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Research report

# Increased water temperature renders single-housed C57BL/6J mice susceptible to antidepressant treatment in the forced swim test

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**Abstract**

To investigate genotype × environment interactions in the forced swim test, we tested the influence of water temperature (20 °C, 25 °C, 30 °C) on floating behaviour in single-housed male C57BL/6J and BALB/c mice. We observed a contrasting relationship between floating and water temperature between the two strains, with C57BL/6J floating more and BALB/c floating less with increasing water temperature, independent of the lightening conditions and the time point of testing during the animals' circadian rhythm. Both strains showed an inverse relationship between plasma corticosterone concentration and water temperature, indicating that the differences in stress coping are unrelated to different perception of the aversive encounter. Treatment with desipramine (20 mg/kg, i.p.) caused a reduction in immobility time in C57BL/6J mice if the animals were tested at 30 °C water temperature, with no effect at 25 °C and no effects on forced swim stress-induced corticosterone secretion. The same treatment failed to affect floating behaviour in BALB/c at any temperature, but caused a decrease in plasma corticosterone levels. Taken together we demonstrate that an increase in water temperature in the forced swim test exerts opposite effects on floating behaviour in C57BL/6J and BALB/c and renders single-housed C57BL/6J mice, but not BALB/c mice, susceptible to antidepressant-like behavioral effects of desipramine.


*Keywords:* Depression; Stress coping; Desipramine; Inbred mice; Isolation; Corticosterone

## 1. Introduction

Since its establishment 30 years ago [1], the forced swim test has become one of the standard procedures for the testing of antidepressant-like activity of pharmacological compounds [2,3]. In its classical version, rats and mice are either once (mice) or twice, on 2 consecutive days (rats), placed into a cylinder filled with water [1,4]. Animals show typical behavioural responses to the unpleasant environment, which can be classified as (i) immobility (floating, except for movements necessary to maintain balance), (ii) swimming (movement of at least the two hind legs, with no paw breaking the water surface) and (iii) struggling (the forepaws break the water surface, usually at the walls). Mice and rats show a decrease in struggling and, correspondingly, an increase in floating over the time course of the first exposure (6 min) and from the first to the second exposure (day 1 to day 2). The increased immobility time is particularly sensitive to a variety of antidepressants, including selective serotonin and noradrenaline re-uptake inhibitors and tricyclic antidepressants [3,9], which cause a significant decrease in floating. Therefore, increased immobility time is often regarded as an indicator of behavioural despair or even "depression-like behaviour". However, the acute effects of antidepressants may also reflect increased arousal, and behavioural changes observed from the first to the second FST exposure could also mirror cognitive processes [5] and interindividual differences in stress coping [6]. Moreover, a variety of environmental factors affect forced swimming behaviour and the individual susceptibility to antidepressant treatment. For mice, these factors include single housing and water temperature (for review see [7]). Several experimental procedures require single housing of male mice, such as chronic mild stress protocols [8], animal models of post-traumatic stress disorder [9] or experiments, for which mice have to undergo surgery (e.g. microdialysis experiments). Hence, the failure to decrease the floating time of single-housed male mice

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by antidepressant treatment [10] provides a major shortcoming of the forced swim test.

The present study was designed to assess genotype × environment interactions in the forced swim test for single-housed male mice of the two inbred strains C57BL/6J and BALB/c at different water temperatures either during the inactivity phase or during the activity phase of the animals' circadian rhythm. In addition, we used these two strains, which markedly differ in their stress-coping behaviour as a result of complex genotype × environment interactions [11,12], to study the efficiency of treatment with the tricyclic antidepressant desipramine at different water temperatures.

## 2. Material and methods

### 2.1. Animals

Male C57BL/6J@Crl (B6J) and BALB/cAnNCrl (BALB/c) mice were purchased from Charles River (Borchern, Germany) at an age of 6 to 8 weeks. Animals were housed singly in type II Macrolon cages under standard laboratory conditions (12-h light/12-h dark cycle, 22 ± 1 °C, 55 ± 5% humidity, food and water ad libitum) for 14 days before starting the experiments. Experiments were approved by the Committee on Animal Health and Care of the local governmental body of Bavaria and performed in strict compliance with the EEC recommendations for the care and use of laboratory animals (86/609/CEE).

### 2.2. Forced swim test

Mice were transferred to the experimental room at least 2 h before testing. All mice were tested either on 2 consecutive days with one exposure per day (Experiment 1) or with a single exposure (Experiment 2). Each exposure began with insertion of the mouse into a 5 l glass beaker (height: 27 cm, diameter: 16.5 cm) that was filled with tap water to a height of 15 cm. The test lasted for 6 min and ended with the removal of the animal. Animals' behaviour was videotaped for subsequent off-line analysis. Water was changed between subjects. Immobility time was scored from the videotapes by trained observers who were blind to water temperature and the drug treatment, by pressing preset keys on a computer keyboard, using a customized freeware software (EVENTLOG; Robert Hendersen 1986). The resulting ethograms were further processed by customized software (Winrat Vers. 2.31; Heinz Barthelmes, MPI Munich). A mouse was judged to be immobile (i.e. floating) when it stopped any movements except those that were necessary to keep its head above water.

