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# Stressors can affect immobility time and response to imipramine in the rat forced swim test

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ABSTRACT

We subjected Wistar rats to the forced swim test (FST) to compare the effects of two doses of imipramine in physically stressed rats (P: unavoidable electric footshocks), emotionally stressed rats (E: odors), or non-stressed rats (C). Stress or control sessions lasted 35 days. Drug treatments began on day 21 and continued for the next 14 days. E rats were placed for 10 min, once per day for 35 days, in a small non-movement-restricting cage impregnated with urine collected from a P rat. E and P rats exhibited opposite changes in locomotion. After 21 days of stress sessions, P rats displayed the longest immobility times in the FST, followed by E rats. In the P group, on day 7 of treatment (day 28 of the study), imipramine (2.5 mg/kg) reduced immobility time to baseline values. In the E group, immobility time decreased only after 14 days of treatment with the low imipramine dose. The high dose of imipramine (5.0 mg/kg) reduced immobility time at day 7 of treatment in all groups. In conclusion, physical and emotional stress similarly increased immobility time in the FST, but emotional stress appears to be more resistant to imipramine treatment.



## 1. Introduction

A wide range of imipramine doses (5–45 mg/kg) injected one to three times before testing reduces immobility time in the forced swim test (FST) in rats (Kelliher et al., 2003; Kúsmider et al., 2006; Porsolt et al., 1978; Rénéric et al., 2002; Takamori et al., 2001), an intriguing effect because antidepressant efficacy in human becomes apparent only after several weeks of treatment (Antelman and Gershon, 1998; Antelman et al., 2000). One might surmise that the acute effect of antidepressants on monoamine turnover may underlie their clinical actions (Cryan et al., 2005; Detke et al., 1997; Elhwuegi, 2004), but a relatively low dose of clomipramine (2.5 mg/kg) increased the firing rate of lateral septal nucleus neurons in Wistar rats only after 14 days of treatment (Contreras et al., 1990). Likewise, a low dose of fluoxetine (1 mg/kg) only increased the neuronal firing rate of lateral septal nucleus neurons and reduced immobility time in the FST after 3 weeks of treatment (Contreras et al., 2001). Therefore, long-onset plastic changes may occur in several neural functions to establish clinically relevant antidepressant actions (Reid and Stewart, 2004).

Several experimental conditions prior to a FST session increase total immobility time, including prior subordination stress (Rygula et al., 2005), inescapable stress (Weiss et al., 1981), or social isolation (Yates et al., 1991). In fact, repeated exposure to FST decreases motivation and perseverance in subsequent tests (Willner et al., 1987) and reduces struggling (Kitada et al., 1981). Most antidepressants reduce total immobility time in the FST in rats previously subjected to a cold environment, cages with restricted movement, or electric footshocks (Borsini et al., 1989) compared with control groups. Antidepressants also reduce immobility time in non-stressed rats subjected to FST compared with saline-treated rats (Barros and Ferigolo, 1998; Drago et al., 2001; Kitamura et al., 2004; Lucki, 1997). We recently demonstrated that emotional stress produced by daily exposure for 3 weeks to odors originating from physically stressed rats increased immobility time in the FST and elicited changes in the open field test (OFT) in both groups (Gutiérrez-García et al., 2007). Therefore, we hypothesized that increased immobility time in the FST produced by long-term emotional or physical stress may be modified by a regimen of long-term, low-dose imipramine treatment compared with long-term, high-dose imipramine treatment. In the present study, we subjected different groups of rats to either physical stress (P: unavoidable electric footshocks) or emotional stress (E: exposure to urine odors from P rats) for 21 days. Different groups of P or E rats subsequently received one of two doses of imipramine during the next 14 days. We compared our results with control groups that were not exposed to stress but received saline or imipramine

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treatment. We found that physical or emotional stress produced a similar increase in immobility time in the FST. A relatively high dose of imipramine restored control values after 7 days of treatment, and a lower dose of imipramine produced comparable results but only after 14 days of treatment, particularly in the E group.

## 2. Materials and methods

### 2.1. Animals and housing

A total of 56 male Wistar rats (Center UNAM, Harlan, México), 3 months of age and weighing 300–350 g at the time of testing, were included in the experiments. The rats were randomly assigned to one of eight groups. Rats were housed eight per cage in acrylic translucent cages (45×33×30 cm) under a 12 h/12 h light/dark cycle (lights on at 7:00 AM; <100 lx) with *ad libitum* access to food and water. The rats were handled daily, once per day, beginning 1 week before the experiment to reduce some of the possible effects of stress produced by handling during experimentation. All experimental procedures were performed between 9:00 AM and 12:00 PM. All animal procedures adhered to the general principles of laboratory animal care (NIH, publication 85-23 1985).

### 2.2. Apparatus

We used a slightly modified version of the two-compartment box described by van den Berg et al. (1998). The glass box (30×25 cm at the base, 30 cm height) contained a stainless steel grid floor (0.5 cm diameter bars, spaced 1.3 cm apart). An opaque Plexiglas plate (0.2 cm thickness) divided the cage into two compartments (safe and shock compartments: each 15 cm×12.5 cm base, 30 cm height). Another Plexiglas plate covered the floor of the safe compartment to prevent electric footshocks. Only one side of the cage was used at a time, depending on the experimental group (i.e., physical or emotional stress). An electric stimulator (Grass Instruments S44, Quincy, MA, USA) coupled in series to a stimulus isolation unit (SIU5, Grass Instruments, Quincy, MA, USA) and to a constant current unit (CCU1A, Grass Instruments, Quincy, MA, USA) provided electrical pulses (1 mA, direct current, 0.5 s, 0.5 c/s, 10 min) to the grid of the shock compartment. The apparatus was sound-attenuated.

