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DO DIFFERENT CAGING SYSTEMS AFFECT THE OUTCOME OF BEHAVIORAL TESTS IN C57BL/6J MALE MICE?

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INTRODUCTION

Housing can affect the **health** and **well-being** of laboratory mice.

→ May affect the **outcome** and the **reliability** of the experimental results.

The purpose of this study was to assess the role of the caging system in the **exploratory** and **anxiety-related behavior** of mice.

Laboratory mice housing systems

Laboratory mice cages must:

- ✓ be constructed of safe, durable materials;
- ✓ be kept clean;
- ✓ be maintained in good repair;
- ✓ be secure and escape-proof;
- ✓ protect mice from climatic extremes;
- ✓ not cause injury to mice;
- ✓ be large enough for the number of animals held; and
- ✓ be compatible with the behavioral needs of the mice.
- ✓ provide the availability to observe the animals readily.

Open Top Cages – OTCs

- ✓ Free exchange of air;

- ✓ The intracage environment is influenced
  - by the animal room's environment (macroenvironment);
  - the frequency of bedding changes, and the
  - housing density.

- ✓ Allows cage-to-cage and room-to-cage transmission of airborne pathogens;
- ✓ Does not protect staff from exposure to animal-related aeroallergens.

Individually Ventilated Cages – IVCs

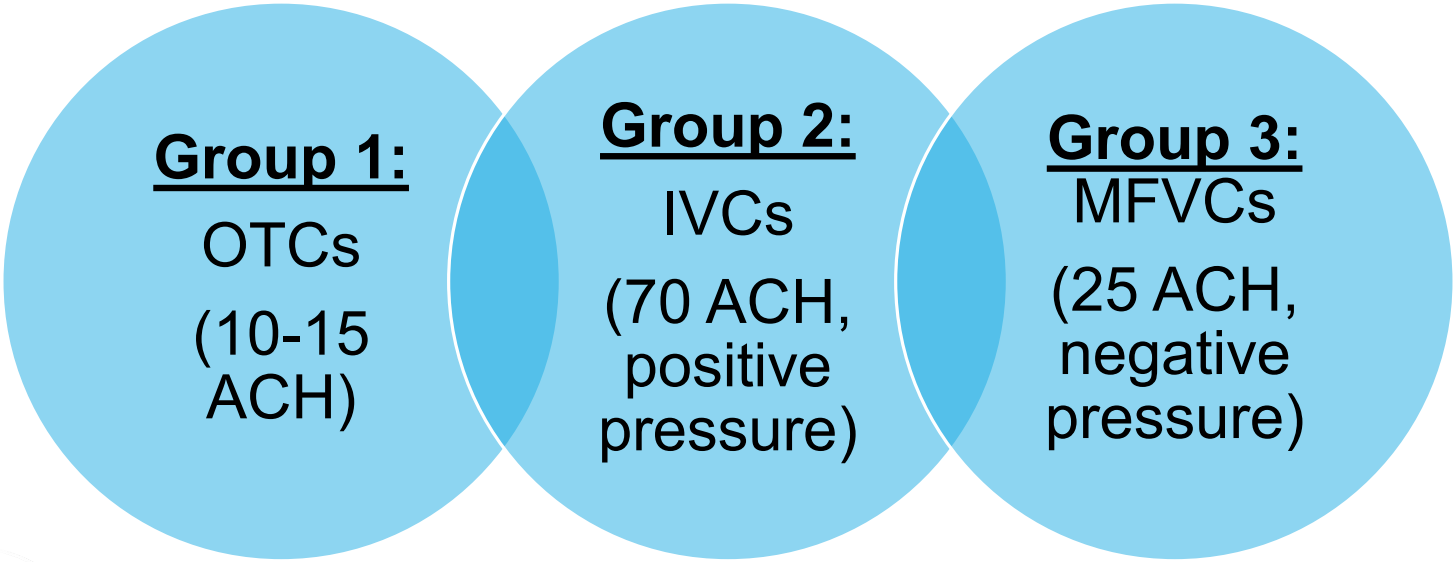
- ✓ Provide a significant barrier to the spread of infectious agents;
- ✓ Protect staff from aeroallergens and zoonotic agents (quarantine);
- ✓ Improve the air quality in the cage (high-efficiency particulate filtration of the incoming air-HEPA);
- ✓ Reduce the frequency of cage and bedding changes;
- ✓ Negative or positive intracage pressure can be defined;
- ✓ Ventilation rates may vary from 25 to 120 air changes per hour (ACH).

Motor Free Ventilated Cages – MFVCs

- ✓ Connected directly to the exhaust of the room's heating, ventilation, and air conditioning (HVAC) unit;
- ✓ Protect the animals against pathogens;
- ✓ Protect the staff against animal-related aeroallergens;
- ✓ Operate only under negative pressure;
- ✓ Ventilation rates (20–25 ACH) can't be modified.

