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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

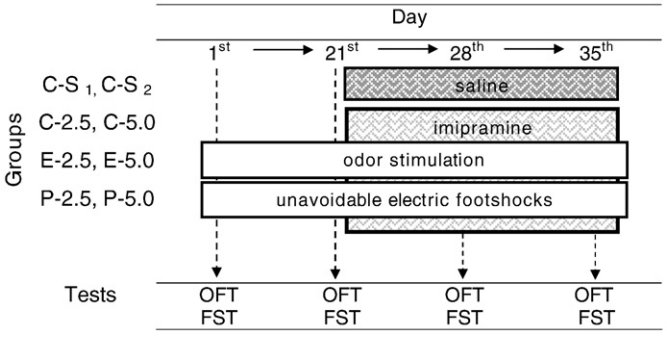

Fig. 1. Experimental design. Two sets of Wistar rats were randomly assigned to eight groups. Two control saline groups (C-S_1, C-S_2) were not subjected to any stress during the 35 days of the study and were injected with saline during the last 14 days of the study. Two control, non-stressed, imipramine-treated 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.

their disparate results until they reach the same conclusions. The observers are constantly trained to attain the same observations and are trained to use the same criteria for analysis. In recent protocols, we have used automated systems that clearly show the concordance of results with the trained observers.

2.5. Statistical analysis

The main difference between drug treated groups was the dose of imipramine injected during the last 14 days of the study. Therefore, the data followed a normal distribution. We used two-way, repeated-measures analysis of variance (ANOVA), with dose of imipramine (2.5 mg/kg: groups C-S_1, C-2.5, E-2.5, P-2.5; 5 mg/kg: groups C-S_2, C-5.0, E-5.0, P-5.0) as one factor and days of study (stress or non-stress exposure: 1 and 21 days; stress or non-stress sessions plus saline or imipramine treatment: days 28 and 35 of the study) as another factor. When at least one of the factors was significant ($P \leq 0.05$), follow-up Holm–Sidak *post hoc* tests were performed. Data are expressed as mean ± SEM.

3. Results

3.1. Open field test

3.1.1. Imipramine 2.5 mg/kg

Two-way, repeated-measures ANOVA revealed a significant main effect of group ($F_{(3,54)} = 7.294$, $P < 0.001$). Square crossings were significantly lower ($P < 0.05$, Holm Sidak *post hoc* test) in the C-2.5 (24.8 ± 1.54) and P-2.5 (23.5 ± 1.92) groups compared with the C-S_1 (34.4 ± 2.25) and E-2.5 (29.5 ± 2.09) groups.

The days of study factor also reached statistical significance ($F_{(3,54)} = 9.047$, $P < 0.001$). For all groups, square crossings were highest on day 21 of the study (32.5 ± 1.99) compared with day 1 (28.8 ± 2.50) and decreased by day 28 (27.0 ± 1.68) and day 35 (24.4 ± 2.01) of the study (i.e., during imipramine or saline treatments).

A significant group × days of study interaction was observed ($F_{(9,54)} = 2.584$, $P < 0.01$), revealing differences in square crossings between groups subjected to stress (i.e., no stress, shock, or odor) and groups subjected to stress plus imipramine or saline treatment (Fig. 2). Similarly, square crossings in the C-2.5 group, which did not receive stress but was subjected to a low dose of imipramine (2.5 mg/kg), displayed no significant changes in square crossings during the study, regardless of test repetition or treatment condition. In the E-2.5 group, square crossings significantly increased on day 21 of the study ($P < 0.05$, Holm Sidak *post hoc* test) and returned to baseline values during the 2.5 mg/kg imipramine treatment. In contrast, square crossings in the P-2.5 group decreased on 21 day of the study ($P < 0.05$, Holm Sidak *post hoc* test) and decreased further during low-dose imipramine treatment.

3.1.2. Imipramine 5.0 mg/kg

Two-way, repeated-measures ANOVA revealed a significant main effect of group ($F_{(3,54)} = 6.402$, $P < 0.004$). Holm Sidak *post hoc* tests revealed a significant difference ($P < 0.05$) between the C-S_2 group (31.2 ± 1.86) and P-5.0 group, which had the lowest number of square crossings (18.8 ± 3.16). Although the C-5.0 (23.1 ± 2.25) and E-5.0 (27.1 ± 2.36) groups exhibited a decrease in square crossings, the effect did not reach statistical significance.

The overall analysis of the days of study factor also reached significance ($F_{(3,54)} = 17.977$, $P < 0.001$). Significantly decreased square crossings were observed on day 28 (20.9 ± 17.0) and day 35 (17.0 ± 2.05) of the study ($P < 0.05$, Holm Sidak *post hoc* test) compared with day 1 and day 21 of testing (32.8 ± 2.42 and 29.6 ± 2.40, respectively).

A significant group × days of study interaction also was observed ($F_{(9,54)} = 5.308$, $P < 0.001$). Although square crossings in the C-S_2 group (non-stressed, saline-treated) were not significant, the C-5.0 group exhibited a decrease in square crossings on day 7 of 5.0 mg/kg imipramine treatment (i.e., on day 28 of the study; $P < 0.05$, Holm Sidak *post hoc* test), with no further changes observed. Similar to E-2.5 rats, the E-5.0 group displayed an increase in square crossings on day 21 of the study ($P < 0.05$, Holm Sidak *post hoc* test) while being subjected to emotional stress. The higher dose of imipramine reduced square crossings (days 28 and 35 of the study; $P < 0.05$, Holm Sidak *post hoc* test). Similar to P-2.5 rats, the P-5.0 group displayed a decrease in square crossings on day 21 of the study compared with day 1 ($P < 0.05$, Holm Sidak *post hoc* test), and the higher dose (5 mg/kg) of imipramine produced the lowest observed square crossings ($P < 0.05$, Holm Sidak *post hoc* test) (Fig. 2).

