Damanhur Journal of Veterinary Sciences 13 (1), (2025) 52-57

Damanhur Journal of Veterinary Sciences

Journal homepage: https://djvs.journals.ekb.eg/

E-ISSN 2636-3003 | ISSN 2636-3011

# Effect of Acute isolation on some welfare indices in Mice

**Safinaz A. Ibrahim[1], Abdelwahab A. Alsenosy[2*], Mona E. Metwali1, Sherif Z. El Kholya[1], and Usama E. Mahrous[1]**

[1]Department of Animal and poultry Behaviour and Management, Faculty of Veterinary Medicine, Damanhur University, Egypt
[2]Department of Biochemistry, Faculty of Veterinary Medicine, Damanhur University, Egypt

**Abstract**

In adherence to the Guide for the Care and Use of Laboratory Animals, this study investigated the effects of acute stress on some behavioural indices.

A total of forty male (n=20) and female (n=20) mice, aged 4 weeks old and weigh 15-25 g were provided from Faculty of Agriculture, Alexandria university were videotaped in the mice' home cages to monitor their behaviour.

The obtained results showed mild differences in feeding, grooming, still alert, rearing, sniffing, and burrowing and bar chewing stereotype behaviours. Although drinking, movement, sleeping, allo-grooming and aggressive behaviours did not show marked improvement during the acute stress phase.

**Keywords:** Acute isolation, Mice, Behaviour

[*]**Correspondence**: Abdelwahab A. Alsenosy
Department of Biochemistry, Faculty of Veterinary Medicine, Damanhur University, Egypt.
Email: dr_alsenosy_2010@vetmed.dmu.edu.eg

P ISSN: 2636-3003
EISSN: 2636-2996
DOI: 10.21608/djvs.2025.295007.1132
Received: June 3, 2024; Received in revised form: September 23, 2024; accepted: January 20, 2025
Editor-in-Chief: Ass. Prof .Dr/Abdelwahab A. Alsenosy (editor@vetmed.dmu.edu.eg )

## 1.Introduction

Mice and rats make up approximately 95% of all laboratory animals, where mice consider the most commonly used animal model, since, they are mammals and their organ systems are very similar to organ systems in humans in terms of shape, structure, and physiology **(Hickman et al., 2017).**

Stress is a complex phenomenon that has been investigated from many different perspectives. Many researchers view stress theory as having evolved from a specific response for each specific stimulus through a standardized general adaptation syndrome for a wide variety of stressors to a newly hypothesized model that suggests a standardized physiological response for psychological stressors and specific homeostatic responses for physical stressors. Stressors can originate from within an individual (endogenous) or from the environment (exogenous) **(Friend, 1991).**

The housing condition of laboratory rodents has been demonstrated to induce changes in their behaviour, physiology and pathology which in turn could deteriorate not only the animal welfare but also, the quality of scientific data. An important aspect of the housing conditions is the social environment (e.g. single versus group housing). Moreover, numerous studies ascertained positive effects of enriched environments on the well-being of laboratory animals including behavioural, physiological and neurochemical parameters **(Abou-Elnaga et al., 2019).**

Acute stress, occurring at a single day and lasting minutes to hours, promotes short-term physiological, immunological, and neurological adaptation and stability. Chronic stress, which lasts several hours every day for a period of weeks or months may have deleterious consequences and is recognized as a risk factor for many diseases and psychiatric disorders **(Dudek et al., 2021).**

This study underscores the impact of acute stress of social isolation on behavioural parameters of caged mice, highlighting its significance on altering mice welfare.

## 2.Materials and Methods

The study was accomplished at research unit of Department of Animal and Poultry Behaviour and Management, Faculty of Veterinary Medicine, Damanhur University during the period from May until August to investigate the effect of isolation stress versus environmental enrichment on some welfare

indices of mice furthermore diurnal behavioural patterns of mice.

*2.1. Animals*

A total of forty male (n=20) and female (n=20) mice, aged 4 weeks old and weigh 15-25 g were provided from Faculty of Agriculture, Alexandria university.