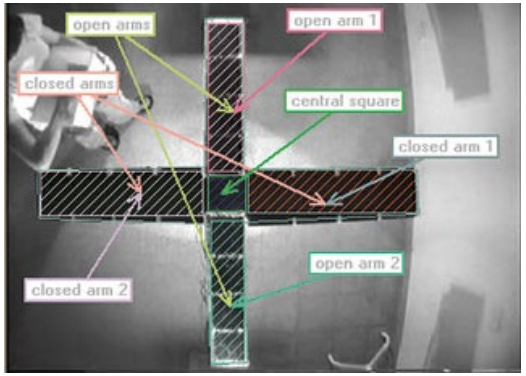
MATERIALS AND METHODS

- ✓ Thirty six 25-days-old C57BL/6, male mice were randomly divided into three equal groups (n = 12):



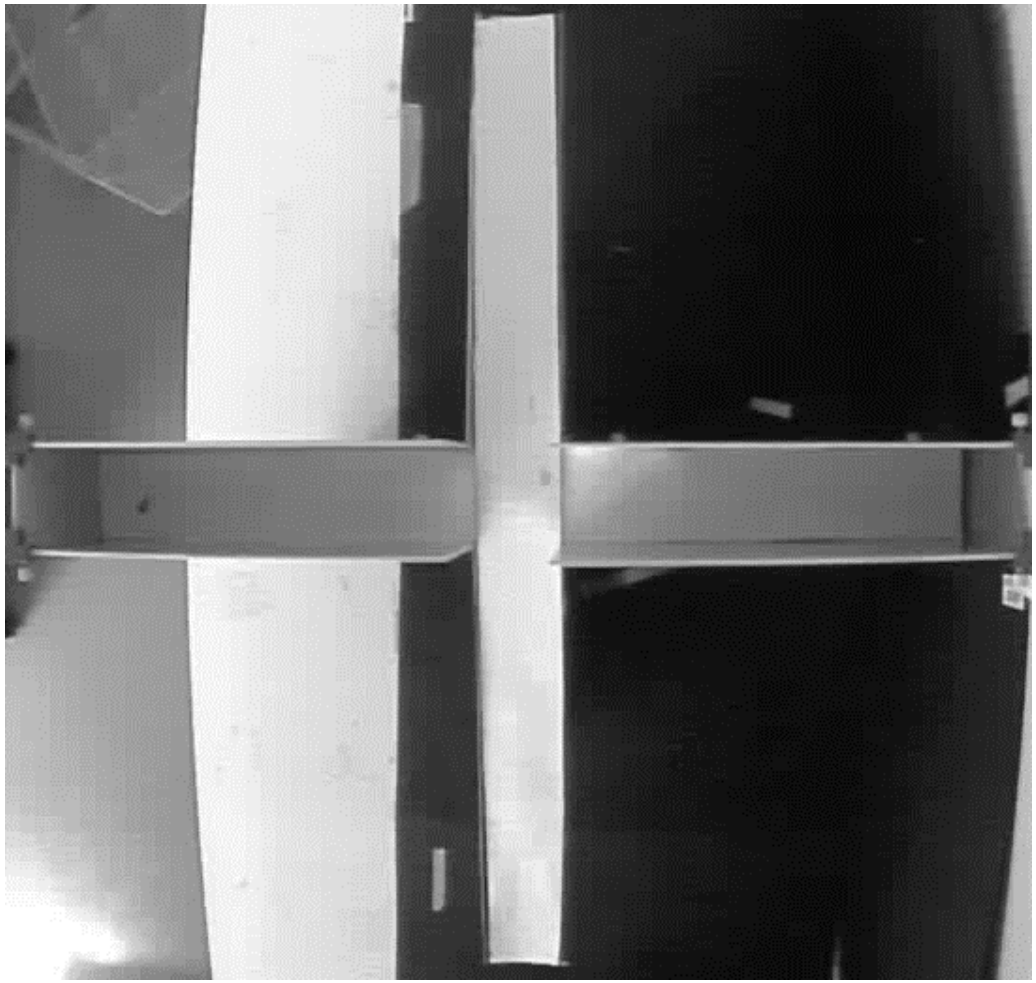
Mice were housed for two months prior to behavioral testing.

Elevated Plus Maze test – EPM

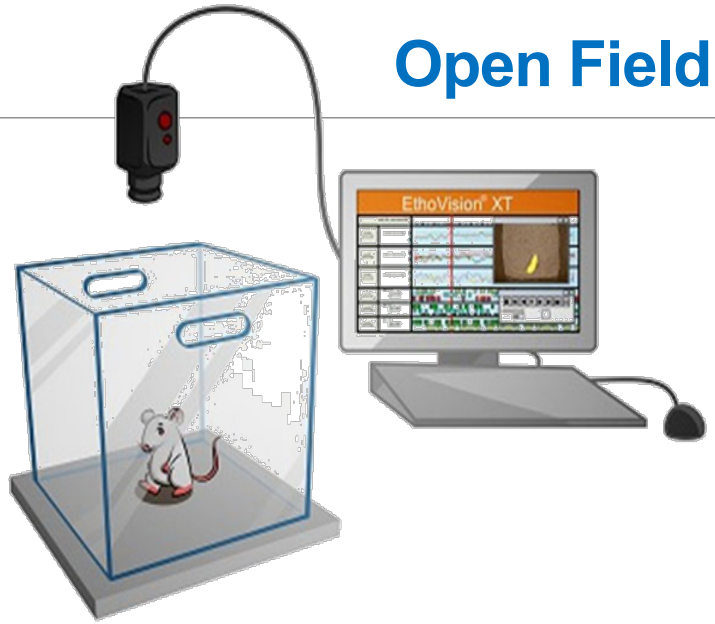


- The following parameters were evaluated:
  (i) time spent in open and closed arms (seconds),
  (ii) number of entries into the open and closed arms, and
  (iii) locomotor activity measured as the total distance travelled (cm).
  Anxiety-like behavior of each mouse was determined based on the number of entries and/or the time spent in the open arms.

