Albert Einstein College of Medicine
Dominick P. Purpura Department of Neuroscience
1410 Pelham Pkwy S K912F Bronx, NY 10461
718 - 430 4042
mgulin@aecom.yu.edu

Dr. Maria Gulinello
Behavioral Core Facility
Albert Einstein College of Medicine

Behavioral Core Protocols and Training

Marble Burying

The marble burying test is a useful model of neophobia [1], anxiety [1-7] and obsessive-compulsive behavior [8-11]. It has also been proposed that the test may have predictive validity for the screening of novel antidepressants [12-15], anxiolytics [15, 16] and antipsychotics [17-19].

This behavior belongs is probably a type of defensive burying typical of rodents [20]. It does not seem that marbles are specifically aversive [6], although making them so by coating them or pairing them with aversive substances does increase burying [21, 22]. This is consistent with the fact that electrified probes are buried more than innocuous ones. Factors such as novelty or hedonic value of the substances to be buried, however, do not seem to be primary modulators in the amount of burying [23].

C57/B mice can be expected to bury roughly 75% of the marbles in a 30 min period, though this may vary with the strain, age and sex [24].

The neuronal circuitry of this behavior has not been clearly elucidated. The hippocampus and septum are likely to be important, since lesions in these areas reduce digging [24-26]

Procedure

Fill the cage approximately 5-10 cm deep with wood chip bedding, lightly tamped down to make a flat, even surface. The bedding substrate can be reused if it is flattened and firmed down again between mice; reuse of bedding does not seem to affect the burying/digging performance of subsequently tested mice.

Place a regular pattern of glass marbles on the surface, evenly spaced, each about 4 cm apart.

Place one animal in each cage and leave for 30-60 min.

Count the number of marbles buried (to 2/3 their depth) with bedding.

Young adult mice (2–4 months old) will probably dig better than mice more than one year old. Assess whether the laboratory is quiet enough; the mice should be undisturbed. In particular, eliminate potential sources of ultrasound such as running water and computer monitors. Avoid testing on cage-cleaning days, as the mice become very active re-exploring their new cages, and subsequently become rather quiescent and less responsive to the stimulation of new bedding in the test situation.

In strains of mice with low burying behavior (possibly FVB and 129), the marbles can be coated with Tabasco sauce to increase burying.

Validation –
pharmacological
[2-5, 17, 27-30]

physiological
(altered heart rate and piloerection etc) [31]

stressors increase
[32]

Relationship to risk assessment ?
[33, 34]

Comparison to other tests
[1, 4, 17, 26, 35]

Other
[36-42]

Defensive burying

Defensive burying [20, 41] is refers to the typical rodent behavior of displacing bedding material with vigorous treading-like movements of their forepaws and shoveling movements of their heads directed towards a variety of noxious stimuli that pose a near and immediate threat, such as a wall-mounted electrified shock-prod [43]. This can occur spontaneously [23], but is increased when or when the target stimulus is paired with aversive stimuli in a conditioning paradigm [22, 44, 45]. This is considered to be an anxiety-like behavior [46] but may also in some circumstances reflect fear [47-49] or pain [50, 51] depending on the methods to induce burying.

Validation –
Pharmacological
[44, 46, 52-58]

physiological
[58-60]

stressors increase
[32, 58, 61-63]

Relationship to risk assessment ?
[49]

Comparison to other tests
[26, 51, 55, 56, 59, 64-69]

Brain regional involvement
[68-77]

Strain differences
[49, 78]

Procedural issues
[41, 76, 79, 80]

Other
[50, 67, 81-86]