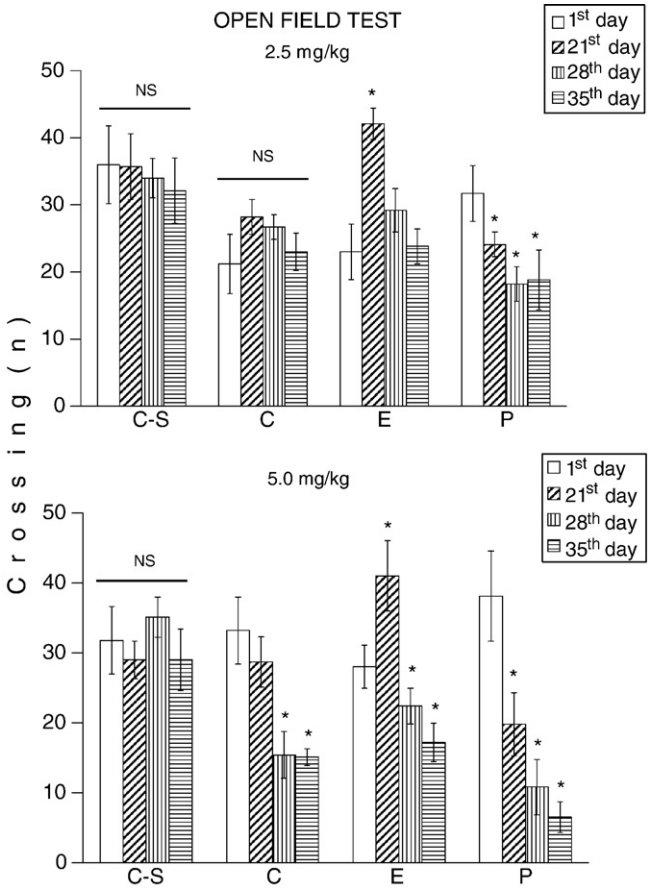

Fig. 2. Open field test. Square crossings were unchanged throughout the test in both C-S groups. The lower dose of imipramine (2.5 mg/kg) did not alter square crossings in the C-2.5 group, but the high dose of imipramine reduced square crossings in the C-5.0 group. Both the E-2.5 and E-5.0 groups displayed the highest number of square crossings on day 21 of the study. Only the 5.0 mg/kg dose of imipramine restored square crossings values to those observed on day 1 of the study. In contrast, in the P-2.5 and P-5.0 groups, square crossings decreased on day 21 of the study, and the two tested doses of imipramine reduced square crossings further. *$P < 0.05$, compared with day 1 of the study for each group. $^{+}P < 0.05$, compared with day 21 of the study (before treatments) (Holm Sidak *post hoc* test).

3.2. Forced swim test

3.2.1. Imipramine 2.5 mg/kg

Analysis of total immobility time by two-way, repeated-measures ANOVA revealed significant main effects of group ($F_{(3,54)} = 3.242$, $P < 0.04$). Compared with the C-S_1 group (37.3 ± 3.65 s), the lowest immobility times occurred in the C-2.5 group (27.0 ± 3.22 s; $P < 0.05$, Holm Sidak *post hoc* test), and the highest immobility times occurred in the E-2.5 group (48.8 ± 5.70 s, $P < 0.05$), followed by the P-2.5 group (61.0 ± 9.57 s; $P < 0.05$).

A significant main effect of days of study also was observed ($F_{(3,54)}$=3.423, P<0.04). Compared with day 1 of the study (39.3±4.12 s), the highest immobility times were observed on day 21 of the study (58.7±9.35 s; P<0.05, Holm Sidak *post hoc* test). Immobility time decreased on day 28 of the study (42.4±5.71 s) and decreased further on day 35 (33.7±4.67 s, P<0.05) to values that were comparable to day 1 of the study.

A significant group×days of study interaction was observed ($F_{(9,54)}$=3.162, P<0.004), although immobility time was not significantly different in the C-S_1 group (non-stressed, saline-treated). The C-2.5 group displayed immobility times that were similar to the C-S_1 group on days 1 and 21 of the study. When subjected to the low imipramine dose (2.5 mg/kg), immobility time in the C-2.5 group decreased during treatment (Fig. 3), but the effect was not significant. Immobility times increased in the E-2.5 group on day 21 of the study (P<0.05, Holm Sidak *post hoc* test), and imipramine treatment returned immobility times to baseline values only on day 35 of the study, with no significant changes prior to day 35. Similarly, immobility times increased in the P-2.5 group on day 21 of the study when animals were subjected to physical stress (P<0.05), and 7-day imipramine treatment restored immobility times to baseline values on day 28 of the study.