Elevated Plus Maze test



Open Field test

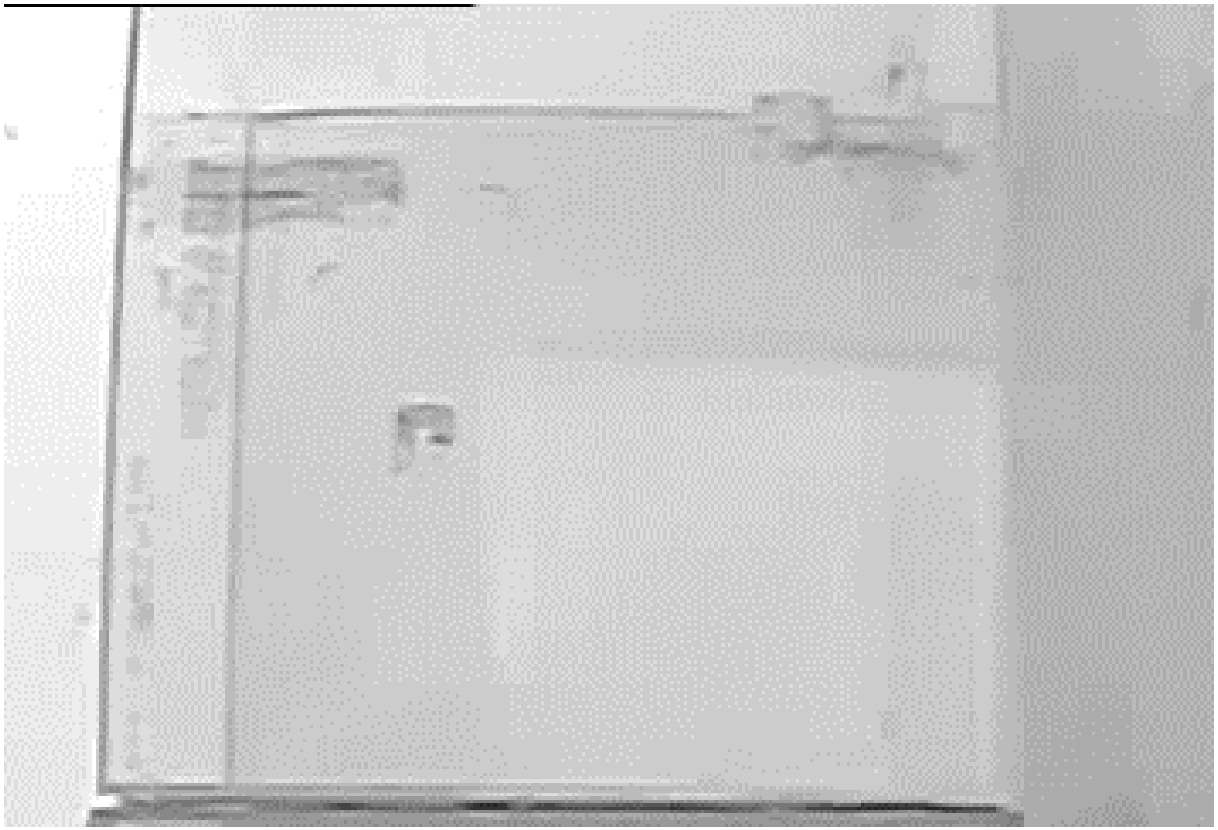


- The following parameters were evaluated:

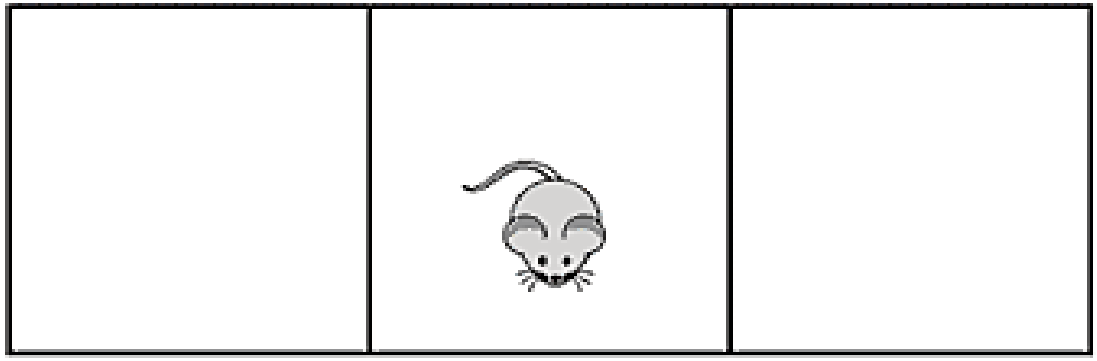
(i) locomotor activity, as measured by the total distance travelled (cm) and

(ii) anxiety-related behavior, as measured by the total amount of time a mouse spent in the central area of the arena (20 cm× 20 cm).