References

1. Ho, Y.-J., J. Eichendorff, and R.K.W. Schwarting, *Individual response profiles of male Wistar rats in animal models for anxiety and depression.* Behavioural Brain Research, 2002. **136**(1): p. 1.
2. Borsini, F., J. Podhorna, and D. Marazziti, *Do animal models of anxiety predict anxiolytic-like effects of antidepressants?* Psychopharmacology (Berl), 2002. **163**(2): p. 121-41.
3. Archer, T., et al., *Marble burying and spontaneous motor activity in mice*: *interactions over days and the effect of diazepam.* Scand J Psychol, 1987. **28**(3): p. 242-9.
4. Brodkin, J., et al., *Anxiolytic-like activity of the mGluR5 antagonist MPEP*: *A comparison with diazepam and buspirone.* Pharmacology Biochemistry and Behavior, 2002. **73**(2): p. 359.
5. Broekkamp, C.L., et al., *Major tranquillizers can be distinguished from minor tranquillizers on the basis of effects on marble burying and swim-induced grooming in mice.* Eur J Pharmacol, 1986. **126**(3): p. 223-9.
6. Njung'e, K. and S.L. Handley, *Evaluation of marble-burying behavior as a model of anxiety.* Pharmacol Biochem Behav, 1991. **38**(1): p. 63-7.
7. Nicolas, L.B., Y. Kolb, and E.P. Prinssen, *A combined marble burying-locomotor activity test in mice*: *a practical screening test with sensitivity to different classes of anxiolytics and antidepressants.* Eur J Pharmacol, 2006. **547**(1-3): p. 106-15.
8. Gyertyan, I., *Analysis of the marble burying response*: *marbles serve to measure digging rather than evoke burying.* Behav Pharmacol, 1995. **6**(1): p. 24-31.
9. Londei, T., A.M. Valentini, and V.G. Leone, *Investigative burying by laboratory mice may involve non-functional, compulsive, behaviour.* Behav Brain Res, 1998. **94**(2): p. 249-54.
10. Woods-Kettelberger, A., et al., *Animal models with potential applications for screening compounds for the treatment of obsessive-compulsive disorder.* Expert Opin Investig Drugs, 1997. **6**(10): p. 1369-81.
11. Hedlund, P.B. and J.G. Sutcliffe, *The 5-HT7 receptor influences stereotypic behavior in a model of obsessive-compulsive disorder.* Neuroscience Letters, 2007. **414**(3): p. 247-251.
12. Dekeyne, A., *Behavioural models for the characterisation of established and innovative antidepressant agents.* Therapie, 2005. **60**(5): p. 477-84.
13. Dourish, C.T., C. McNicoll, and A. Fletcher, *The 5-HT1A Receptor Antagonist WAY-100635 enhances the Behavioural Effects of SSRIs.* European Neuropsychopharmacology, 1996. **6**(Supplement 3): p. 16.
14. Harasawa, T., et al., *Role of serotonin type 1A receptors in fluvoxamine-induced inhibition of marble-burying behavior in mice.* Behav Pharmacol, 2006. **17**(7): p. 637-640.
15. Palucha, A. and A. Pilc, *Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs.* Pharmacology & Therapeutics, 2007. **115**(1): p. 116-147.
16. Shimazaki, T., M. Iijima, and S. Chaki, *Anxiolytic-like activity of MGS0039, a potent group II metabotropic glutamate receptor antagonist, in a marble-burying behavior test.* European Journal of Pharmacology, 2004. **501**(1-3): p. 121-125.
17. Li, X., D. Morrow, and J.M. Witkin, *Decreases in nestlet shredding of mice by serotonin uptake inhibitors*: *comparison with marble burying.* Life Sci, 2006. **78**(17): p. 1933-9.
18. Matsushita, M., et al., *Perospirone, a novel antipsychotic drug, inhibits marble-burying behavior via 5-HT1A receptor in mice*: *implications for obsessive-compulsive disorder.* J Pharmacol Sci, 2005. **99**(2): p. 154-9.
19. Egashira, N., et al., *Involvement of the sigma1 receptor in inhibiting activity of fluvoxamine on marble-burying behavior*: *Comparison with paroxetine.* European Journal of Pharmacology, 2007. **563**(1-3): p. 149-154.
20. Pinel, J.P. and D. Treit, *Burying as a defensive response in rats.* Journal of Comparative and Physiological Psychology, 1978. **92**(4): p. 708-712.

