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Protocol status: Working
We use this protocol and it's
working.

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🌐 Marble Burying

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ABSTRACT

Description: Marble burying is a typical behavior in mice that has
been shown to be sensitive to differences based on strain, hippocampal lesions,
and biological/chemical treatments[1,2,3,4]. The marble burying assay
is quick and requires no specialized equipment to perform. Marble burying
relies on the assessment of naturalistic behaviors that have been attributed to
anxiety-like or OCD-like movements[5]. In this assay experimenters will allow mice to freely
bury marbles placed on top of bedding, mice will be given a determined amount of time to
bury these marbles (30 minutes used by our lab) and the number of marbles remaining
uncovered will be quantified and compared between genotypes, treatment groups, ages.
Overall, the lack of marble burying is indicative of alterations to natural instincts of mice,
suggesting perturbations to neurological systems.

MATERIALS

1. Fish tank marbles or similar (one flat edge and one rounded)
https://www.chewy.com/underwater-treasures-decorative/dp/560318.
2. Clean mouse cages (static rack style with no auto-water opening).
3. Clean mouse bedding.
4. 2-3 Blinded evaluators.

|	Set-up
1	The night before assessment gather and autoclave enough standard mouse cages for each mouse to be assessed.
2	Autoclave enough marbles to have 20 per cage (marbles should be flat on one side and rounded on the other, see materials tab for reference).
3	Day of assessment fill each cage with 1.5" of sterile unscented mouse bedding.
4	Arrange the marbles in each cage so that the flat edge is contacting the bedding and rounded edge exposed. The arrangement should be uniform in each cage and follow what is seen in Figure 1).
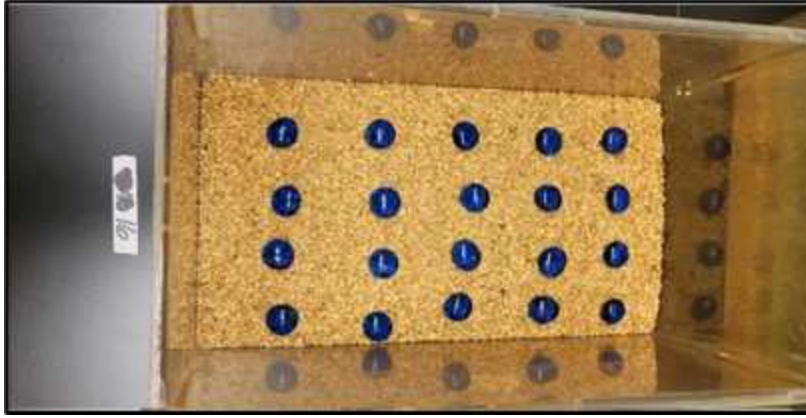
Figure 1. Example layout for marbles in each cage prior to assessment.

5   Place each cage on the bench of the behavior room (or any space free of noise and commotion) with a numeric label associated with each cage (1-n).

Acclimation

6   Once all cages are set up, bring mice up from the vivarium to acclimate (at least 1h)

7   Determine the order of the mice for each cage, making note of this order in your lab notebook.

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8   After acclimation, start to place the mice 1-by-1 into the labeled cages. Mice should be placed into the corner of each cage (remain consistent), making sure to gently place the mouse into the bedding to avoid from disturbing the marbles.

9   As soon as the first mouse is in the cage, start a timer for 30 minutes and continue to place the rest of the mice into their cages.

10   After 30 minutes is up, remove mice from their cages in the same order they were placed in. Again, do so cautiously to avoid from disturbing the bedding or marbles at this step.

11   Once all mice are removed, take a photo of each cage, with the respective cage number visible.

12   Send images to 2-3 independent reviewers who are blinded to the order of mice.

Scoring

13   Have each reviewer record the number of "non-buried" marbles in each cage. "Non-buried" marbles are those that are >25% visible.

14   Average the amount of marbles buried between mice of the same genotype, treatment, age, etc. and compare between groups.