

Losing our marbles

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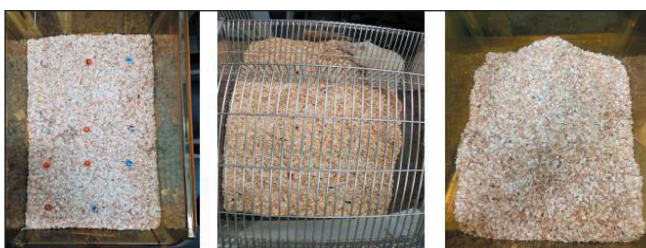
Introduction

Marble Burying Test (MBT) is frequently used to assess anxiety, OCD and repetitive behaviour in laboratory rodents by analysing their normal digging and burying behaviour. Rats bury non-noxious objects such as marbles and food and have been shown in different studies to approach and interact with marbles by sniffing and then deliberately burying the marbles by forward movement of the snout and forepaws.¹

MBT is a non-regulated behavioural test which allows students at King's College London who are undertaking the animal module course as part of their Pharmacology degree to evaluate the housing and enrichment of rats and evaluate their behaviour and how they differ between individuals in a thought provoking practical. Students observe the animals in their home cage and look at their normal behaviour before placing them in the test cage to evaluate how this impacts how they behave.

Aims

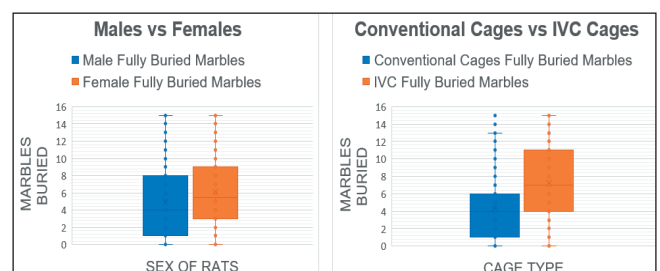
- Students to observe and record behaviour.
- Does the type of caging; conventional or individually ventilated cage (IVC) impact the rats marble burying behaviour?
- Is there a difference between males and females in their burrowing behaviour?



Method

- Cages were filled with wood chip bedding 5cm deep. Bedding was reused between experiments and flattened down between tests. Each rat returned to the same test cage.
- 15 glass marbles of different colours and patterns were used and placed 4cm apart within the cage.
- 2 cohorts of males and females (IVC 2 males and 2 females and conventional 3 males and 3 females) were used and the same cohort was used throughout the study.
- 1 Sprague Dawley (SD) rat (6-weeks-old at the start of the study) was placed in each cage and left in a quiet, undisturbed room for 30 minutes once daily at 3pm for 3 weeks, followed by a 2 week break before retesting for a further 3 weeks. This continued for 4 months.
- Marbles were counted after each burying session. A fully buried marble counted as the whole marble being covered by the substrate and if the marble was covered by two-thirds of substrate then this was partially buried.
- Whilst not on study the SD rats were housed in pairs, kept on a 12-hour light/dark cycle and had access to food and water ad libitum.

Results



Data was analysed by conducting a statistical T-Test. This provided a statistically significant finding ($p=7.10E-12$) showing rats housed in IVCs buried more marbles than that of those housed in conventional cages.

Furthermore, there is statistical significance ($p=5.65E-05$) finding that female rats bury more marbles than male rats regardless of the cage type.

The MBT provides students with an opportunity to observe any stressors that may occur. The timing of the study provided us with a great opportunity to assess whether moving from conventional caging to IVCs caused behavioural changes. Further studies need to be conducted to draw conclusions.

The QR code displayed at the top left corner will show you a short clip of the interactions between one of the test rats and marbles.



Discussion

The aim of the study was for the students to observe and record behaviours in the rats, to look at the different caging types and to see if there was a difference between males and females in their burrowing behaviour of non-toxic objects such as marbles. We will explore our findings here.

One theory of the rats burying more marbles in IVCs compared to conventional caging could be because the rats are being placed into a new cage environment which is not mimicking their home cage.

The number of marbles buried within the MBT did not diminish throughout the test therefore we can rule out that the rats became habituated during the test and exposure times as marble burying is consistent with repetitive behaviour or compulsion as well as repetitive digging behaviour.

Steroid hormones, progesterone and oestrogen could be influencing the approach/anxiety behaviour in females, thus impacting on the number of marbles that the females are burying.²

Students provided highly positive feedback on this practical such as 'I hadn't thought about the behavioural aspect of animal research before the marble burying practical' and the enjoyment of performing a hands-on practical with different species.

Acknowledgements

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References

- ¹ <https://www.nature.com/articles/nprot.2006.20>
- ² [Progesterone and estrogen influence impulsive burying and avoidant freezing behavior of naturally cycling and ovariectomized rats – ScienceDirect](#)