### 2.3. Experimental groups

The study included a total of 35 days of daily, once-per-day, 10-min sessions of stress or non-stress conditions. After 21 days of daily exposure to stress or non-stress, the rats received a daily injection of saline or imipramine 1 h before the 10-min stress session during the last 14 days of the study. Rats from two control groups (C-$S_1$, $n$=7; C-$S_2$, $n$=7) were individually placed in the safe compartment and were not exposed to physical or emotional stress. Rats from the C-$S_1$ and C-$S_2$ groups were injected with saline (0.9% NaCl; 0.2 ml, i.p.). In the remaining groups, we tested two doses of imipramine (2.5 mg/kg, i.p., $n$=21; 5.0 mg/kg, i.p., $n$=21; 2 ml/kg body weight; Sigma, St. Louis, MO, USA) dissolved in saline solution (0.9% NaCl) and injected daily during the last 14 days of the study. Rats from the control imipramine-treated groups (C-2.5, $n$=7; C-5.0, $n$=7) also underwent 10-min sessions (safe compartment) devoid of any sensorial stimulation for 35 days and received corresponding doses of imipramine during the last 14 days of the study. The rats from the physically stressed groups (P-2.5, $n$=7; P-5.0, $n$=7) received unavoidable electric footshocks once per day for 35 days. When rats received footshocks, they spontaneously urinated. After each footshock session, we collected the urine (0.1–0.6 ml) with a clean 1 ml syringe, and the footshock-exposed rats were returned to their home cage. The P groups also received their respective imipramine treatment during the last 14 days of the study. After each 10-min physical stress session, the urine collected from the P-2.5 or P-5.0 rat was distributed on the floor of the safe compartment a few seconds before placing a rat from the emotionally stressed groups into that compartment (E-2.5, $n$=7; E-5.0, $n$=7). Rats from the E groups did not receive electric footshocks (Fig. 1).

### 2.4. Behavioral tests

Behavioral tests were performed on days 1, 21, 28, and 35 of the stress or non-stress sessions. From days 21 to 35 of the study, the rats also received daily saline or imipramine treatment 1 h before stress or non-stress sessions.

#### 2.4.1. Open field test

The open field test is commonly used in combination with the FST to eliminate nonspecific effects of antidepressant treatments (Borsini et al., 1985; Briones-Aranda et al., 2005; Contreras et al., 2001; Martínez-Mota et al., 2008; Porsolt et al., 1978; Wieland and Lucki, 1990). On the days of recording, immediately after being subjected to stress or non-stress sessions, each rat was individually placed in an acrylic box (44×33×20 cm), with the floor divided into 12 squares (11×11 cm each) for a 5-min videotaped OFT session. Square crossings were counted when an animal passed from one square to another with its hind legs. After each test, we carefully cleaned and deodorized the box with a cleaning solution (ammonia 0.5%, ethanol 15%, extran 10%, isopropyl alcohol 5%, Pinol® 19%, water 50.5%). All tests were performed during the light period of the light/dark cycle. The testing room was illuminated with white light (40 lx) from a tungsten lamp placed 2 m above the OFT and FST devices. Once the OFT session concluded, the FST session began.

#### 2.4.2. Forced swim test

The 5-min FST consisted of placing each rat individually in a rectangular pool (50×30 base area, 60 cm height) filled with water to a depth of 24 cm (25±1 °C). Immobility was assumed when a rat floated without displacements and only moved to maintain its nostrils above the water surface or when it touched the bottom of the pool for more than 2 s, making at least two points of contact (i.e., with one or both hind paws and its tail). After each test, the pool was cleaned and filled with clean water. We do not accept discrepancies between observers. The observers obtain their first results separately. In cases of dissimilar results, the observers view the videotaped tests together and discuss

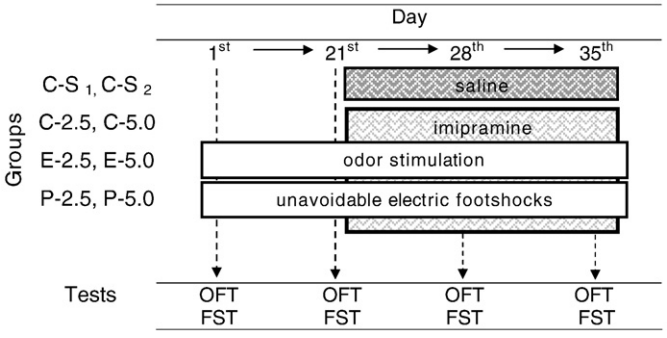


**Fig. 1.** Experimental design. Two sets of Wistar rats were randomly assigned to eight groups. Two control saline groups (C-$S_1$, C-$S_2$) were not subjected to any stress during the 35 days of the study and were injected with saline during the last 14 days of the study. Two control, non-stressed, imipramine-treated groups received imipramine at a dose of 2.5 mg/kg, i.p. (C-2.5) or 5.0 mg/kg, i.p. (C-5.0) during the last 14 days of the study. Two groups were exposed to emotional stress throughout the study and received imipramine at a dose of 2.5 mg/kg, i.p. (E-2.5) or 5.0 mg/kg, i.p. (E-5.0) during the last 14 days of the study. Two additional groups were exposed to electric footshocks stress throughout the study and received imipramine 2.5 mg/kg, i.p. (P-2.5) or 5.0 mg/kg, i.p. (P-5.0) during the last 14 days of the study. Behavioral tests (open field test, OFT; forced swim test, FST) were performed on the first day of the study and again at the end of days 21, 28, and 35 of the study.