

Marble Burying Test Analysis in Terms of Biological and Non-Biological Factors

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Abstract

Obsessive Compulsive Disorder is a mental disorder characterized by obsessions and/or compulsions. In order to understand underlying the mechanism of obsessive compulsive disorder, scientists apply animal tests. Animal tests of obsessive compulsive disorder (OCD) include genetic, pharmacological and behavior tests. Marble burying test is unconditioned OCD test. Our aim is to investigate marble burying test in terms of biological and non-biological factors.

Keywords: Marble Burying Test, Obsessive Compulsive Disorder, Rodent's Behavior

INTRODUCTION

Psychiatric disorders can effect social life further these disorders put a strain burden on. Obsessive Compulsive Disorder (OCD) is psychiatric disorder characterized by obsessions and/or compulsions. Obsessions have some features such as persistent and recurrent thoughts, impulses. Compulsion is composed of repetitive behaviors [1]. Observation of obsessions include fear of contamination, fear of harming self or others, moral and symmetry. Compulsions have some behaviors such as excessive washing or grooming, counting, checking, telling or confessing, repeating, and hoarding [2].

OCD is a severe psychiatric disorder and according to World Health Organization; OCD is one of the world's ten leading causes of illness-related disability [3]. OCD has a lifespan prevalence of 0.8-2% [4]. Although OCD is a public health problem, its neurobiology is not well understood.

In order to enlighten OCD mechanisms, scientists generally use animal behavior models. Although OCD is difficult to model in animals, a lot of models have been developed recent years. Marble burying test is commonly used as OCD animal test.

MARBLE BURYING TEST

Neophobia is a fear of new or strange objects. Neophobia is seen in rodents to noxious and harmless objects. Rodents exhibit various type of specific behaviors such as digging, burrowing, burying, rearing, hoarding and grooming.

Rodents dig in to bedding material to find food or hoard food. Other reason can array for saving offspring from predator, for nesting [5]. Noxious object trigger also digging and burying. Moreover rodents bury carrion of other rodents in some cage.

Grooming is a stereotype behavior showed increasingly under the anxiety or stress in rodents. Further some animal can pluck fur. For instance mice with disruptions of Hoxb8 exhibit excessive grooming compared to control. Trichotillomania can develop in Hoxb8 mutant mice [6].

Rearing behavior is the indicator of locomotor activity

and scout around. If anxiety level decreases, rearing behavior increases contrarily.

The cage is filled approximately 5 cm deep with bedding materials such as sawdust, husk and corncob. Warm lightly tamped down to make a flat, even surface. Marble is placed intermittently in the cage.

Subjects are placed in each cage and the test timer is started. Researcher can observe digging, grooming, rearing and burying behavior. Marble burying parameters are digging latency, burying latency, number of marbles buried, rearing latency and rearing number, grooming latency and grooming time.

Marble Burying Test Analysis In Terms of Biological Factors

Species

According to PUBMED Data, 156 articles were analyzed in terms of species. Our results suggest that all experiments base on rodent familia. This feature of marble burying test is not similar to other anxiety tests such as open field test, elevated plus maze and light dark box test. Species choosing results indicate that 87.82% mice, 11.53% rat, 0.64% mice and rat. It is actually seen that mice were most frequently preferred in Marble Burying Test (MBT). Strain of rats and mice vary significantly including Wistar Albino, Brattleboro, Nile Grass, Sprague Dowley, Wistar Kyoto, Lewis rat; Swiss Albino, CD1, C57Bl/6J, BALB/c, Swiss Webster Albino, NMRI mice and other species.