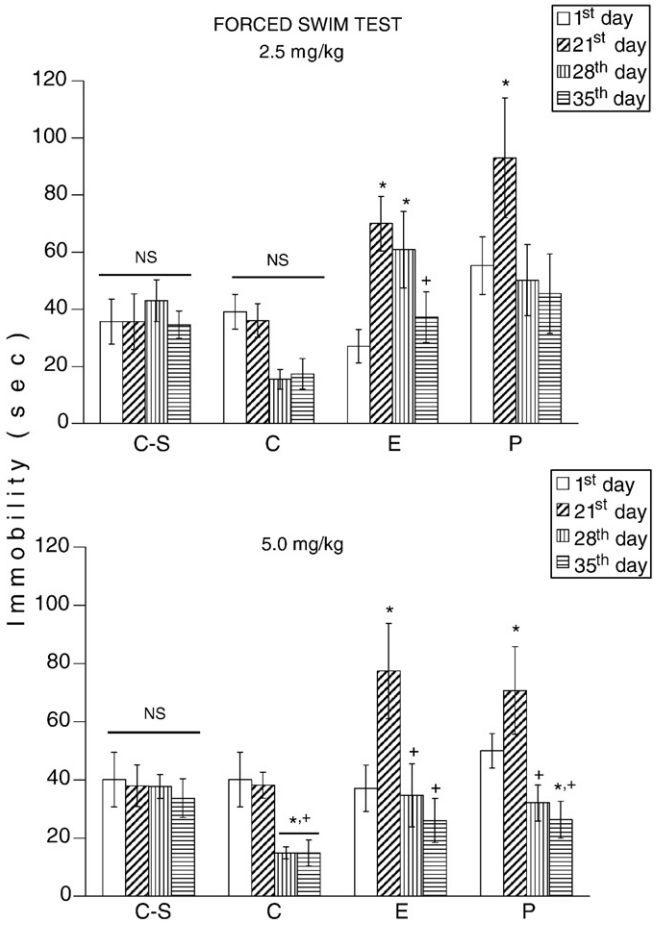

Fig. 3. Forced swim test. Immobility time remained unchanged throughout the test in both C-S groups. The high dose of imipramine (5.0 mg/kg) significantly reduced immobility time in the C-5.0 group. The highest immobility times were observed in emotionally stressed and physically stressed groups on day 21 of the study (before treatments). In the E-2.5 group treated with 2.5 mg/kg imipramine, immobility time was reduced only at the second week of treatment (day 35 of the study). A similar reduction was observed in the P-2.5 group after the 7-day treatment. The low dose of imipramine (2.5 mg/kg) restored immobility times in the P-2.5 group during the first week of treatment (day 28 of the study) to values observed on day 1 of the study. The high dose of imipramine (5.0 mg/kg) reduced immobility times at the first week of treatment to values that were lower than on the first day of treatment. *P<0.05, compared with the first day of the study for each group. +P<0.05, compared with day 21 of the study (before treatments) (Holm Sidak *post hoc* test).

3.2.2. Imipramine 5.0 mg/kg

Two-way, repeated-measures ANOVA did not find a significant main effect of group ($F_{(3,54)}$=2.292, P=0.113). The C-S_2 group (37.4±3.37 s), C-5.0 group (27.0±3.59 s), and stressed groups (E-5.0: 43.8±6.53 s; P-5.0: 44.8±5.47 s) displayed similar immobility times.

However, the factor days of study reached statistical significance ($F_{(3,54)}$=24.281, P<0.001). Higher immobility time (56.1±6.60 s) was observed on day 21 of the study (P<0.05, Holm Sidak *post hoc* test) than on day 1 (41.8±4.02 s), day 28 (29.8±3.57 s), and day 35 (25.2±3.26 s). Overall, the lowest immobility times were observed on days 28 and 35 of the study (i.e., during saline or imipramine treatment; P<0.05).

A significant group×days of study interaction was observed ($F_{(9,54)}$=2.948, P<0.006). Similar to C-S_1 rats, the C-S_2 group did not exhibit differences in immobility time throughout the study. However, the high imipramine dose (5.0 mg/kg) significantly (P<0.05) decreased immobility time in the C-5.0 group on day 7 of treatment (day 28 of the study), with no further changes occurring. The highest immobility times were observed in the E-5.0 and P-5.0 groups on day 21 of the study (P<0.05). Seven days of 5 mg/kg imipramine treatment (day 28 of the study) restored immobility times to levels observed on day 1 of the study in both stressed groups (Fig. 3).

4. Discussion

The aim of the present study was to verify that emotional or physical stress produces similar results in the FST (Gutiérrez-García et al., 2007) and to compare two regimens of imipramine treatment in non-stressed and saline-treated rats. The most important findings were that physical or emotional stress produced differential effects in the OFT but similar results in the FST before imipramine treatment. Twenty-one days of emotional or physical stress increased total immobility time in the FST, suggesting behavioral "despair." The two doses of imipramine (2.5 and 5.0 mg/kg) produced differential time-dependent results. Baseline values of immobility time were restored in the emotionally stressed group only at the second week of treatment with the lower imipramine dose. The high imipramine dose restored immobility time in all groups after 7 days of treatment.

