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Short communication

Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks

F.E. Harrison[a,b,*], A.H. Hosseini[c], M.P. McDonald[a,c,d]

[a] Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, United States
[b] Department of Medicine, Vanderbilt University, Nashville, TN 37232, United States
[c] Program in Neuroscience, Vanderbilt University, Nashville, TN 37232, United States
[d] Departments of Neurology and Anatomy & Neurobiology, University of Tennessee Health Science Center, Memphis, TN 38163, United States

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ABSTRACT

The effects of abnormally high or low stress on learning are well established. The Barnes maze and Morris water maze are two commonly used tests of spatial memory, of which the water maze is considered more stressful; however, until now this has not been demonstrated empirically. In the present study, mice matched for performance on commonly used anxiety tasks were trained on either the Barnes maze or water maze or received no cognitive testing. Water-maze training induced greater increases in plasma corticosterone than did Barnes maze training, assessed 30 min after the final session. Importantly, spatial learning was inversely correlated with corticosterone levels in the water maze but not the Barnes maze, suggesting that performance on the water maze may be more affected by test-induced stress even within wild-type subjects of the same age and gender. These findings are important when considering the appropriate cognitive tasks for any experiment in which stress responses may differ systematically across groups.

The Barnes maze [3] and Morris water maze [19] are similar tasks in that they both measure the ability of a mouse to learn and remember the location of a target zone using a configuration of distal visual cues located around the testing area [11,23]. Both tasks rely on hippocampal-dependent spatial reference memory and on the inherent tendencies of the subjects to escape from an aversive environment [2,27]. It has been suggested that the Barnes maze is less anxiogenic [13,21]; however, we know of no data that support this assumption.

Innate anxiety and cognitive ability differ considerably among mouse strains [5,13,18] highlighting the fact that the selection of a background strain and the choice of behavioural tasks are critical to the outcome of an experiment. The present experiment was designed to determine whether water maze and Barnes maze performance induce differential stress reactions, as assessed biochemically by serum corticosterone. Rats exhibit a robust increase in corticosterone on the first day of water maze testing that is stable and only slightly diminished throughout days of testing [1]. No such data are available for the Barnes maze. The demonstration of differential stress responses in mice performing the two behavioural tasks would have implications for the choice of background strain as well as the appropriate cognitive and control tasks to use for a particular study.

The subjects were thirty 7-week-old male, C57BL/6J mice obtained from Jackson Laboratory (Bar Harbor, ME, USA; stock #000664) and left undisturbed for 1 week before testing began. Mice were housed five per cage until 1 day before testing in the Barnes or water maze, when mice were singly housed to eliminate any additional stress that may arise from test order within the cage as other mice are removed for testing and then returned. Although individual housing itself may be stressful [9], all mice were treated identically and thus the effect of housing stress was constant across groups. Mice were housed in a temperature-controlled room under a 12-h light/dark cycle with free access to food and water. All procedures were approved by the Vanderbilt University Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

* Corresponding author at: Vanderbilt University, Division of Diabetes, Endocrinology & Metabolism, 7465 Medical Research Building IV, 2213 Garland Avenue, Nashville, TN 37232-0475, United States. Tel.: +1 615 936 1660; fax: +1 615 936 1667.
E-mail address: Fiona.Harrison@Vanderbilt.edu (F.E. Harrison).

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Anxiety was tested as previously described, in a standard elevated-plus maze [4,12,22] followed by the light–dark activity task [4,12,22] on separate days during the first week. In the elevated plus maze time spent in closed arms was calculated as the percent of total time on arms excluding time in the central area [7,8]. The number of entries into arms and distance traveled were also recorded. In the light–dark task the measures of interest were latency to enter the dark compartment, time spent within each area, transitions between areas, and total distance traveled.

Social dominance was assessed in the second week using the tube test [14,15,17]. On the day before testing each mouse was given three habituation sessions to become accustomed to the act of running into the tube. During the test two mice from the same home-cage were placed at either end of a 30-cm long × 3.5-cm diameter clear tube. A subject was declared a "winner" when its opponent backed out of the tube. Each mouse was tested against each of the other four individuals from within its cage and trials were repeated with mice beginning from the opposite ends to avoid position bias. A score of 1 was awarded to the winner of a bout and a score of 0 was given to the loser. Thus each mouse was assigned a dominance score of between 0 (no wins) and 8 (no losses). The mouse with the highest score in each cage was assigned the 'dominant' status, if the highest score was shared between two mice they were designated 'co-dominant', and the lowest scoring mouse was the 'subordinate' mouse.