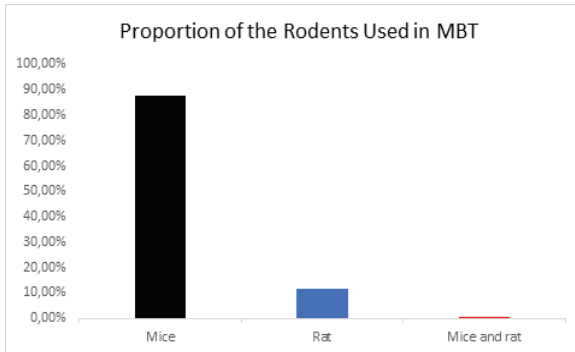


Figure 1. The most frequently used rodents are mice as seen in the graph [7].

Table 1. The strains of the rats were presented in the table.

Strain of Rats	Researchers
Wistar Albino Rat	Pandey et al, 2009 [8].
Brattleboro Rat	Fodor et al, 2016 [9].
Nile Grass Rat	Ikeno et al, 2016 [10].
Sprague Dowley Rat	Mannucci et al, 2012 [11].
Wistar Kyoto Rat	Burke et al, 2016 [12].
Lewis Rat	Lotan et al, 2014 [13].

Table 2. The strains of the mice were presented in the table.

Strain of Mice	Researchers
Swiss Albino Mice	Salunke et al, 2014 [14].
CD1 Mice	Hodgson et al, 2007 [15].
C57Bl/6J Mice	Popova et al, 2011 [16].
BALB/c Mice	Farley et al, 2010 [17].
Swiss Webster albino Mice	Chioca et al, 2013 [18].
NMRI Mice	Andreassen et al, 2013 [19].

Gender

Gender choosing results point out that 66.66% male, 17.30% female and male, 8.33% female, %7.70% ambiguous. In MBT male subjects were used more than female subjects. The reason of preferring male subjects may cause from hormonal waves of female subjects and thus these waves trigger of basal anxiety level. This feature of marble burying test is similar to other anxiety tests such as open field test, elevated plus maze and light dark box test. Interestingly some researchers did not notify the gender of the subjects.

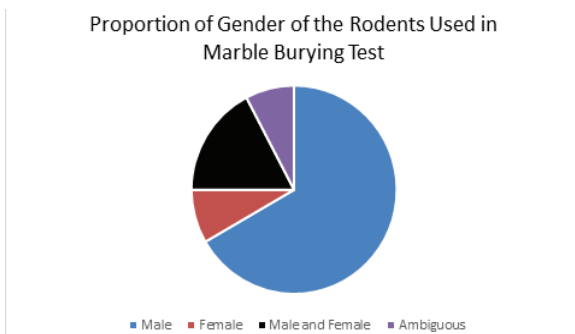


Figure 2. The most frequently preferred gender is male subject as seen in the graph [7].

Age and Numbers of Subjects in Each Group

According to the type of the research, age of the subjects vary significantly. Range of age is 1-32 weeks. Range of numbers of subjects in each group is 6-20. After analyzing the PUBMED Data, we noticed that researchers have not used specific age of rodent.

Table 3. Range of ages of the subjects were presented in the table.

Range of Age	Researchers
1 week	Beis et al, 2011 [20].
3 weeks	Sawin et al, 2014 [21].
6 weeks	Burke et al, 2016 [12].
8 weeks	Chikahisa et al, 2007 [22].
8-12 weeks	Arora et al, 2013 [23].
12-24 weeks	Elston et al, 2014 [24].
32 weeks	De Filippis et al, 2014 [25].

Table 4. Range of numbers of subjects were presented in the table.

Range of Numbers of Subjects	Researchers
6-8	Salunke et al, 2014 [14].
8	Dagyttè et al, 2011 [26].
8-12	Young et al, 2006 [27].
15	Weidner et al, 2014 [28].
20	Onksen et al, 2011 [29].

Marble Burying Test Analysis In Terms of Non-Biological Factors

MBT Time Frequency

Test time frequency percentage found out the fact that 69.87% 30 minutes, 9.61% 20 minutes, 8.33% 15 minutes, 5.12% 10 minutes, the other time 7.05%. MBT are conducted usually 30 minutes. The other anxiety tests such as elevated plus maze, open field test, light dark box, hole board test or locomotor activity tests (rota rod test and/or open field test) can combine with MBT [30 31]. Our observations sign that different anxiety or depression animal test combinations result in fatigue, so different test time can be applied or reduced test time. For instance light dark box test can be performed dark season in a day (20:00-24:00).

