# Effect of 5-HT$_{1A}$ Receptor Agonists on Marble Burying and Stress-Induced Hyperthermia in Mice

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## Introduction

- Marble burying and stress-induced hyperthermia (SIH) are tests that have good predictive validity for anxiety.
- In humans as well as rodents, physiological stress causes an acute rise in body temperature. GABA-ergic and serotonergic mechanisms underlie SIH since benzodiazepines and 5-HT$_{1A}$ receptor agonists have been reported to decrease SIH (Olivier et al., 1998, 2002). Furthermore 5-HT$_{1A}$ receptor KO mice that show enhanced anxiogenic and stress-like responses are insensitive to anxiogelytic effects of the 5-HT$_{1A}$ receptor agonist flesinoxan (Pattij et al., 2002), confirming the involvement of 5-HT$_{1A}$ systems in this phenomenon.
- Spontaneous burying of glass marbles have been used as an index of anxiolytic drug effects in mice. Acute injection of benzodiazepines will decrease marble burying. However, this test is also sensitive to antidepressant compounds like SSRIs (Nicholas and Prinssen, 2006).

### *HYPOTHESIS*

*SIH and marble burying tests have predictive validity as anxiety tests and can be used to screen novel compounds with 5-HT$_{1A}$ receptor mechanisms.*

## Methods

### Animals

Male C57Bl/6 mice from Jackson Laboratories (Bar Harbor, Maine) were used in this study. Upon receipt, mice were assigned unique identification numbers (tail marked) and were group housed in polycarbonate cages with filter tops. All animals remained housed in groups of four during the remainder of the study. Mice were maintained on a 12 hr /12 hr light/dark cycle with the light on at 6:00 a.m EST. The room temperature was maintained between 20 and 23°C with a relative humidity maintained between 30% and 70%. Chow and water were provided *ad libitum* for the duration of the study. Prior to testing, all mice were examined on a regular basis, handled, and weighed to assure adequate health and suitability. In each test, animals were randomly assigned across treatment groups. Animals were not disturbed between test days.

### Stress-Induced Hyperthermia

The SIH test is based upon the principle that mice have a natural hyperthermic response to stress, which reflects the level of anxiety. The test involves two measures of rectal temperature repeated in the same animal with a 10-minute interval. On the day prior to testing, the mice were brought to the experimental room one hour before scheduled lights out and singly housed overnight with food and water ad libitum. On the morning of the experiment, animals were first injected with either vehicle, buspirone, 8-OH DPAT, WAY100635 or a combination of buspirone + WAY and 8-OH DPAT+WAY. One hour following injection, each animal was removed from the holding cage and held in a supine position and his rectal temperature was measured by slowly inserting a rectal probe into the animal's rectum at a length of approximately 0.5 cm. The rectal probe is attached to a PhysiTemp digital thermometer (Fisher Scientific) which provides temperature readings at 0.1°C accuracy. The probe remained inside the animal for approximately 5 seconds or until body temperature reached stability. This temperature was recorded as the baseline rectal temperature (T1). The animal was immediately placed back to the holding cage and after a 10-min interval the 2nd rectal temperature (T2) was taken using the same procedure as in measuring T1. Upon completion of the two rectal temperature measures, the animal was returned to the home cage and then later returned to the colony room. Before each insertion, the rectal probe was cleaned with an alcohol pad and lubricated with sterile K-Y jelly.

### Marble Burying

Marble burying is a test that is sensitive to anxiolytic agents. Mice were first injected with either vehicle, buspirone, 8-OH DPAT, WAY100635 or a combination of buspirone + WAY and 8-OH DPAT+WAY and placed in holding cages for 30 min, following which they were placed in cages containing 5 cm of bedding and 20 black glass marbles arranged in 5 rows of 4 marbles. Following a 30 min test, the number of unburied marbles were counted. A marble was considered buried if it was two third of the way in the bedding.

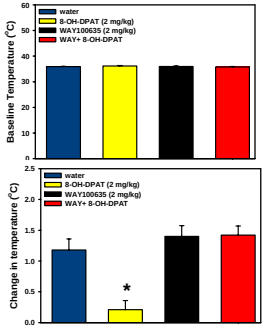
### Open Field

The open field chambers are plexiglas square chambers (27.3 x 27.3 x 20.3 cm; Med Associates Inc., St Albans, VT) surrounded by infrared photobeam sources (16 x 16 x 16). The enclosure was configured to split the open field into a center and periphery zone and the photocell beams were set to measure activity in the center and in the periphery of the OF chambers. Distance traveled was measured from consecutive beam breaks and was the index for activity. Mice were injected with vehicle or buspirone and placed in the OF chambers for a 60 min test session.

### Statistical analysis

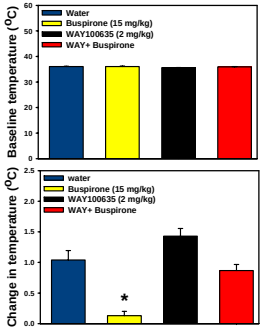
Data analyzed using analysis of variance (ANOVA) or student's t-test followed by Fisher PLSD post hoc test when appropriate. An effect was considered significant if $p < 0.05$.