*2.2. Management:*

The ethical committee of the Faculty of Veterinary Medicine, Damanhour University (DMU-VET-IMED-2022-01) approved the research protocol. All animal handling and procedures were carried out following national animal care and welfare.

Mice were housed in clean and well ventilated propylene cages with wire mesh cover that had been previously disinfected using alcohol 70% and well-bedded with clean saw dust.

Mice were kept under natural cycle of light (12-hours light /12-hours dark) without using artificial lighting program.

Mice were demounted once weekly from cages for cleaning and removing of fecal matter. Moreover, feeders and drinkers were taken out and immersed in potassium permanganate solution overnight, then dried and used.

Mice were fed a commercial broiler starter ration from EL-Eman Company, Egypt containing 23% crude protein, 2.97% fat and 3.81% crude fiber and tap water was continuously available through glasses with nibbles.

Different cage labels were used for marking groups, remarking was applied at intervals when labels diminished.

Medicaments used were Omigran powder as a source of vitamin and electrolyte mixture for three consecutive days in the second week. Moreover, detox liquid was given for three consecutive days in the 4th week to provide antitoxin protection.

*2.3. Experimental procedures:*

Effect of acute social isolation:

Forty mice were allotted into 4 groups represented by:

a. Group 1: Ten socially isolated males (one mouse/cage sized 10*22*28 cm)
b. Group 2: Ten socially housed males (control) allotted into 2 subgroups each one five mice/cage (sized 18*28*40 cm).
c. Group 3: Ten socially isolated females (one mouse/cage sized 10*22*28 cm).
d. Group 4: Ten socially housed females (control) allotted into 2 subgroups each one five mice/cage (sized 18*28*40 cm).

The effect of acute isolation was investigated for 3 consecutive days (from age of 30 days until age of 33 days). They had free access to feed and water i.e ad-libtum feeding 100 g / group.

*2.4. Behavioural observation:*

Behavioural observations were carried out to study the effect of acute isolation. Mice were observed 4 times per day early morning (6-6:20 min), early afternoon (12- 12:20), early evening (6-6:20) and late evening (12-12:20).

The observed behavioural patterns were defined by (www.mousebehaviour.org):

| Behaviour | Description |
|---|---|
| Feeding : | Handling/manipulating/ingesting food item (including bedding/nesting material and/or fecal pellets. |
| Drinking : | Drinking from bowl or drinker. |
| Sleep : | Lying with eyes closed. |
| Self-grooming | Licking and nibbling directed towards all of own body surfaces carried out using the mouth/tongue and/or front paws. |
| Gnawing | Using teeth to gnaw and chew at objects within the cage, excluding cage bars |
| Movement | Walking, hopping, running or jumping |
| Stationary alert | Alert (eyes open) but with no directed attention, while sitting, standing, or leaning against the cage side or an object within the cage. Body relaxed and immobile. |
| Rearing | Sitting on hind limbs with forepaws off the ground, and stretching up without leaning on cage side or any other object. |
| Sniffing | Sniffing movements with nose applied to any part of the cage/air/bedding material/nesting material/ground/ other objects within the cage. |
| Burrowing | Digging in the floor substrate/litter material with forepaws, interspersed with kicking movements of hind legs. |
| Allo-groom | Gentle grooming (sometimes reciprocal) of any part of another individual. Engaging in allo-grooming is voluntary and may be solicited |
| Agonism | Pushing/boxing/pawing one another with forepaws; chasing or being chased; biting or being bitten; vocalization. |
| Bar-chewing | Using teeth to gnaw/chew/bite repetitively at the wire bars of the cage. |

*2.5. Statistical analysis*

Data were collected and analysed by statistical software SPSS version. The data were analysed two way analysis of variance with the following model

$$X_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$$

Where: $X_{ijk}$ = An observational data; $\mu$= Overall mean; $A_i$=effect of $i^{th}$ housing system; $B_j$ = Effect of $j^{th}$ sex; $(AB)_{ij}$= Effect due to interaction between housing system and sex and $e_{ijk}$=random error.