21. Wilkie, D.M., A.J. MacLennan, and J.P. Pinel, *Rat defensive behavior: burying noxious food.* J Exp Anal Behav, 1979. **31**(3): p. 299-306.
22. Parker, L.A., *Defensive burying of flavors paired with lithium but not amphetamine.* Psychopharmacology, 1988. **96**(2): p. 250-252.
23. Poling, A., J. Cleary, and M. Monaghan, *Burying by rats in response to aversive and nonaversive stimuli.* J Exp Anal Behav, 1981. **35**(1): p. 31-44.
24. Deacon, R.M.J. and J.N.P. Rawlins, *Hippocampal lesions, species-typical behaviours and anxiety in mice.* Behavioural Brain Research, 2005. **156**(2): p. 241-249.
25. Gray, D.S., et al., *Effect of septal lesions on conditioned defensive burying.* Physiol Behav, 1981. **27**(6): p. 1051-6.
26. Dringenberg, H.C., Y. Levine, and J.L. Menard, *Electrical stimulation of dorsal, but not ventral hippocampus reduces behavioral defense in the elevated plus maze and shock-probe burying test in rats.* Behav Brain Res, 2007.
27. Millan, M.J., et al., *Stereospecific blockade of marble-burying behaviour in mice by selective, non-peptidergic neurokinin1 (NK1) receptor antagonists.* Neuropharmacology, 2002. **42**(5): p. 677.
28. Shimazaki, T., M. Iijima, and S. Chaki, *Anxiolytic-like activity of MGS0039, a potent group II metabotropic glutamate receptor antagonist, in a marble-burying behavior test.* European Journal of Pharmacology, 2004. **501**(1-3): p. 121.
29. Shinomiya, K., et al., *Effect of paroxetine on marble-burying behavior in mice.* Methods Find Exp Clin Pharmacol, 2005. **27**(10): p. 685-7.
30. Skalisz, L.L., V. Beijamini, and R. Andreatini, *Effect of Hypericum perforatum on marble-burying by mice.* Phytother Res, 2004. **18**(5): p. 399-402.
31. Lahdesmaki, J., et al., *Behavioral and neurochemical characterization of [alpha]2A-adrenergic receptor knockout mice.* Neuroscience, 2002. **113**(2): p. 289.
32. Chotiwat, C. and R.B.S. Harris, *Increased anxiety-like behavior during the post-stress period in mice exposed to repeated restraint stress.* Hormones and Behavior, 2006. **50**(3): p. 489-495.
33. Hashemi, E., et al., *Gabrb3 gene deficient mice exhibit increased risk assessment behavior, hypotonia and expansion of the plexus of locus coeruleus dendrites.* Brain Research, 2007. **1129**: p. 191-199.
34. Pinel, J.P., et al., *Rat (Rattus norvegicus) defensive behavior in total darkness: risk-assessment function of defensive burying.* J Comp Psychol, 1994. **108**(2): p. 140-7.
35. Hodgson, R.A., et al., *Comparison of the V1b antagonist, SSR149415, and the CRF1 antagonist, CP-154,526, in rodent models of anxiety and depression.* Pharmacology Biochemistry and Behavior, 2007. **86**(3): p. 431-440.
36. Dawson, P.A., S.E. Steane, and D. Markovich, *Behavioural abnormalities of the hyposulphataemic Nas1 knock-out mouse.* Behavioural Brain Research, 2004. **154**(2): p. 457.
37. Yamada, K., et al., *Decreased marble burying behavior in female mice lacking neuromedin-B receptor (NMB-R) implies the involvement of NMB/NMB-R in 5-HT neuron function.* Brain Research, 2002. **942**(1-2): p. 71.
38. Schneider, T. and P. Popik, *Attenuation of estrous cycle-dependent marble burying in female rats by acute treatment with progesterone and antidepressants.* Psychoneuroendocrinology, 2007. **32**(6): p. 651-659.
39. Uday, G., et al., *LHRH antagonist attenuates the effect of fluoxetine on marble-burying behavior in mice.* European Journal of Pharmacology, 2007. **563**(1-3): p. 155-159.
40. Egashira, N., et al., *Impaired social interaction and reduced anxiety-related behavior in vasopressin V1a receptor knockout mice.* Behavioural Brain Research, 2007. **178**(1): p. 123-127.
41. Pinel, J.P., et al., *Development of defensive burying in Rattus norvegicus: experience and defensive responses.* J Comp Psychol, 1989. **103**(4): p. 359-65.
42. Saadat, K.S., et al., *The acute and long-term neurotoxic effects of MDMA on marble burying behaviour in mice.* J Psychopharmacol, 2006. **20**(2): p. 264-71.
43. De Boer, S.F. and J.M. Koolhaas, *Defensive burying in rodents: ethology, neurobiology and psychopharmacology.* European Journal of Pharmacology, 2003. **463**(1-3): p. 145.