We used the i.p. route of administration for saline and imipramine injections. However, when long-term drug administration in rodents employs subcutaneously implanted osmotic pumps, the release of drug solutions for extended periods of time is continuous and at controlled, stable, and constant rates over time (Bruins Slot et al., 2002a). The osmotic pump also allows for long-term drug administration without daily, or more frequent, handling of animals. To avoid such handling appears particularly relevant in animal models of chronic pain (Bruins Slot et al., 2002b), arthritis (Bervoets and Colpaert, 1984), and antidepressants (Allison et al., 1995; Cryan et al., 2005; Mitchell and Fletcher, 1993; Uys et al., 2006). However, Bruins Slot et al. (2002b) stated some concerns that need to be addressed before using osmotic pumps. First, the amount of compound that can be administered is limited by the compound's solubility in its vehicle. Second, a constant rate of drug delivery can be achieved only insofar as the drug solution remains stable at body temperature for the duration of infusion. Third, the dose administered should be pharmacologically active and sufficiently well tolerated (i.e., produce no undesirable effects). A daily i.p. injection, in contrast, guarantees stable plasma levels of any drug. This is important because our general hypothesis is precisely that antidepressants (and perhaps many other drugs) act in the long-term as a direct consequence of such stable plasma levels. Supporting this hypothesis, we have repeatedly reported that daily i.p. administration of vehicle or repeated FST does not modify immobility

time (Estrada Camarena et al., 2002; Gutiérrez-García et al., 2007; Martínez-Mota et al., 1999). Similarly, Hilakivi-Clarke (1992) reported that the FST is sensitive to stressors, but administration of antidepressant compounds or handling does not significantly influence the results. Therefore, we opted to use the i.p. route, not only because of the aforementioned reasons, but also because it is less expensive than osmotic pumps.

In the FST, the main measure is immobility time. Immobility time is reduced by the administration of clinically effective antidepressants, and the time is used as an indicator of behavioral "despair." Depression per se is impossible to determine in rodents and is a complex disease comprising many signs and symptoms. One must measure only one of the signs of depression (e.g., "despair" reflected in immobility time). Of course, immobility time may be influenced by exploratory activity, motionlessness, and restlessness. Therefore, the OFT is a useful tool for discarding any influence of motility on immobility time in the FST. This is why we (and many other researchers) employ the OFT prior to the FST, but not vice versa, given that the FST is a stressful experience that may influence the OFT. Although antidepressants typically reduce or do not affect locomotion, they effectively reduce immobility time (Alonso et al., 1991; Detke et al., 1995) or increase the latency to the first episode of immobility in the FST in non-stressed rats (Contreras et al., 1998; Espejo and Miñano, 1999). The P and E groups were subjected to different types of stressors, physical or emotional, respectively, with opposite changes in square crossings in the OFT, results that are consistent with previous reports (Kikusui et al., 2001; MacKay-Sim and Laing, 1981; Zalaquett and Thiessen, 1991). Physical stress produced by single or repeated footshocks frequently leads to freezing (Inoue et al., 1994) or reduces locomotion in the OFT for up to 4 weeks (van Dijken et al., 1991, 1992). Pijlman et al. (2003) concluded that physical stress decreases locomotion, and emotional stress (e.g., witnessing footshock sessions) increases locomotion. We recently also reported similar differential effects in response to physical or emotional stress in the OFT. Increased anxiety-like behavior, measured in the defensive burying test after a single session of emotional stress, also was observed and was attributable to a urine ketonic compound, 2-heptanone, contained in the urine of physically stressed rats (Gutiérrez-García et al., 2006, Pohorecky et al., 2008). The present study demonstrated that a low dose of imipramine restored baseline values of square crossings in the E group and reduced square crossings more in the P group. High-dose imipramine (5 mg/kg) reduced square crossings from the first week of treatment (day 28 of the study) in all groups to below baseline values. Thus, a low dose of imipramine was sufficient for restoring square crossings after emotional stress, but not after physical stress.

In the CS_1 and CS_2 groups, immobility time in the FST varied nonsignificantly during the 35 days of the study, with no effect of repeated testing. We applied the OFT and FST once every 7 days in all groups. Notably, although the control group (i.e., devoid of stress, injections) was only subjected to the OFT and FST, our results consistently indicated that test repetition did not modify the results, and immobility times remained consistent throughout the 5 weeks of once-per-week OFT followed by FST. Both physically and emotionally stressed rats displayed approximately twice the immobility time observed in the control groups on day 21 of the study prior to initiating drug treatments. The C-2.5 and C-5.0 groups appeared to be more sensitive to imipramine because immobility time decreased from the first week of treatment, particularly in response to the high dose of imipramine, and the low dose of imipramine reduced immobility time in the E group at the second week of treatment. Therefore, the consequence of emotional stress in the FST appears to be more resistant to the effects of imipramine compared with physical stress.

Imipramine at an acute dose of 10 mg/kg administered two or three times reduces immobility time in control non-stressed rats (Barros and Ferigolo, 1998; Kulkarni and Dhir, 2007; Kúsmider et al., 2006; Porsolt et al., 1978; Takamori et al., 2001). However, in our study we compared results from non-stressed rats with physically or emotionally stressed rats and analyzed the data using a two-way ANOVA. Despite a clear reduction of immobility time in the control group, the effect did not reach statistical significance at the imipramine dose of 2.5 mg/kg. Imipramine differentially restored baseline levels of immobility in both stressed groups depending on dose and time. A dose of 2.5 mg/kg restored immobility time to baseline values after 2 weeks of treatment, and a dose of 5.0 mg/kg restored immobility time to baseline values after 1 week of treatment. The anti-immobility effect of imipramine in the control non-stressed group appeared to be masked by the effects of imipramine in the stressed groups. Therefore, a reliable model for studying antidepressants may be the use of long-term observations and long-term treatments (Kúsmider et al., 2006) and comparison of the effects of antidepressants in stressed and non-stressed animals. These comparisons may thus yield results that are comparable to the clinic.