## 3.Results

Table (1): Means and their standard error of the effect of housing system and days of acute isolation on ingestive behaviour of mice

| Item | | Feeding | Drinking |
|---|---|---|---|
| **Housing system** | | | |
| Individual male | | 0.47±0.08 | 0.29±0.08[b] |
| Individual female | | 0.45±0.08 | 0.18±0.08[b] |
| Grouped males | | 0.51±0.19 | 1.06±0.17[a] |
| Grouped females | | 0.66±0.19 | 0.92±0.17[a] |
| **Days of isolation** | | | |
| 1st day | | 0.46±0.13 | 0.34±0.11[b] |
| 2nd day | | 0.60±0.13 | 1.01±0.11[a] |
| 3rd day | | 0.51±0.13 | 0.48±0.11[ab] |
| **Days of isolation*Housing system** | | | |
| 1 | Individual male | 0.28±0.15 | 0.10±0.13[c] |
| 1 | Individual female | 0.38±0.15 | 0.10±0.13[c] |
| 1 | Grouped males | 0.43±0.33 | 0.38±0.29[c] |
| 1 | Grouped females | 0.75±0.33 | 0.80±0.29[b] |
| 2 | Individual male | 0.44±0.15 | 0.41±0.13[c] |
| 2 | Individual female | 0.68±0.15 | 0.13±0.13[c] |
| 2 | Grouped males | 0.60±0.33 | 1.93±0.29[a] |
| 2 | Grouped females | 0.67±0.33 | 1.58±0.29[a] |
| 3 | Individual male | 0.69±0.15 | 0.36±0.13[c] |
| 3 | Individual female | 0.30±0.15 | 0.33±0.13[c] |
| 3 | Grouped males | 0.50±0.33 | 0.88±0.29[b] |
| 3 | Grouped females | 0.55±0.33 | 0.38±0.29[c] |

Means within the same column carry different superscripts are significantly different

Table (2) Means and their standard error of the effect of housing system and days of acute isolation on movement activities and resting behaviour of mice

| Item | | Movement | Still alert | Sleeping |
|---|---|---|---|---|
| **Housing system** | | | | |
| Individual male | | 3.99±0.51[b] | 0.35±0.09 | 0.67±0.05[b] |
| Individual female | | 2.86±0.51[b] | 0.38±0.09 | 0.67±0.05[b] |
| Grouped males | | 7.04±1.15[a] | 0.22±0.20 | 0.83±0.12[a] |
| Grouped females | | 6.88±1.15[a] | 0.63±0.20 | 0.98±0.12[a] |
| **Days of isolation** | | | | |
| 1st day | | 7.83±0.77[a] | 0.78±0.14[a] | 0.56±0.08[b] |
| 2nd day | | 3.33±0.77[b] | 0.14±0.14[b] | 0.86±0.08[a] |
| 3rd day | | 4.42±0.77[b] | 0.26±0.14[b] | 0.93±0.08[a] |
| **Days of isolation*Housing system** | | | | |
| 1 | Individual male | 5.95±0.89 | 0.65±0.16 | 0.40±0.09 |
| 1 | Individual female | 5.68±0.89 | 0.78±0.16 | 0.30±0.09 |
| 1 | Grouped males | 7.53±1.99 | 0.2±0.35 | 0.63±0.20 |
| 1 | Grouped females | 12.18±1.99 | 1.48±0.35 | 0.93±0.20 |
| 2 | Individual male | 2.05±0.88 | 0.12±0.16 | 0.78±0.09 |
| 2 | Individual female | 1.1±0.89 | 0.07±0.16 | 0.83±0.09 |
| 2 | Grouped males | 5.43±1.99 | 0.18±0.35 | 0.80±0.20 |
| 2 | Grouped females | 4.75±1.99 | 0.2±0.35 | 1.05±0.20 |
| 3 | Individual male | 3.97±0.9 | 0.28±0.16 | 0.82±0.09 |
| 3 | Individual female | 1.8±0.89 | 0.28±0.16 | 0.88±0.09 |
| 3 | Grouped males | 8.18±1.99 | 0.28±0.35 | 1.05±0.20 |
| 3 | Grouped females | 3.73±1.99 | 0.2±0.35 | 0.98±0.20 |