44. Craft, R.M., J.L. Howard, and G.T. Pollard, *Conditioned defensive burying as a model for identifying anxiolytics.* Pharmacol Biochem Behav, 1988. **30**(3): p. 775-80.
45. Treit, D., J.P. Pinel, and H.C. Fibiger, *Conditioned defensive burying: a new paradigm for the study of anxiolytic agents.* Pharmacol Biochem Behav, 1981. **15**(4): p. 619-26.
46. Treit, D., *Ro 15-1788, CGS 8216, picrotoxin, and pentylenetetrazol: do they antagonize anxiolytic drug effects through an anxiogenic action?* Brain Res Bull, 1987. **19**(4): p. 401-5.
47. Treit, D., V.M. Lolordo, and D.E. Armstrong, *The effects of diazepam on "fear" reactions in rats are modulated by environmental constraints on the rat's defensive repertoire.* Pharmacol Biochem Behav, 1986. **25**(3): p. 561-5.
48. Hill, M.N., et al., *Endocannabinoids modulate stress-induced suppression of hippocampal cell proliferation and activation of defensive behaviours.* Eur J Neurosci, 2006. **24**(7): p. 1845-9.
49. Yang, M., et al., *The rat exposure test: a model of mouse defensive behaviors.* Physiol Behav, 2004. **81**(3): p. 465-73.
50. Treit, D., *The inhibitory effect of diazepam on defensive burying: anxiolytic vs. analgesic effects.* Pharmacol Biochem Behav, 1985. **22**(1): p. 47-52.
51. Frye, C.A. and A.M. Seliga, *Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats.* Cogn Affect Behav Neurosci, 2001. **1**(4): p. 371-81.
52. Blampied, N.M. and R.C. Kirk, *Defensive burying: effects of diazepam and oxprenolol measured in extinction.* Life Sci, 1983. **33**(8): p. 695-9.
53. Treit, D., J.P. Pinel, and H.C. Fibiger, *The inhibitory effect of diazepam on conditioned defensive burying is reversed by picrotoxin.* Pharmacol Biochem Behav, 1982. **17**(2): p. 359-61.
54. Perrine, S.A., B.A. Hoshaw, and E.M. Unterwald, *Delta opioid receptor ligands modulate anxiety-like behaviors in the rat.* Br J Pharmacol, 2006. **147**(8): p. 864-72.
55. Morilak, D.A., et al., *Role of brain norepinephrine in the behavioral response to stress.* Prog Neuropsychopharmacol Biol Psychiatry, 2005. **29**(8): p. 1214-24.
56. Frye, C.A. and A.M. Seliga, *Olanzapine's effects to reduce fear and anxiety and enhance social interactions coincide with increased progestin concentrations of ovariectomized rats.* Psychoneuroendocrinology, 2003. **28**(5): p. 657-73.
57. Gomez, C., et al., *Rapid anxiolytic activity of progesterone and pregnanolone in male rats.* Pharmacol Biochem Behav, 2002. **72**(3): p. 543-50.
58. Korte, S.M., et al., *Effect of corticotropin-releasing factor antagonist on behavioral and neuroendocrine responses during exposure to defensive burying paradigm in rats.* Physiol Behav, 1994. **56**(1): p. 115-20.
59. Walker, F.R., et al., *Individual differences predict susceptibility to conditioned fear arising from psychosocial trauma.* J Psychiatr Res, 2007.
60. Korte, S.M., et al., *Conditioned neuroendocrine and cardiovascular stress responsiveness accompanying behavioral passivity and activity in aged and in young rats.* Physiol Behav, 1992. **51**(4): p. 815-22.
61. Whiteside, D.A. and L.D. Devenport, *Naloxone, preshock, and defensive burying.* Behav Neurosci, 1985. **99**(3): p. 436-40.
62. Matuszewich, L., et al., *The delayed effects of chronic unpredictable stress on anxiety measures.* Physiol Behav, 2007. **90**(4): p. 674-81.
63. Gutierrez-Garcia, A.G., et al., *A single session of emotional stress produces anxiety in Wistar rats.* Behav Brain Res, 2006. **167**(1): p. 30-5.
64. Arakawa, H., *Ontogenetic interaction between social relationships and defensive burying behavior in the rat.* Physiol Behav, 2007. **90**(5): p. 751-9.
65. Violle, N., et al., *Ethological comparison of the effects of a bovine alpha s1-casein tryptic hydrolysate and diazepam on the behaviour of rats in two models of anxiety.* Pharmacol Biochem Behav, 2006. **84**(3): p. 517-23.
66. Richardson, H.N., et al., *Exposure to repetitive versus varied stress during prenatal development generates two distinct anxiogenic and neuroendocrine profiles in adulthood.* Endocrinology, 2006. **147**(5): p. 2506-17.