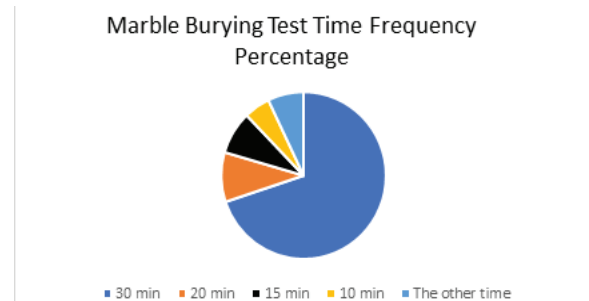


Figure 3. The most frequently preferred test time is 30 min as seen in the graph [7].

Table 5. MBT time frequencies were presented in the table.

MBT Time Frequency	Researchers
30 min	Beis et al, 2011 [20].
20 min	Fodor et al, 2016 [9].
15 min	Bahi et al, 2013 [32].
10 min	Gallo et al, 2014 [33].

Number of Marbles

Range of burying number is 4-25. According to MBT time and dimensions and sizes of MBT apparatus, number of marbles can be increased or decreased. The number of marbles buried (to 2/3, 1/2 depth) with bedding can be counted [10] [19]. Diameter of used marbles are usually 1.5 cm.

Table 6. Number of marbles used in MBT were presented in the table.

Number of Marbles	Researchers
4	Dagytyé et al, 2010 [26].
9	Lotan et al, 2014 [13].
12	Beis et al, 2010 [20].
15	Fodor et al, 2016 [9].
20	Burke et al, 2016 [12].
24	Young et al, 2006 [27].
25	Pandey et al, 2009 [8].

Bedding Materials

Husk, sawdust and corncob are used as bedding materials [31] [34] [35]. Subjects are accustomed to bedding material. Sand or other material can change burying numbers. Bedding materials must alter each experiment, as urination and defecation can effect adversely subjects.

Other Non-Biological Parameters

Lighting is of significance importance for subjects, as high level lighting trigger off stress. The MBT can be enlightened by warm white light at 60-150 lx. The test room must be silent. All behavior must be recorded by camera. Adaptation, handling and administration method of drug treatment can effect test results. Range of test cleaning solutions are 10-70% ethanol. Apparatus must be cleaned by ethanol and dried completely. MBT can performed in temperature (23-25 °C) and damp (50-70%) controlled room. Interestingly there were no any seasonal parameters in literature.

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REFERENCES

[1] Qiu L, Fu X, Wang S, Tang Q, Chen X, Cheng L, Zhang F, Zhou Z, Tian L. 2017. Abnormal regional spontaneous neuronal activity associated with symptom severity in treatment-naive patients with obsessive-compulsive disorder revealed by resting-state functional MRI. *Neurosci Lett.* 15; 640: 99-104.

[2] Wang L, Simpson HB., and Dulawaa SC. Assessing the validity of current mouse genetic models of obsessive-compulsive disorder. 2009. *Behav Pharmacol.* 20(2): 119-133.

[3] Baldwin DS, Anderson IM, Nutt DJ, Bandelow B, Bond A, Davidson JR, den Boer JA, Fineberg NA, Knapp M, Scott J, Wittchen HU. 2005. Evidence-based guidelines for the pharmacological treatment of anxiety disorders: recommendations from the British Association for Psychopharmacology. *J Psychopharmacol.* 19(6):567-96.

[4] Murray, CJ, Lopez, AD. 1996. The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Cambridge, MA: Harvard University Press.

[5] Deacon RM J. 2006. Digging and marble burying in mice: simple methods for in vivo identification of biological impacts. *Nature Protocols.* 1(1): 122-124.