Means within the same column carry different superscripts are significantly different

Table (3) Means and their standard error of the effect of housing system and days of acute isolation on exploratory behaviours of mice

| Item | | Rearing | Sniffing | Burrowing | Grooming |
|---|---|---|---|---|---|
| **Housing system** | | | | | |
| Individual male | | 4.49±0.70 | 1.98±0.27 | 1.32±0.27 | 2.66±0.34 |
| Individual female | | 3.79±0.70 | 1.32±0.27 | 0.91±0.27 | 3.38±0.34 |
| Grouped males | | 5.72±1.56 | 1.02±0.60 | 1.65±0.61 | 3.52±0.76 |
| Grouped females | | 3.61±1.56 | 0.88±0.60 | 1.65±0.61 | 2.56±0.76 |
| **Days of isolation** | | | | | |
| 1st day | | 6.36±1.04[a] | 1.73±0.4 | 2.24±0.41[a] | 3.20±0.51 |
| 2nd day | | 3.48±1.04[b] | 1.05±0.4 | 0.73±0.41[b] | 2.69±0.51 |
| 3rd day | | 3.36±1.04[b] | 1.12±0.4 | 1.17±0.41[b] | 3.19±0.51 |
| **Days of isolation*Housing system** | | | | | |
| 1 | Individual male | 7.48±1.2 | 3.1±0.46 | 2.08±0.47 | 2.33±0.59 |
| 1 | Individual female | 6.95±1.2 | 1.85±0.46 | 1.68±0.47 | 3.93±0.59 |
| 1 | Grouped males | 5.48±2.69 | 1.2±1.03 | 3.25±1.05 | 4.23±1.32 |
| 1 | Grouped females | 5.52±2.69 | 0.75±1.03 | 1.95±1.05 | 2.33±1.32 |
| 2 | Individual male | 2.63±1.19 | 1.22±0.46 | 0.49±0.46 | 2.39±0.58 |
| 2 | Individual female | 2.33±1.2 | 0.85±0.46 | 0.53±0.47 | 3.3±0.59 |
| 2 | Grouped males | 6.08±2.69 | 0.98±1.03 | 0.33±1.05 | 2.68±1.32 |
| 2 | Grouped females | 2.9±2.69 | 1.18±1.03 | 1.6±1.0 | 2.4±1.32 |

| | | | | 5 | |
|---|---|---|---|---|---|
| 3 | Individual male | 3.36±1.22 | 1.62±0.47 | 1.38±0.47 | 3.26±0.6 |
| 3 | Individual female | 2.1±1.2 | 1.25±0.46 | 0.53±0.47 | 2.9±0.59 |
| 3 | Grouped males | 5.6±2.69 | 0.88±1.03 | 1.38±1.05 | 3.65±1.32 |
| 3 | Grouped females | 2.4±2.69 | 0.72±1.03 | 1.4±1.05 | 2.95±1.32 |

Means within the same column carry different superscripts are significantly different