Open Field test



Three-Chamber Sociability and Social Novelty test



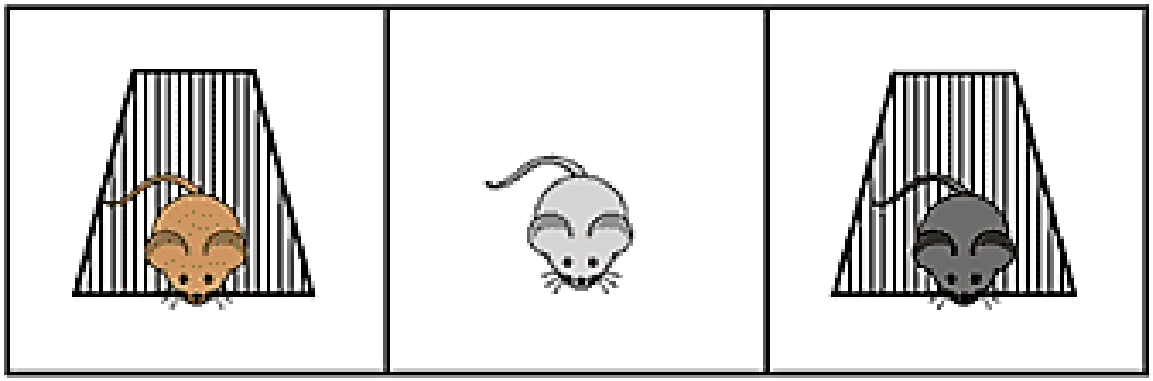
Habituation: Empty Apparatus

Rodents normally prefer to **spend more time with another rodent** (sociability) and will **investigate a novel intruder more** so than a familiar one (social novelty).

Three-Chamber Sociability and Social Novelty test

Sociability: Novel Object; Mouse 1

Three-Chamber Sociability and Social Novelty Test



Social Novelty: Mouse 1; Mouse 2

The following parameters were evaluated:

(i) Time spent in each chamber (s).

(ii) The number of entrances in each chamber during the second phase.

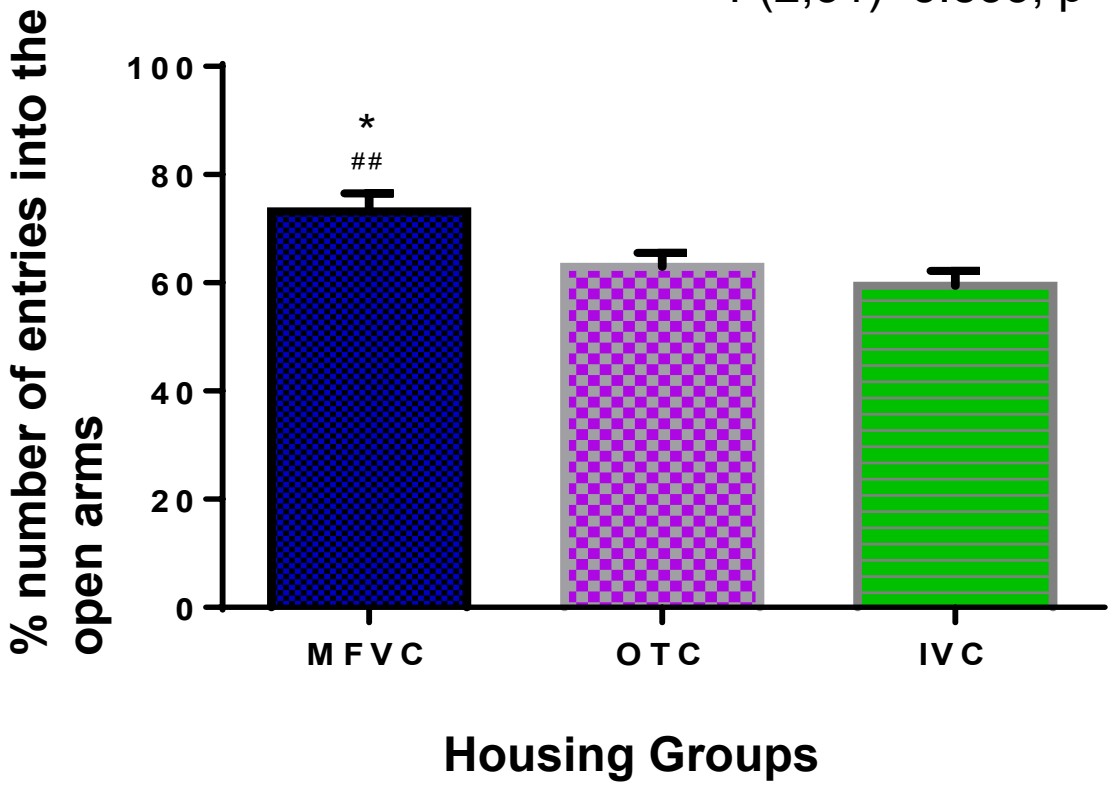
(iii) The number of entrances in each chamber during the third phase.