An acute session of stress produces differential effects on cerebral functioning compared with repeated sessions. For example, a single session of emotional stress increases serotonin (5-hydroxytryptamine, 5-HT) release in the amygdala and prefrontal cortex (Kawahara et al., 1993), and a single footshock session produces a marked increase in extracellular norepinephrine concentrations in the prefrontal cortex (Dazzi et al., 2002). Nearly any acute session of stress increases corticosterone secretion (Ishikawa et al., 1992; Mercier et al., 2003). In the C-2.5 and C-5.0 groups, imipramine may have acted via adaptive changes produced by the FST-generated stress that caused many functional changes, including reduced neuronal firing rate in lateral septal neurons (Contreras et al., 2004), long-lasting decreased extracellular levels of 5-HT in lateral septum and amygdala neurons that receive fibers from the dorsal raphe nucleus (Kirby et al., 1995), increased 5-HT release in the hippocampus, corpus striatum, amygdala, and prefrontal cortex (Rueter and Jacobs, 1996), and increased 5-HT turnover in the frontal cortex and amygdala (Connor et al., 2000; Dazzi et al., 2002).

Numerous studies using both electrophysiological and biochemical techniques have confirmed that imipramine, similarly to other antidepressants, exerts differential long-term actions compared with short-term effects (Blier and de Montigny, 1983; Cryan et al., 2005; Detke et al., 1997). For example, long-term but not acute administration of imipramine (20 mg/kg, 14 consecutive daily doses) normalized olfactory bulbectomy-induced hyperactivity in rats 10 weeks after treatment had ceased (Breuer et al., 2007). Additionally, an acute action of imipramine consists of an increase in the sensitivity of 5-HT-mediated responses, and long-term imipramine decreases the responsiveness of β-adrenoceptors without changing the responses mediated by 5-HT receptors in the hippocampus (Beck and Halloran, 1989). As a result, the effects of imipramine and possibly other antidepressants may differ depending on the type and duration of stress. In the present study, the groups that were subjected to emotional stress were more resistant to the low dose of imipramine. Immobility time in these animals was restored only after the second week of imipramine treatment.

Emotional stress produces acute mild enhancement of norepinephrine turnover in the hypothalamus and amygdala, an enhancement that increases with repeated exposure to stress (Iimori et al., 1982), and repeated FST sessions increase the number of frontal cortical $5\text{-}HT_{2A}$ receptors (Kitamura et al., 2004). Both effects on neurotransmission are reversed by imipramine treatment (Kitamura et al., 2004; Ossowska et al., 2002). Additionally, long-term stress produces other changes, including downregulation of the secretory peptide brain-derived neurotrophic factor and decreased expression of the transcriptional regulator calcium/cyclic adenosine monophosphate response-element binding protein, specifically within the dentate gyrus (Greisen et al., 2005; Gronli et al., 2006; Tafet and Bernardini, 2003). Long-term stress also produces retraction of apical

dendrites of pyramidal neurons (Magarinos and McEwen, 1995; Magarinos et al., 1996) and reorganization of synaptic structures at mossy fiber terminals (Magarinos et al., 1997). Significant differences exist when subjecting an experimental animal to the action of any antidepressant, effects that depend on previous stress exposure (Dal-Zotto et al., 2000; Drago et al., 2001). Thus, in long-term emotionally or physically stressed animals, imipramine may act on systems that are functionally different from a non-stressed subject.

We did not measure plasma corticosterone levels in the present study. Nearly all stressful situations increase plasma corticosterone, but individual differences may occur in the integration of the response to stress depending on the intensity of the stressor (Pitman et al., 1988). Additionally, repeated exposure to the same stressor does not always result in a reduction of the adrenocorticotropic response (Martí and Armario, 1998). Isolation (Gavrilovic and Dronjak, 2005) and inescapable footshock (Itoh et al., 2003) increase plasma corticosterone. However, repeated FST does not produce changes (Gavrilovic and Dronjak, 2005) or only slightly modifies the corticosterone response (Dal-Zotto et al., 2000). In experimental designs similar to those used in the present study, plasma corticosterone levels increased in rats subjected to sensory stimulation (e.g., sounds, vocalizations, and odors) that originate from a rat subjected to stress (Ishikawa et al., 1992). Therefore, although we did not measure plasma corticosterone directly, corticosterone levels may be assumed to increase based on previous studies in which exposure to emotional or physical stress increased plasma corticosterone levels (Blandino et al., 2006; Ishikawa et al., 1992). Furthermore, corticosterone secretion is well known to follow an ultradian rhythm (Haller et al., 2000), with the highest plasma cortisol levels occurring early in the morning in humans (Cutolo et al., 2006) and other mammals (Kalsbeek et al., 2008) before awakening, an effect that is rarely accompanied by signs of anxiety. Plasma corticosterone or cortisol, therefore, may peak to prepare an individual for the day or in response to an emergency (Wüst et al., 2000), and cortisol is well known as an anti-inflammatory endocrine hormone.