Table (4) Means and their standard error of the effect of housing system and days of acute isolation on social behaviour of mice

| Item | | Mutual grooming | Aggression | Bar-mouthing |
|---|---|---|---|---|
| **Housing system** | | | | |
| Individual male | | 0.00±0.00[b] | 0.00±0.00[c] | 0.11±0.05 |
| Individual female | | 0.00±0.00[b] | 0.00±0.00[c] | 0.00±0.00 |
| Grouped males | | 0.23±0.03[a] | 0.13±0.02[a] | 0.03±0.11 |
| Grouped females | | 0.17±0.03[a] | 0.06±0.02[b] | 0.01±0.11 |
| **Days of isolation** | | | | |
| 1st day | | 0.08±0.02 | 0.02±0.01 | 0.02±0.07 |
| 2nd day | | 0.10±0.02 | 0.08±0.01 | 0.05±0.07 |
| 3rd day | | 0.12±0.02 | 0.05±0.01 | 0.04±0.07 |
| **Days of isolation*Housing system** | | | | |
| 1 | Individual male | 0.13±0.09 | 0.00±0.00 | 0.00±0.00 |
| 1 | Individual female | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 1 | Grouped males | 0.00±0.00 | 0.18±0.05 | 0.08±0.18 |
| 1 | Grouped females | 0.00±0.00 | 0.13±0.05 | 0.00±0.00 |
| 2 | Individual male | 0.00±0.00 | 0.00±0.00 | 0.15±0.08 |
| 2 | Individual female | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 2 | Grouped males | 0.02±0.20 | 0.1±0.05 | 0.03±0.18 |
| 2 | Grouped females | 0.13±0.20 | 0.3±0.05 | 0.03±0.18 |
| 3 | Individual male | 0.44±0.09 | 0.00±0.00 | 0.18±0.08 |
| 3 | Individual female | 0.13±0.09 | 0.00±0.00 | 0.00±0.00 |
| 3 | Grouped males | 0.08±0.20 | 0.4±0.05 | 0.00±0.00 |
| 3 | Grouped females | 0.13±0.20 | 0.07±0.05 | 0.00±0.00 |

Means within the same column carry different superscripts are significantly different

**Figure 1:** The effect of housing system and acute isolation on ingestive behaviour of mice.

**Figure 2:** The effect of housing system and acute isolation on movement activities and resting behaviour of mice.

**Figure 3:** The effect of housing system and acute isolation on exploratory behaviour of mice.

**Figure 4:** The effect of housing system and acute isolation on social behaviour of mice.

## 4. Discussion

*4.1. Ingestive behaviour:*

Feed and water intake in mice seem to be following a pattern with small, frequent meals associated with drinking. Feed intake is dependent on housing temperature and strain and two-thirds of their food is eaten during the dark phase **(Jensen, et al., 2013).** Regarding to acute isolation of mice results in table (1) and Figure (1) revealed that there was not significant effect on feeding behaviour between groups. This finding agree with **Muroy et al. (2016)** and **Ely et al. (1997)** study in rats. While it disagree with **Vallès et al. (2000)** who observed that acute immobilization stress lead to a decrease in feed intake and body weight one day post stress. This result might be attributed to differences between the strain of the laboratory animal model or the intensity and duration of stress exposure. However, drinking behaviour was found to be higher in socially housed mice of both sexes than socially isolated mice (1.06±0.17) and (0.92±0.17) versus (0.29±0.08) and (0.18±0.08) respectively. This finding was in close accordance with **Baker et al. (2006)** who concluded an overall reduction in fluid intake after stress exposure

*4.2. Movement and resting behaviour:*

Motor activity expression is essential for both animal and human species to explore their environment for food and social interaction. The control of these diverse but directed movements relies on highly integrated neural networks, such as satiety signals that trigger the need to search for food, and projections from the motor cortex to local circuits in the spinal cord that generate locomotion **(Kas et al., 2009).**

Analysing the influence of acute isolation on home cage behaviours revealed that socially housed mice males and females showed the highest movement activity (7.04±1.15), (6.88±1.15) respectively compared to socially isolated males (3.99±0.51) and females (2.86±0.51) mice groups with no significant difference between sexes. This result agreed with **Moraska and Fleshner, (2001)** and **Shinba et al. (2001)** who reported that acute stressors developed a long term reduction in physical activity.