67. Martinez-Gonzalez, D., et al., *REM sleep deprivation induces changes in coping responses that are not reversed by amphetamine.* Sleep, 2004. **27**(4): p. 609-17.
68. Frye, C.A., S.M. Petralia, and M.E. Rhodes, *Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3[alpha],5[alpha]-THP.* Pharmacology Biochemistry and Behavior, 2000. **67**(3): p. 587.
69. Kopchia, K.L., H.J. Altman, and R.L. Commissaris, *Effects of lesions of the central nucleus of the amygdala on anxiety-like behaviors in the rat.* Pharmacol Biochem Behav, 1992. **43**(2): p. 453-61.
70. Legradi, G., et al., *Microinfusion of pituitary adenylate cyclase-activating polypeptide into the central nucleus of amygdala of the rat produces a shift from an active to passive mode of coping in the shock-probe fear/defensive burying test.* Neural Plast, 2007: p. 79102.
71. Litvin, Y., et al., *CRF type 1 receptors in the dorsal periaqueductal gray modulate anxiety-induced defensive behaviors.* Horm Behav, 2007. **52**(2): p. 244-51.
72. Bondi, C.O., et al., *Noradrenergic facilitation of shock-probe defensive burying in lateral septum of rats, and modulation by chronic treatment with desipramine.* Prog Neuropsychopharmacol Biol Psychiatry, 2007. **31**(2): p. 482-95.
73. Echevarria, D.J., et al., *Administration of the galanin antagonist M40 into lateral septum attenuates shock probe defensive burying behavior in rats.* Neuropeptides, 2005. **39**(5): p. 445-51.
74. Saldivar-Gonzalez, J.A., et al., *Effect of electrical stimulation of the baso-lateral amygdala nucleus on defensive burying shock probe test and elevated plus maze in rats.* Life Sci, 2003. **72**(7): p. 819-29.
75. Reynolds, S.M. and K.C. Berridge, *Fear and feeding in the nucleus accumbens shell: rostrocaudal segregation of GABA-elicited defensive behavior versus eating behavior.* J Neurosci, 2001. **21**(9): p. 3261-70.
76. Lopez-Rubalcava, C., A. Fernandez-Guasti, and R. Urba-Holmgren, *Age-dependent differences in the rat's conditioned defensive burying behavior: effect of 5-HT1A compounds.* Dev Psychobiol, 1996. **29**(2): p. 157-69.
77. Pesold, C. and D. Treit, *The central and basolateral amygdala differentially mediate the anxiolytic effects of benzodiazepines.* Brain Res, 1995. **671**(2): p. 213-21.
78. Capone, F., et al., *Behavioral responses of 129/Sv, C57BL/6J and DBA/2J mice to a non-predator aversive olfactory stimulus.* Acta Neurobiol Exp (Wars), 2005. **65**(1): p. 29-38.
79. Petty-Zirnstein, M.K., K.K. Gustavson, and S.F. Davis, *Defensive burying: the effects of single vs multiple presentation of the aversive stimulus.* J Gen Psychol, 1983. **108**(2d Half): p. 271-5.
80. Saldivar-Gonzalez, A., C. Arias, and R. Mondragon-Ceballos, *Transient emotional changes elicited by intraperitoneal saline injection: effect of naloxone and flumazenil.* Pharmacol Biochem Behav, 1997. **56**(2): p. 211-20.
81. Davis, S.F., V.A. Dickson, and S.A. Moore, *Conditioning and retention of defensive burying as a function of the injection of a central cholinergic stimulant.* J Gen Psychol, 1982. **107**(2d Half): p. 249-54.
82. Kolb, B. and I.Q. Whishaw, *Neonatal Frontal Lesions in the rat: sparing of learned but not species-typical behavior in the presence of reduced brain weight and cortical thickness.* J Comp Physiol Psychol, 1981. **95**(6): p. 863-79.
83. Arakawa, H., *Ontogeny of sex differences in defensive burying behavior in rats: effect of social isolation.* Aggress Behav, 2007. **33**(1): p. 38-47.
84. Menard, J.L. and R.M. Hakvoort, *Variations of maternal care alter offspring levels of behavioural defensiveness in adulthood: evidence for a threshold model.* Behav Brain Res, 2007. **176**(2): p. 302-13.
85. Ahmadiyeh, N., et al., *Maternal behavior modulates X-linked inheritance of behavioral coping in the defensive burying test.* Biol Psychiatry, 2004. **55**(11): p. 1069-74.
86. Fernandez-Guasti, A., G. Roldan-Roldan, and A. Saldivar, *Reduction in anxiety after ejaculation in the rat.* Behav Brain Res, 1989. **32**(1): p. 23-9.