Borsini et al. (1989) consider that a prior stress experience provides useful information for understanding the effects of therapeutic antidepressants. In the present study, we demonstrated that long-term emotional or physical stress similarly increased immobility time in the FST but also elicited some resistance to the effects of imipramine, representing a model that may more closely reflect clinical observations.

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References

Allison K, Ivanová S, Greenshaw AJ. Behavioural response to SKF 38393 and quinpirole following chronic antidepressant treatment. Eur J Pharmacol 1995;277:139–44.
Alonso SJ, Castellano MA, Alfonso D, Rodríguez M. Sex differences in behavioral despair: relationships between behavioral despair and open field activity. Physiol Behav 1991;49:69–72.
Antelman SM, Gershon S. Clinical application of time-dependent sensitization to antidepressant therapy. Prog Neuropsychopharmacol Biol Psychiatry 1998;22:65–78.
Antelman SM, Levine J, Gershon S. Time-dependent sensitization: the odyssey of a scientific heresy from laboratory to the door of the clinic. Mol Psychiatry 2000;5:350–6.
Barros HM, Ferigolo M. Ethopharmacology of imipramine in the forced-swimming test: gender differences. Neurosci Biobehav Rev 1998;23:279–86.
Beck SG, Halloran PM. Imipramine alters β-adrenergic, but not serotonergic, mediated responses in rat hippocampal pyramidal cells. Brain Res 1989;504:72–81.
Bervoets K, Colpaert FC. Respiratory effects of intrathecal capsaicin in arthritic and non-arthritic rats. Life Sci 1984;34:2477–83.
Blandino Jr P, Barnum CJ, Deak T. The involvement of norepinephrine and microglia in hypothalamic and splenic IL-1β responses to stress. J Neuroimmunol 2006;173:87–95.
Blier P, de Montigny C. Electrophysiological investigations of the effect of repeated zimelidine administration on serotonergic neurotransmission in the rat. J Neurosci 1983;3:1270–8.
Borsini F, Lecci A, Sessarego A, Frassine R, Meli A. Discovery of antidepressant activity by forced swimming test may depend on pre-exposure of rats to a stressful situation. Psychopharmacology (Berl) 1989;97:183–8.
Borsini F, Pulvirenti L, Samanin R. Evidence of dopamine involvement in the effect of repeated treatment with various antidepressants in the behavioural "despair" test in rats. Eur J Pharmacol 1985;110:253–6.
Breuer ME, Groenink L, Oosting RS, Westenberg HGM, Olivier B. Long-term behavioral changes after cessation of chronic antidepressant treatment in olfactory bulbectomized rats. Biol Psychiatry 2007;61:990–5.
Briones-Aranda A, Rocha L, Picazo O. Alterations in GABAergic function following forced swimming stress. Pharmacol Biochem Behav 2005;80:463–70.
Bruins Slot LA, Pauwels PJ, Colpaert FC. Sign-reversal during persistent activation in μ-opioid signal transduction. J Theor Biol 2002a;215:169–82.
Bruins Slot LA, Tarayre JP, Koek W, Ribet JP, Colpaert FC. Experimental conditions for the continuous subcutaneous infusion of four central analgesics in rats. Pharmacol Biochem Behav 2002b;72:943–51.
Connor TJ, Kelliher P, Shen Y, Harkin A, Kelly JP, Leonard BE. Effect of subchronic antidepressant treatments on behavioral, neurochemical, and endocrine changes in the forced swim test. Pharmacol Biochem Behav 2000;65:591–7.
Contreras CM, Chacón L, Rodríguez-Landa JF, Bernal-Morales B, Gutiérrez-García AG, Saavedra M. Spontaneous firing rate lateral septal neurons decreases after forced swimming test in Wistar rat. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:343–8.
Contreras CM, Martínez-Mota L, Saavedra M. Desipramine restricts estral cycle oscillations in swimming. Prog Neuropsychopharmacol Biol Psychiatry 1998;22:1121–8.
Contreras CM, Marván ML, Alcalá-Herrera V, Guzmán-Sáenz MA. Chronic clomipramine increases firing rate in the lateral septal nuclei of the rat. Physiol Behav 1990;48:551–4.
