions. Thus, known and potential sex differences in response to housing conditions should be accounted for in experimental and statistical design.

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