RESULTS

All statistical analyses were conducted using one-way of GraphPad Prism V6. Data are expressed as mean ± standard error of the mean (SEM) and statistical significance was set at 5%.

Elevated Plus Maze test

F(2,31)=5.833, p=0.0071



Elevated Plus Maze test

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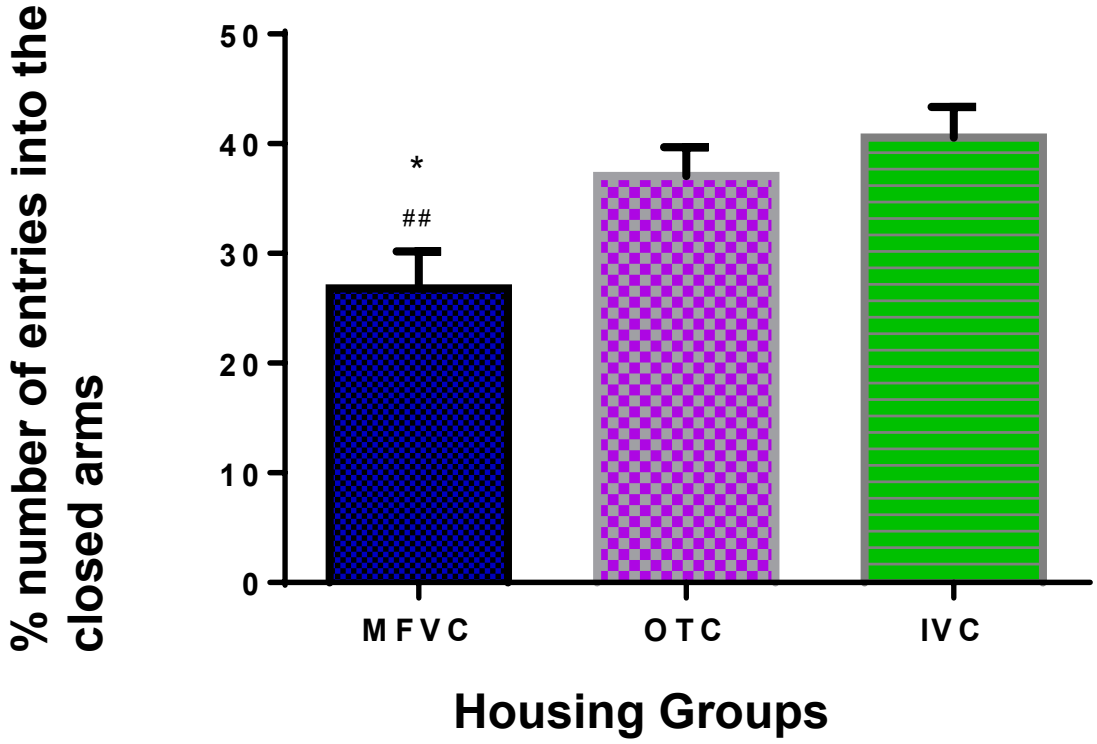
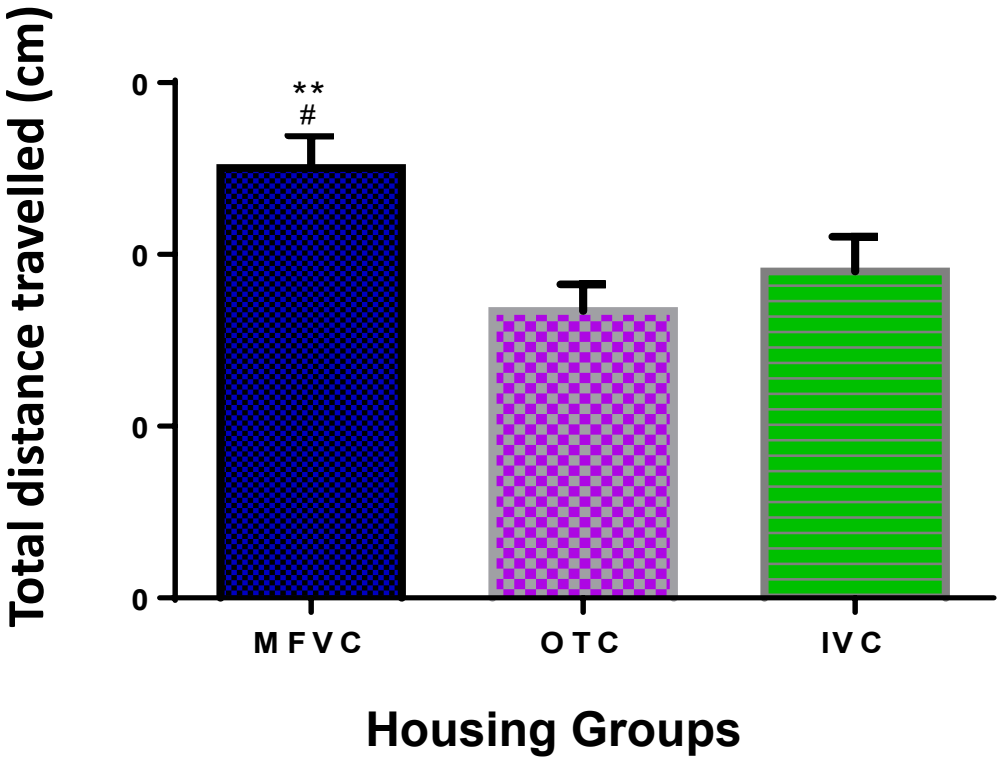