[6] Greer JM, Capecchi MR. 2002. Hoxb8 is required for normal grooming behavior in mice. *Neuron.* 33(1):23 – 34.

[7] Çalışkan H, Çakan O, Uzunkulaoglu M, Kankal S, Özden FM, Cihan KH, Şentunalı B, Zaloglu N. 2017. 2. National Congress on Applied Biological Sciences. Poster Presentation. No: 18.

[8] Pandey DK, Yadav SK, Mahesh R, Rajkumar R. 2009. Depression-like and anxiety-like behavioural aftermaths of impact accelerated traumatic brain injury in rats: a model of comorbid depression and anxiety. *Behav Brain Res.* 205(2):436-42.

[9] Fodor A, Kovács KB, Balázsfi D, Klausz B, Pintér O, Demeter K, Daviu N, Rabasa C, Rotllant D, Nadal R, Zelena D. 2016. Depressive- and anxiety-like behaviors and stress-related neuronal activation in vasopressin-deficient female Brattleboro rats. *Physiol Behav.* 158:100-111.

[10] Ikeno T, Deats SP, Soler J, Lonstein JS, Yan L. 2016. Decreased daytime illumination leads to anxiety-like behaviors and HPA axis dysregulation in the diurnal grass rat (*Arvicanthis niloticus*). *Behav Brain Res.* 300: 77-84.

[11] Mannucci C1, Navarra M, Calzavara E, Caputi AP, Calapai G. 2012. Serotonin involvement in *Rhodiola rosea* attenuation of nicotine withdrawal signs in rats. *Phytomedicine.* 19(12):1117-24.

[12] Burke NN, Coppinger J, Deaver DR, Roche M, Finn DP, Kelly J. 2016. Sex differences and similarities in depressive- and anxiety-like behaviour in the Wistar-Kyoto rat. *Physiol Behav.* 167:28-34.

[13] Lotan D, Cunningham M, Joel D. 2014. Antibiotic treatment attenuates behavioral and neurochemical changes induced by exposure of rats to group a streptococcal antigen. *PLoS One.* 9(6):e101257.

[14] Salunke BP, Umathe SN, Chavan JG. 2014. Experimental evidence for involvement of nitric oxide in low frequency magnetic field induced obsessive compulsive disorder-like behavior. *Pharmacol Biochem Behav.* 122:273-8.

[15] Hodgson RA, Higgins GA, Guthrie DH, Lu SX, Pond AJ, Mullins DE, Guzzi MF, Parker EM, Varty GB. 2007. Comparison of the V1b antagonist, SSR149415, and the CRF1 antagonist, CP-154,526, in rodent models of anxiety and depression. *Pharmacol Biochem Behav.* 86(3):431-40.

[16] Popova NK, Morozova MV, Naumenko VS. 2011. Ameliorative effect of BDNF on prenatal ethanol and stress exposure-induced behavioral disorders. *Neurosci Lett.* 505(2):82-6.

[17] Farley S, Apazoglou K, Witkin JM, Giros B, Tzavara ET. 2010. Antidepressant-like effects of an AMPA receptor potentiator under a chronic mild stress paradigm. *Int J Neuropsychopharmacol.* 13(9):1207-18.

[18]. Chioca LR, Ferro MM, Baretta IP, Oliveira SM,

Silva CR, Ferreira J, Losso EM, Andreatini R. 2013. Anxiolytic-like effect of lavender essential oil inhalation in mice: participation of serotonergic but not GABAA/benzodiazepine neurotransmission. *J Ethnopharmacol.* 147(2):412-8.

[19] Andreasen JT, Redrobe JP, Nielsen EØ, Christensen JK, Olsen GM, Peters D. 2013. A combined $\alpha 7$ nicotinic acetylcholine receptor agonist and monoamine reuptake inhibitor, NS9775, represents a novel profile with potential benefits in emotional and cognitive disturbances. *Neuropharmacology.* 73:183-91.