Contreras CM, Rodríguez-Landa JF, Gutiérrez-García AG, Bernal-Morales B. The lowest effective dose of fluoxetine in the forced swim test significantly affects the firing rate of lateral septal nucleus neurones in the rat. J Psychopharmacol 2001;15:231–6.
Cryan JF, Page ME, Lucki I. Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. Psychopharmacology (Berl) 2005;182:335–44.
Cutolo M, Sulli A, Pizzorni C, Secchi ME, Soldano S, Seriolo B, et al. Circadian rhythms: glucocorticoids and arthritis. Ann N Y Acad Sci 2006;1069:289–99.
Dal-Zotto S, Martí O, Armario A. Influence of single or repeated experience of rats with forced swimming on behavioural and physiological responses to the stressor. Behav Brain Res 2000;114:175–81.
Dazzi L, Ladu S, Spiga F, Vacca G, Rivano A, Pira L, et al. Chronic treatment with imipramine or mirtazapine antagonizes stress- and FG7142-induced increase in cortical norepinephrine output in freely moving rats. Synapse 2002;43:70–7.
Detke MJ, Johnson J, Lucki I. Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. Exp Clin Psychopharmacol 1997;5:107–12.
Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl) 1995;121:66–72.
Drago F, Nicolosi A, Micale V, Lo Menzo G. Placebo affects the performance of rats in models of depression: is it a good control for behavioral experiments? Eur Neuropsychopharmacol 2001;11:209–13.
Elhwuegi AS. Central monoamines and their role in major depression. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:435–51.
Espejo EF, Miñano FJ. Prefrontocortical dopamine depletion induces antidepressant-like effects in rats and alters the profile of desipramine during Porsolt's test. Neuroscience 1999;88:609–15.
Estrada Camarena E, Contreras CM, Saavedra M, Luna-Baltazar I, López-Rubalcava C. Participation of the lateral septal nuclei (LSN) in the antidepressant-like actions of progesterone in the forced swimming test (FST). Behav Brain Res 2002;134:175–83.
Gavrilovic L, Dronjak S. Activation of rat pituitary-adrenocortical and sympatho-adrenomedullary system in response to different stressors. Neuro Endocrinol Lett 2005;26:515–20.
Greisen MH, Altar CA, Bolwig TG, Whitehead R, Wörtwein G. Increased adult hippocampal brain-derived neurotrophic factor and normal levels of neurogenesis in maternal separation rats. J Neurosci Res 2005;79:772–8.
Gronli J, Bramham C, Murison R, Kanhema T, Fiske E, Bjorvatn B, et al. Chronic mild stress inhibits BDNF protein expression and CREB activation in the dentate gyrus but not in the hippocampus proper. Pharmacol Biochem Behav 2006;85:842–9.
Gutiérrez-García AG, Contreras CM, Mendoza-López MR, Cruz-Sánchez S, García-Barradas O, Rodríguez-Landa JF, et al. A single session of emotional stress produces anxiety in Wistar rats. Behav Brain Res 2006;167:30–5.
Gutiérrez-García AG, Contreras CM, Mendoza-López MR, García-Barradas O, Cruz-Sánchez JS. Urine from stressed rats increases immobility in receptor rats forced to swim: role of 2-heptanone. Physiol Behav 2007;91:166–72.
Haller J, Halasz J, Mikics E, Kruk MR, Makara GB. Ultradian corticosterone rhythm and the propensity to behave aggressively in male rats. J Neuroendocrinol 2000;12:937–40.
Hilakivi-Clarke LA. Injection of vehicle is not a stressor in Porsolt's swim test. Pharmacol Biochem Behav 1992;42:193–6.
Iimori K, Tanaka M, Kohno Y, Ida Y, Nakagawa R, Hoaki Y, et al. Psychological stress enhances noradrenaline turnover in specific brain regions in rats. Pharmacol Biochem Behav 1982;16:637–40.
Inoue T, Tsuchiya K, Koyama T. Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. Pharmacol Biochem Behav 1994;49:911–20.
Ishikawa M, Hara C, Ohdo S, Ogawa N. Plasma corticosterone response of rats with sociopsychological stress in the communication box. Physiol Behav 1992;52:475–80.