In the light, rats tend to sleep with curled-up body and closed eyes, while in the dark they tend to sleep more stretched out and often with open eyes. These differences in posture may be caused by the differences in light intensity **(Van Betteray et al., 1991).**

Results in table (2) and Figure (2) showed that socially housed mice of both sexes recorded higher sleeping behaviour since, means were (0.83±0.12) in males and (0.98±0.12) in females compared to those subjected to acute isolation (0.67±0.05) in both sexes. The same result obtained by **Gargiulo et al. (2021)** and **Koehl et al. (2002)** who found that acute restraint stress increased wakefulness and decreased non-rapid eye movement sleep in rats during light phase. This could be a response to mice experiencing stress may be in a state of hyper-arousal, leading to difficulty in initiating or maintaining sleep.

In the light, rats tend to sleep with curled-up body and closed eyes, while in the dark they tend to sleep more stretched out and often with open eyes. These differences in posture may be caused by the differences in light intensity **(Van Betteray et al., 1991).**

*4.3. Exploratory behaviour and body care activities::*

When faced with an unfamiliar environment or object, animals often exhibit behaviour patterns that broadly can be termed exploration, such as locomoting around the environment, orientating towards novelty, and touching or sniffing novel objects **(Glickman and Sroges, 1966).**

Results summarized in table (3) and Figure (3) revealed that acute isolation of mice had no significant difference on both rearing and sniffing behaviours. This finding is close to **Mercier et al, (2003)** who reported that no changes in exploratory behaviour for animals when they subjected to stress. Although, **(Heinz et al., 2021)** found that anxiety-like behaviour of rodents was frequently accompanied by reduced exploratory behaviour.

Grooming is an innate behaviour conserved across animal species, including mammals. Mouse grooming serves many adaptive functions such as coat and body care, stress reduction, de-arousal, social functions, thermoregulation, nociception, as well as other functions. It is regulated by specific brain circuits and is sensitive to stress **(Kalueff et al., 2010).**

Acute isolation of mice unlikely to have a pronounced effect on grooming behaviour of mice, the data illustrated in Table (3) showed that short term isolation didn't significantly alter grooming behaviour in isolated male and female mice (2.66±0.34) (3.38±0.34) respectively compared to socially housed mice males and females (3.52±0.76) (2.56±0.76) respectively, however, **Rojas-Carvajal and Brenes (2020)** noted that foot shock stress slightly decreased complex grooming sequences but increased cephalic grooming.

*4.4. Social behaviours:*

Social interactions between animals are affected by the number of animals associated together in a common group. Social interactions in such circumstances are affected by the position of the interacting animal in the dominant order of the group **(Arnold and Pahl, 1974).**

Socially housed mice during the course of acute isolation experiment (Table 4 and Figure 4) showed highest aggressive behaviour in socially housed males (0.13±0.02) compared to socially housed females (0.06±0.02). This difference in aggressive behaviour could be attributed to testosterone hormones of males, which more prone to exhibit aggressive behaviours, especially when establishing social hierarchies or defending territories. On the other hand, the estrogen and progesterone in females have a moderating effect leading to less aggression. Moreover, housing male's mice sporadically had a bad impact on their abnormal behaviour where mice Table (4) showed higher levels of bar mouthing than other groups either sporadic females or grouped males or females.

4.5 Abnormal behaviors:

Stereotypic behaviors are rarely observed in the wild and their presence in captive environments is associated with the unnatural evolution of species-specific behaviors. Thus, it is considered as a coping mechanism for reducing the magnitude of environmental induced stress (Polverino et al., 2015). With regard to the effect of acute isolation on induction of bar-mouthing behavior in mice we found no significant difference between groups. This may be attributed to the short duration and low intensity of the stress applied. Particularly, such behaviors usually appear after experiencing boredom or perceiving stress as a threatening.

**Conflict of interest**: There are no conflicts of interest stated by the authors.

**Acknowledgement**: Special thanks to assistances in animal and poultry behaviour department for their help in completing this study.

**Funding**: This research did not receive any specific grant from public, commercial, or not-for-profit funding agencies.

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