### 2.3. Drug treatment

Desipramine hydrochloride (Sigma–Aldrich, Steinheim, Germany) was dissolved in vehicle solution (2% dimethylsulfoxide in 0.9% saline) and injected at a dose of 20 mg/kg in a volume of 10 ml/kg body weight i.p. 30 min prior to the forced swim test.

### 2.4. Plasma corticosterone

Ten minutes after FST onset mice were decapitated and trunk blood was collected in pre-chilled tubes containing EDTA. Blood samples were centrifuged for 15 min at 2500 × *g* at 4 °C. Plasma samples were stored in aliquots at −80 °C until assay. Plasma corticosterone was measured by a commercially available RIA kit (MP Biomedicals, Eschwege, Germany) as described elsewhere [13], according to manufacturer's instructions.

### 2.5. Experiment 1

Both B6J and BALB/c were randomly assigned to one out of three groups that were tested at water temperatures of 20 °C, 25 °C or 30 °C ($n = 10$, each). Animals were tested on 2 consecutive days during the light phase of the circadian cycle under 500 lx.

### 2.6. Experiment 2

Both B6J and BALB/c were randomly assigned to one out of four groups ($n = 10$–13) which were treated either with desipramine (20 mg/kg) or with vehicle 30 min prior to FST exposure at 25 °C or 30 °C warm water [2 × 2 × 2 design with strain (B6J, BALB/c) × temperature (25 °C, 30 °C) × drug (vehicle, desipramine)]. Trunk blood was collected 10 min after the onset of FST exposure. Animals were tested during the dark phase of their circadian cycle under red light.

### 2.7. Data analysis

Floating time was analyzed in 1 min intervals. In addition, we calculated the total floating duration shown during the last 4 min of the exposure and normalized the data to the total observation interval (240 s). Inter-observer variability was assessed for the total immobility times of 20 mice with two additional observers, and proven for reliability by linear correlation ($r^2 > 0.930$, $p < 0.0001$). Statistical analysis was performed by analyses of variance (ANOVA) as indicated in the text, followed by Newman–Keuls *post hoc* test if appropriate. Statistical significance was accepted if $p < 0.05$. In *Experiment 1*, behavioural data from day 2 of one B6J mouse are missing because of problems with video recording.

## 3. Results

### 3.1. Experiment 1: Influence of water temperature

As shown in Fig. 1A, floating behaviour of B6J mice clearly depended on the water temperature both at day 1 [*Temperature*: $F_{2,27} = 9.47$, $p < 0.001$; 2-way ANOVA (*Temperature*, *Interval*) for repeated measures (*Interval*)] and at day 2 (*Temperature*: $F_{2,26} = 11.9$, $p < 0.001$), with maximal floating at 30 °C and minimal floating at 20 °C (d1: 25 °C < 30 °C, $p = 0.011$; 20 °C < 30 °C, $p < 0.001$; d2: 20 °C < 25 °C, $p = 0.002$; 20 °C < 30 °C, $p < 0.001$; Newman–Keuls post hoc test). Also BALB/c mice floated differently in response to the different water temperatures both at day 1 (*Temperature* × *Interval*: $F_{10,135} = 5.30$, $p < 0.0001$) and at day 2 (*Temperature* × *Interval*: $F_{10,135} = 3.88$, $p < 0.0005$). This time, however, floating was maximal at 20 °C and minimal at 30 °C (see Fig. 1B for results of post hoc test).

A direct comparison of the floating behaviour shown by the two strains during the last 4 min of the exposure by 2-way ANOVA revealed a significant major effect of *Strain* at day 1 ($F_{1,54} = 13.3$, $p = 0.0006$) as well as significant *Strain* × *Temperature* interactions both at day 1 ($F_{2,54} = 9.48$; $p = 0.0002$) and at day 2 ($F_{2,53} = 12.8$, $p < 0.0001$), thus confirming the divergent response of the two strains to alterations in water temperature (Fig. 2).

### 3.2. Experiment 2: Response to desipramine

Desipramine caused a slight reduction in floating in B6J mice if tested under 25 °C [*Drug*: $F_{1,21} = 6.36$, $p = 0.019$; *Drug* × *Interval*: $F_{5,105} = 2.86$, $p = 0.018$; 2-way ANOVA (*Drug*, *Interval*) for repeated measures (*Interval*); Fig. 3A] and a strong reduction if tested under 30 °C (*Drug*: $F_{1,22} = 11.5$, $p = 0.002$; *Drug* × *Interval*: $F_{5,110} = 5.86$, $p < 0.0001$; Fig. 3A). In BALB/c,