Itoh T, Abe K, Tokumura M, Horiuchi M, Inoue O, Ibii N. Different regulation of adenylyl cyclase and rolipram-sensitive phosphodiesterase activity on the frontal cortex and hippocampus in learned helplessness rats. Brain Res 2003;991:142–9.
Kalsbeek A, Verhagen LA, Schalij I, Foppen E, Saboureau M, Bothorel B, et al. Opposite actions of hypothalamic vasopressin on circadian corticosterone rhythm in nocturnal versus diurnal species. Eur J Neurosci 2008;27:818–27.
Kawahara H, Yoshida M, Yokoo H, Nishi M, Tanaka M. Psychological stress increases serotonin release in the rat amygdala and prefrontal cortex assessed by in vivo microdialysis. Neurosci Lett 1993;162:81–4.
Kelliher P, Kelly JP, Leonard BE, Sánchez C. Effects of acute and chronic administration of selective monoamine re-uptake inhibitors in the rat forced swim test. Psychoneuroendocrinology 2003;28:332–47.
Kikusui T, Takigami S, Takeuchi Y, Mori Y. Alarm pheromone enhances stress-induced hyperthermia in rats. Physiol Behav 2001;72:45–50.
Kirby LG, Allen AR, Lucki I. Regional differences in the effects of forced swimming on extracellular levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. Brain Res 1995;682:189–96.
Kitada Y, Miyauchi T, Satoh A, Satoh S. Effects of antidepressants in the rat forced swimming test. Eur J Pharmacol 1981;72:145–52.
Kitamura Y, Araki H, Nagatani T, Takao K, Shibata K, Gomita Y. Influence of imipramine on the duration of immobility in chronic forced-swim-stressed rats. Acta Med Okayama 2004;58:271–4.
Kulkarni SK, Dhir A. Effect of various classes of antidepressants in behavioral paradigms of despair. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:1248–54.
Kúsmider M, Faron-Górecka A, Dziedzicka-Wasylewska M. Delayed effects of antidepressant drugs in rats. Behav Pharmacol 2006;17:641–9.
Lucki I. The forced swimming test as a model for care and component behavioral effects of antidepressant drugs. Behav Pharmacol 1997;8:523–32.
MacKay-Sim A, Laing DG. The sources of odors from stressed rats. Physiol Behav 1981;27:511–3.
Magarinos AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors. Neuroscience 1995;69:83–8.
Magarinos AM, McEwen BS, Flügge G, Fuchs E. Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. J Neurosci 1996;16:3534–40.
Magarinos AM, Verdugo JM, McEwen BS. Chronic stress alters synaptic terminal structure in hippocampus. Proc Natl Acad Sci USA 1997;94:14002–8.
Martí O, Armario A. Anterior pituitary response to stress: time-related changes and adaptation. Int J Dev Neurosci 1998;16:241–60.
Martínez-Mota L, Contreras CM, Saavedra M. Progesterone reduces immobility in rats forced to swim. Arch Med Res 1999;30:286–9.
Martínez-Mota L, Cruz-Martínez JJ, Márquez-Baltazar S, Fernández-Guasti A. Estrogens participate in the antidepressant-like effect of desipramine and fluoxetine in male rats. Pharmacol Biochem Behav 2008;88:332–40.
Mercier S, Canini F, Buguet A, Cespuglio R, Martin S, Bourdon L. Behavioural changes after an acute stress: stressor and test types influences. Behav Brain Res 2003;139:167–75.
Mitchell PJ, Fletcher A. Venlafaxine exhibits pre-clinical antidepressant activity in the resident-intruder social interaction paradigm. Neuropharmacology 1993;32:1001–9.
NIH. Guide for the Care and Use of Laboratory Animals. Publication 85-23. Washington DC: National Academy Press; revised 1985.
Ossowska G, Nowak G, Klenk-Majewska B, Danilczuk Z, Zebrowska-Lupina I. Effect of imipramine on brain D-1 and 5-HT-2A receptors in a chronic unpredictable stress model in rats. Pol J Pharmacol 2002;54:89–93.
Pijlman FT, Herremans AH, van de Kieft J, Kruse CG, van Ree JM. Behavioural changes after different stress paradigms: prepulse inhibition increased after physical, but not emotional stress. Eur Neuropsychopharmacol 2003;3:369–80.
Pitman DL, Ottenweller JE, Natelson BH. Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: chronic stress and habituation. Physiol Behav 1988;43:47–55.
Pohorecky LA, Blakley GG, Ma EW, Soini HA, Wiesler D, Bruce KE, et al. Social housing influences the composition of volatile compounds in the preputial glands of male rats. Horm Behav 2008;53:536–45.
Porsolt RD, Bertin A, Jalfre M. "Behavioural despair" in rats and mice: strain differences and the effects of imipramine. Eur J Pharmacol 1978;51:291–4.
Reid IC, Stewart CA. Brain plasticity and antidepressant treatments: new cells, new connections. Neurotox Res 2004;6:483–9.
Rénéric JP, Bouvard M, Stinus L. In the rat forced swimming test, chronic but not subacute administration of dual 5-HT/NA antidepressant treatments may produce greater effects than selective drugs. Behav Brain Res 2002;136:521–32.
Rueter LE, Jacobs BL. A microdialysis examination of serotonin release in the rat forebrain induced by behavioral/environmental manipulations. Brain Res 1996;739:57–69.
Rygula R, Abumaria N, Flugge F, Fuchs E, Ruther E, Havemann-Reinecke U. Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav Brain Res 2005;162:127–34.
Tafet GE, Bernardini R. Psychoneuroendocrinological links between chronic stress and depression. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:893–903.
Takamori K, Yoshida S, Okuyama S. Effect of ACTH on the imipramine- and desipramine-induced decrease in duration of immobility time as measured in a rat forced swimming test. Life Sci 2001;69:1891–6.
Uys JD, Muller CJ, Marais L, Harvey BH, Stein DJ, Daniela WM. Early life trauma decreases glucocorticoid receptors in rat dentate gyrus upon adult re-stress: reversal by escitalopram. Neuroscience 2006;137:619–25.
van den Berg CL, Lamberts RR, Wolterink G, Wiegant VM, van Ree JM. Emotional and footshock stimuli induce differential long-lasting behavioural effects in rats: involvement of opiods. Brain Res 1998;799:6-15.
van Dijken HH, Van der Hyeden JA, Mos J, Tilders FJ. Long-lasting behavioural changes after a single footshock stress session: a model of depression? In: Olivier B, Mos J, Slangen JL, editors. Animal models in psychopharmacology. Boston: Birkhäuser; 1991. p. 231–6.
van Dijken HH, Van der Heyden JA, Mos J, Tilders FJ. Inescapable footshocks induce progressive and long-lasting behavioural changes in male rats. Physiol Behav 1992;51:787–94.
Weiss JM, Goodman PA, Losito BG, Corrigan S, Charry JM, Bailey WH. Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. Brain Res Rev 1981;3:167–205.
Wieland S, Lucki I. Antidepressant-like activity of 5-HT_{1A} agonists measured with the forced swim test. Psychopharmacology (Berl) 1990;101:497–504.
Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 1987;93:358–64.
Wüst S, Wolf J, Hellhammer DH, Federenko I, Schommer N, Kirschbaum C. The cortisol awakening response: normal values and confounds. Noise Health 2000;2:79–88.
Yates G, Panksepp J, Ikemoto S, Nelson E, Conner R. Social isolation effects on the "behavioral despair" forced-swimming test: effect of age and duration of testing. Physiol Behav 1991;49:347–53.
Zalaquett C, Thiessen D. The effects of odors from stressed mice on conspecific behavior. Physiol Behav 1991;50:221–7.