## Figure 1: The 5-HT$_{1A}$ receptor antagonist WAY 100635 reverses the decrease in SIH induced by 8-OH-DPAT



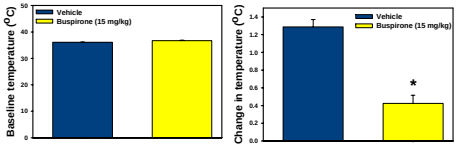

*Mice were injected with either water, 8-OH-DPAT (2 mg/kg) and/or WAY100635 (2 mg/kg) 60 min prior to taking the first body temperature. Data represent mean ± SEM of 10 mice/group*

## Figure 2: The 5-HT$_{1A}$ receptor antagonist WAY 100635 reverses the decrease in SIH induced by buspirone



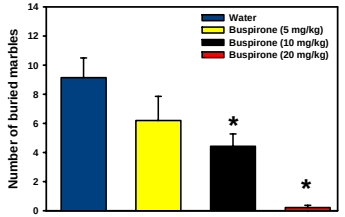

*Mice were injected with either water, buspirone (15 mg/kg) and/or WAY100635 (2 mg/kg) 60 min prior to test. Data represent mean ± SEM of 9-10 mice/group*

## Figure 3: Chronic treatment with buspirone does not cause tolerance to its anxiolytic effects in SIH



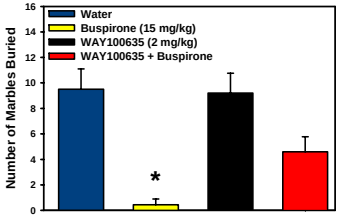

*Mice were injected i.p. with either vehicle (5% PEG200, 5% Tween 80 in saline) or buspirone (15 mg/kg) for 14 days prior to testing. Data represent mean ± SEM of 8 mice/group.*

## Figure 4: Buspirone dose dependently decreases marble burying.



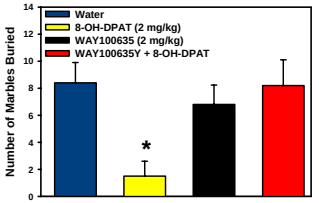

*Mice were injected i.p. with either water or buspirone 30 min prior to testing. Data represent mean ± SEM of 9-15 mice/group.*

## Figure 5: WAY 100635 partially reverses the decrease in marble burying induced by buspirone.



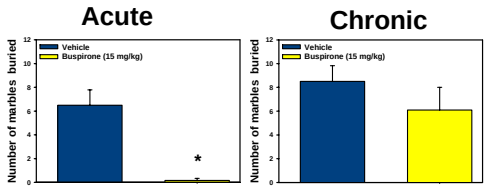

*Mice were injected i.p. with either water, buspirone (15 mg/kg) and or WAY100635 (2 mg/kg) 30 min prior to testing. Data represent mean ± SEM of 9-10 mice/group.*

## Figure 6: WAY 100635 reverses the decrease in marble burying induced by 8-OH-DPAT



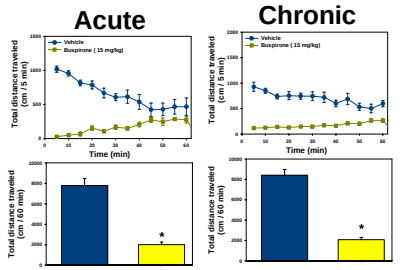

*Mice were injected i.p. with either water, 8-OH-DPAT (2 mg/kg) and or WAY100635 (2 mg/kg) 30 min prior to testing. Data represent mean ± SEM of 10 mice/group.*

## Figure 7: Chronic treatment with buspirone causes tolerance to its anxiolytic effects in marble burying




*Mice were injected either acutely or chronically with vehicle or buspirone (15 mg/kg) 30 min prior to test. Data represent mean ± SEM of 6 - 10 mice/group.*

## Figure 8: Chronic treatment with buspirone does not cause tolerance to its locomotor effects




*Mice were injected acutely or chronically with vehicle or buspirone (15 mg/kg) and placed in the open field chambers 30 min after injection. Data represent mean ± SEM of 8-10 mice/group.*

## Summary

- In C57Bl/6J mice the 5-HT1A receptor agonist 8-OH DPAT and the partial agonist buspirone decrease SIH. The effect is reversed with the 5-HT1A receptor antagonist WAY100635.
- In marble burying pretreatment with the 5-HT$_{1A}$ receptor agonist 8-OH DPAT and the partial agonist buspirone decreased the number of marbles buried in C57Bl/6J mice. WAY100635 fully reversed the anxiolytic effects of 8-OH-DPAT but only showed partial reversal of buspirone's effect.
- C57Bl/6J mice chronically treated with buspirone did not develop tolerance to its anxiolytic effects in the SIH paradigm. On the other hand tolerance was seen in the marble burying test which was not a result of mice developing tolerance to the locomotor activity effects of buspirone.
- These data show that both tests have predictive validity as 'anxiety' mouse models to screen compounds that work via 5-HT$_{1A}$ mechanisms.

## References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- Olivier B, Zethof TJJ, van der Heyden JAM (1998) The anxiolytic effects of flesinoxan in the individually housed stress-induced hyperthermia paradigm are 5-HT1A receptor mediated. Eur J Pharmacol 342:177-182.
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