[20] Beis D, Schwarting RK, Dietrich A. 2011. Evidence for a supportive role of classical transient receptor potential 6 (TRPC6) in the exploration behavior of mice. *Physiol Behav.* 102(2):245-50.

[21] Sawin EA, Murali SG, Ney DM. 2014. Differential effects of low-phenylalanine protein sources on brain neurotransmitters and behavior in C57Bl/6-Pah(enu2) mice. *Mol Genet Metab.* 111(4):452-61.

[22] Chikahisa S, Sano A, Kitaoka K, Miyamoto K, Sei H. 2007. Anxiolytic effect of music depends on ovarian steroid in female mice. *Behav Brain Res.* 179(1):50-9.

[23] Arora T, Bhowmik M, Khanam R, Vohora D. 2013. Oxcarbazepine and fluoxetine protect against mouse models of obsessive compulsive disorder through modulation of cortical serotonin and CREB pathway. *Behav Brain Res.* 247:146-52.

[24] Elston TW, Pandian A, Smith GD, Holley AJ, Gao N, Lugo JN. 2014. Aniracetam does not alter cognitive and affective behavior in adult C57BL/6J mice. *PLoS One.* 6;9(8):e104443.

[25] De Filippis B, Nativio P, Fabbri A, Ricceri L, Adriani W, Lacivita E, Leopoldo M, Passarelli F, Fuso A, Laviola G. 2014. Pharmacological stimulation of the brain serotonin receptor 7 as a novel therapeutic approach for Rett syndrome. *Neuropsychopharmacology.* 39(11):2506-18.

[26] Dągtyć G, Crescente I, Postema F, Seguin L, Gabriel C, Mocaër E, Boer JA, Koolhaas JM. 2011. Agomelatine reverses the decrease in hippocampal cell survival induced by chronic mild stress. *Behav Brain Res.* 218(1):121-8.

[27] Young R, Batkai S, Dukat M, Glennon RA. 2006. TDIQ (5,6,7,8-tetrahydro-1,3-dioxolo[4,5-g]isoquinoline) exhibits anxiolytic-like activity in a marble-burying assay in mice. *Pharmacol Biochem Behav.* 84(1):62-73.

[28] Weidner KL, Buenaventura DF, Chadman KK. 2014. Mice over-expressing BDNF in forebrain neurons develop an altered behavioral phenotype with age. *Behav Brain Res.* 15;268:222-8.

[29] Onksen JL, Brown EJ, Blendy JA. 2011. Selective deletion of a cell cycle checkpoint kinase (ATR) reduces neurogenesis and alters responses in rodent models of behavioral affect. *Neuropsychopharmacology.* 36(5):960-9.

[30] Gupta D, Radhakrishnan M, Kurhe Y. 2014. Anxiolytic-like effects of alverine citrate in experimental mouse models of anxiety. *Eur J Pharmacol.* 742:94-101.

[31] Colla AR, Rosa JM, Cunha MP, Rodrigues AL. 2015. Anxiolytic-like effects of ursolic acid in mice. *Eur J Pharmacol.* 758:171-6.

[32] Bahi A. 2013. Individual differences in elevated plus-maze exploration predicted higher ethanol consumption and preference in outbred mice. *Pharmacol Biochem Behav.* 105: 83-8.

[33] Gallo I, Rattazzi L, Piras G, Gobbetti T, Panza E, Perretti M, Dalley JW, D'Acquisto F. 2014. Formyl peptide receptor as a novel therapeutic target for anxiety-related disorders. *PLoS One.* 17;9(12):e114626.

[34] Savignac HM, Kiely B, Dinan TG, Cryan JF. 2014. Bifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice. *Neurogastroenterol Motil.* 26(11):1615-27.

[35] Schwartz JJ, Careaga M, Chang C, Onore CE, Ashwood P. 2015. Allergic fetal priming leads to developmental, behavioral and neurobiological changes in mice. *Transl Psychiatry.* 7;5:e543.