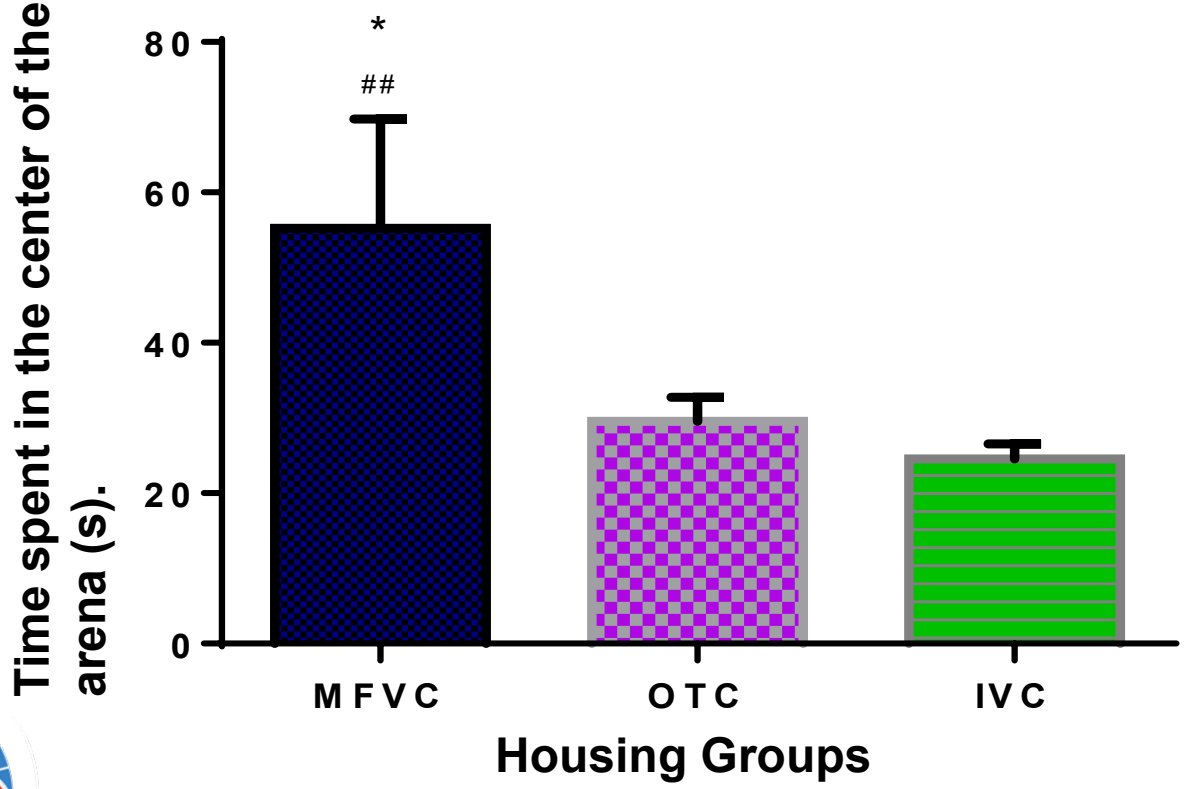
Open Field test

F(2, 16) = 4.808, p= 0.0232

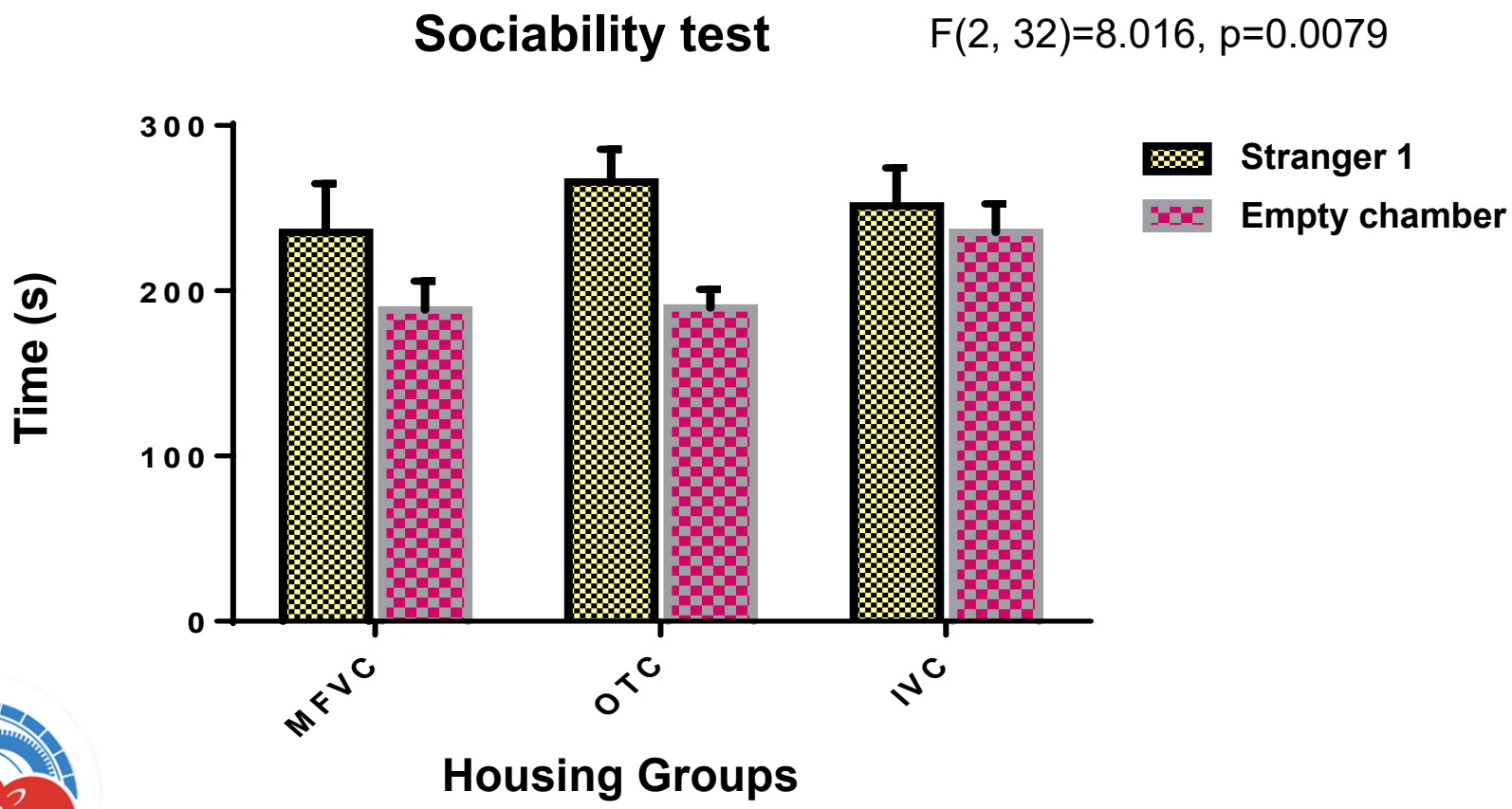


Open Field test

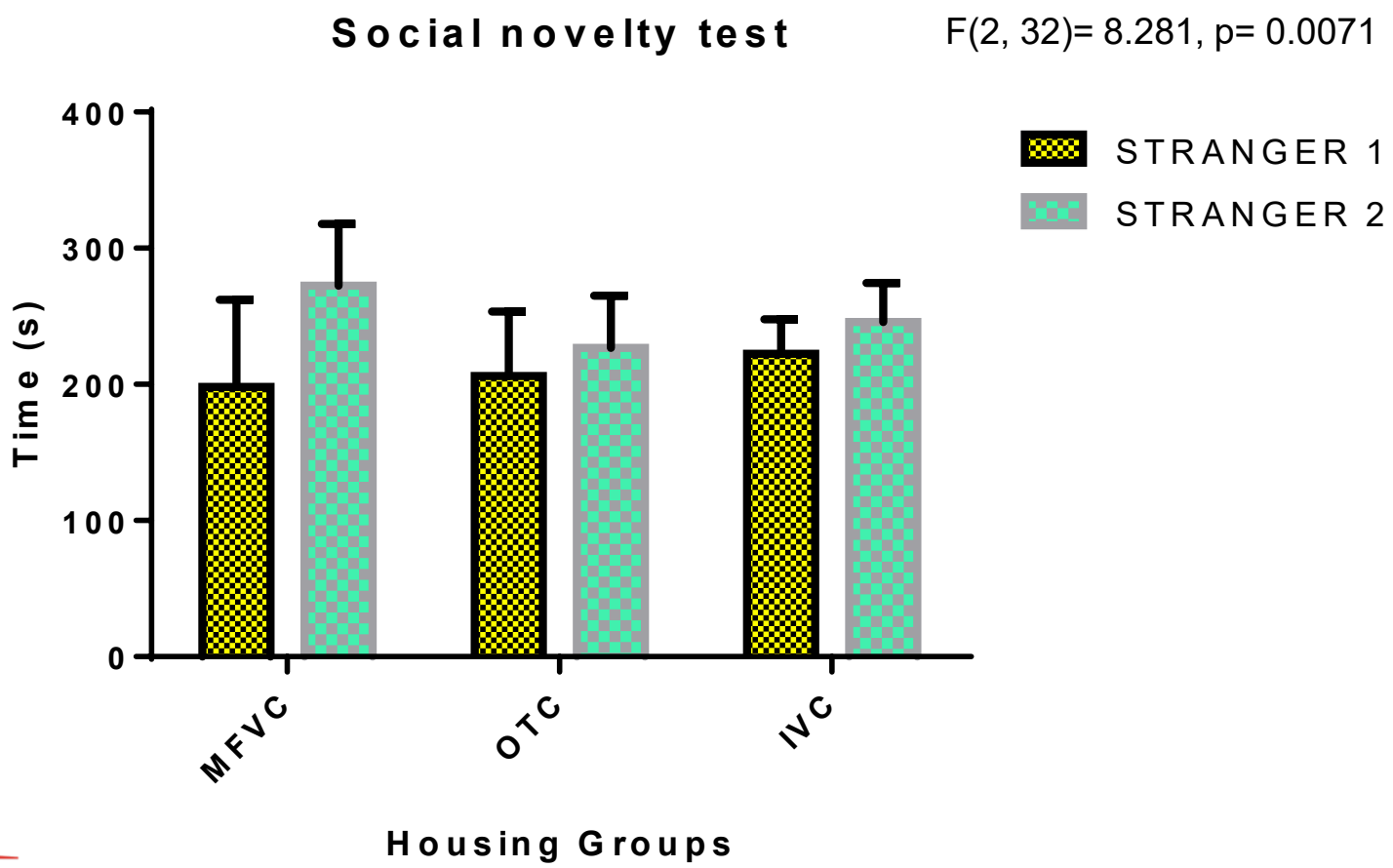
F(2,16) = 5.006, p= 0.0205



Three-Chamber Sociability and Social Novelty test



Three-Chamber Sociability and Social Novelty test

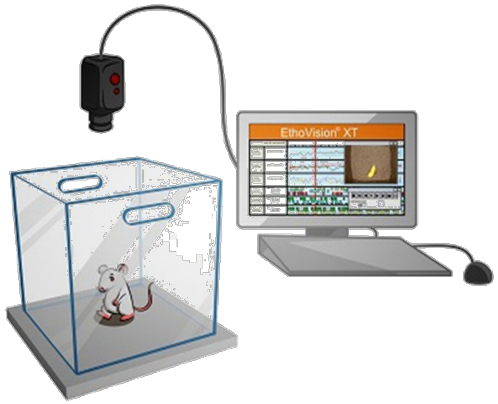


DISCUSSION (I)

- Results from the **Elevated Plus Maze test** revealed that mice housed in the MFVCs showed increased exploratory and less anxiety-like behavior.

→ Tend to show **higher locomotor activity** and **spend more time in the center of the arena** compared to OTC and IVC housed mice.