Mice were then matched according to an overall anxiety and dominance score calculated using *Z*-scores to combine performance on the anxiety-related measures in the elevated-plus and light–dark tasks and the social dominance test. *Z*-scores were calculated for time in closed arms and entries into closed arms in the plus maze and time in dark and latency to enter the dark in the light/dark task. Three groups were created: Water maze, Barnes maze, or Naïve (no further testing) with 10 mice per group such that the mean *Z*-score for each group did not differ [$F_{2,27} = .001$; $p = .998$] and with one dominant, two co-dominant and two subordinate mice in each group.

The Barnes and water mazes were used to assess spatial learning, as previously described in detail [4,11,12,22]. In order to make fair comparisons between the two tasks, our usual procedures were modified to equate the number and duration of trials and number of testing days. The two mazes were of equal diameter (92 cm) and positioned in the same place in the same testing room so that visible spatial cues as well as other environmental factors (background noise and light level) were identical between the two tasks. Both tasks were conducted by the same experimenter 1 week apart but at the same time of day. For each task, six 1-min trials were run per day for 5 days, conducted in a massed fashion so that each mouse completed all of its daily trials before the next mouse was tested. Spatial learning in the Barnes maze was assessed using total and primary errors (errors committed before the first encounter with the escape hole). Escape latency and path length were also measured. In the water maze, escape latency and path length as well as mean distance from the platform (search error) were the variables of interest. Swim speed and swimming in the periphery (outer 8 cm of the pool) were also assessed in the water maze as non-cognitive control factors. On each testing day, the 10 mice in the test group were transported in their home cages approximately 10 m on a cart from the colony room to the corridor outside the testing room, where they remained for the duration of the session. At the same time, five Naïve control mice were yoked to five of the tested mice. Yoked Naïve mice were transported with test mice to control for anxiety responses associated with removing the cages from the housing room and transporting them to the testing room.

Thirty minutes following the last trial on the fifth day of cognitive testing, mice were briefly anaesthetized using isoflurane and then sacrificed by decapitation. Trunk blood was collected and stored on wet ice for 1 h, then spun in a centrifuge at 13,000 rpm for 20 min. Serum was collected from each sample and stored at −80 °C until used for analysis of corticosterone levels. Corticosterone was measured by radioimmunoassay [25] using an ImmuChem Double Antibody Corticosterone Kit (MP Biomedicals, Solon, OH, USA; Cat #07-120103) by the Vanderbilt Hormone Assay Core. Mice were killed at 30 min after testing when serum corticosterone levels were expected to still be elevated even after 5 days of testing [1]. Although corticosterone levels may vary over time between testing and death, all animals were sacrificed at the same time interval following testing and thus any post-test variations in corticosterone magnitude was consistent across groups. Although corticosterone is not the only biological indicator of stress (e.g. adrenocorticotrophic hormone, adrenaline) it is a reliable marker that has often been used to indicate the stress following behavioural tasks (e.g. [1,2]).

Statistical analyses were conducted using SPSS 14.0 for Windows. Corticosterone level was analyzed by univariate ANOVA with behavioural group (Water maze, Barnes maze or Naïve) as the between-groups factor. Follow-up comparisons were made using Bonferroni corrected *t*-tests. Task acquisition across the 5 days in the Barnes maze and water maze was analyzed separately within each task by repeated-measures ANOVA with session as the repeated measure. Comparisons of day 5 escape latencies between Barnes maze and water maze were determined using an independent-samples *t*-test. Relationships between corticosterone and behaviour, and among measures in the anxiety, social dominance, and spatial learning tasks were determined using bivariate correlations.

1. Anxiety and social dominance

None of the anxiety-related measures in the elevated-plus maze was significantly correlated with any of the anxiety-related measures in the light/dark test (r's < .272, p's > .146), suggesting that they represent independent aspects of the psychological construct "anxiety". The score on the social dominance tube test was unrelated to any of the anxiety measures in either test (r's < .310, p's > .095).

2. Spatial learning

Mice improved significantly on both spatial learning tasks across the 5 days of testing. The water maze measures of search error, escape latency, and total path length all decreased, indicating good spatial learning [$Fs_{4,36} > 11.7$, p's < .001]. Similarly, total and primary errors decreased significantly in the Barnes maze [$Fs_{4,36} > 14.915$, p's < .001]. Escape latency and path length, which sometimes correlate with error measures in the Barnes maze, also decreased across training [$Fs_{4,36} > 16.959$, p's < .001]. On Day 5, escape latencies on the Barnes and water mazes were not statistically different, indicating that mice in each group spent approximately the same amount of time in the testing environments [$t(18) = .454$, $p = .466$].

3. Anxiety and spatial learning

To evaluate the relationship between stress and learning, correlative analyses were conducted on data from water-maze and Barnes-maze groups. In the Barnes maze, the unitary measures of escape latency and path length were both correlated with percent closed-arm entries in the plus maze (r's > .685, p's < .03). In the water maze, path length was associated with percent time in dark in the light–dark test ($r = .739$, $p = .015$). None of the other pre-test anxiety measures was significantly correlated with any other mea-