DISCUSSION (II)



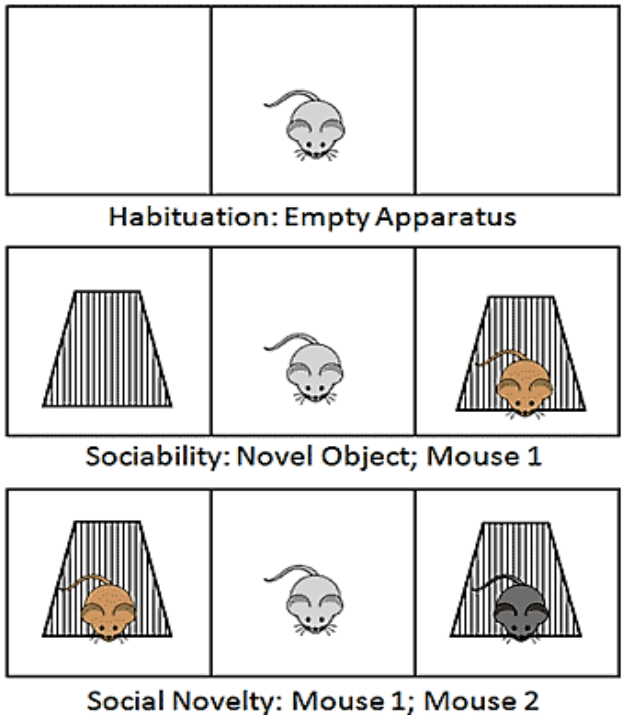
- Results from the **Open Field test** revealed that mice housed in the MFVCs showed increased exploratory and less anxiety-like behavior.

→ Tend to spend **more time in the central area** and **to express higher locomotor activity** compared to OTC and IVC caged mice.

DISCUSSION (III)

- The **Three Chamber Sociability and Social Novelty test** showed that mice of all three caging systems equally preferred to spend more time with another mouse (sociability) and to investigate a novel mouse more than a familiar one (social novelty).

→ No significant difference was observed between the mice of the three groups MFVC, OTC and IVC.



CONCLUSIONS

- It is concluded that different caging systems may influence the exploratory and anxiety-like behavior of laboratory mice.

Differences related to:

- ✓ The design of the cages.
- ✓ Air changes per hour.
- ✓ Noise.
- ✓ Pherormones.

- It is essential to take into account the housing conditions when designing and performing experimental protocols as well as when reporting, analyzing, and systematically reviewing the results of behavioral testing in mice.

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