

## Antidepressant behavioral effects of duloxetine and amitriptyline in the rat forced swimming test<sup>1</sup>

Efeitos antidepressivos da duloxetina e da amitriptilina no teste do nado forçado em ratos

Honório Sampaio Menezes<sup>1</sup>, Bárbara Beatriz Moreira Bueno<sup>II</sup>, Leandro Ciulla<sup>II</sup>, Alexandre Schuh<sup>II</sup>, Fernanda de Freitas Luz<sup>II</sup>, Rafael José Vargas Alves<sup>III</sup>, Milena Pacheco Abegg<sup>III</sup>, Sílvia Leticia Merceo Bacchi Cirino<sup>III</sup>

<sup>1</sup> PhD, Assistant Professor, Cardiology University Foundation (IC/FUC), Porto Alegre and Lutheran University of Brazil (ULBRA), Canoas, RS, Brazil.

<sup>II</sup> Researcher of Experimental Medicine Group, ULBRA, Canoas, RS, Brazil.

<sup>III</sup> Graduate student, Experimental Medicine Group, ULBRA, Canoas, RS, Brazil.

### ABSTRACT

**Purpose:** To compare the effects of the antidepressant drugs duloxetine and amitriptyline on depressive behaviors in rats. **Methods:** Fifteen male Wistar rats were given systemic injections of duloxetine, amitriptyline or saline prior to a Forced Swimming Test (FST). Immobility and number of stops were measured. Data were analyzed by one-way ANOVA and Kruskal-Wallis. **Results:** Rats given injections of duloxetine displayed fewer stops than the amitriptyline and control group ( $p < 0.05$ ). The control group and Amitriptyline showed no difference ( $p=0.8$ ). **Conclusion:** Duloxetine reduced depressive behaviors in the Forced Swimming Test been more effective than amitriptyline.

**Key words:** Physiological Effects of Drugs. Depression. Animal Experimentation. Rats.

### RESUMO

**Objetivo:** Comparar o efeito antidepressivo da droga cloridrato de duloxetina com a amitriptilina. **Métodos:** O teste do nado forçado, teste comportamental que avalia a atividade antidepressiva em ratos, foi utilizado em 15 ratos Wistar, machos adultos, divididos em três grupos iguais: duloxetina, amitriptilina e controle. Os dados foram analisados pelo teste One-way ANOVA e Kruskal-Wallis. **Resultados:** Houve diferença significativa entre o número de paradas ( $p < 0,05$ ) entre os grupos duloxetina e amitriptilina e o grupo controle. Grupo amitriptilina e controle não apresentaram diferença ( $p=0,8$ ). **Conclusão:** A duloxetina reduziu o comportamento depressivo sendo mais efetiva do que a amitriptilina.

**Descritores:** Efeitos Fisiológicos de Drogas. Depressão. Experimentação Animal. Ratos.

<sup>1</sup>Research performed at Experimental Animal Cardiology University Foundation (IC/FUC), Porto Alegre and Lutheran University of Brazil (ULBRA), Canoas, RS, Brazil.

### Introduction

The distinction between the normal depression feeling and the pathology that requires medical intervention is extremely hard to those not specialized in mental health<sup>1</sup>. Besides the traditional psychiatric evaluation, a safe diagnosis of depression must comprise an assessment of physical symptoms as well, once the patients may not emphasize their emotional symptoms, tending to limit their complaints to back, neck or shoulder pain. In fact, it was observed that 69% of the patients suffering from depression reported the presence of physical symptoms as their main complaint<sup>2</sup>.

Depression has been described as normal alterations in humor as well as a group of specific disturbances. Sadness and unhappy feelings are common in situations where one faces loss, separation, failure, or interpersonal conflicts. The biological etiology of depression is hypothesized to be due to a deficiency in

monoaminergic neurotransmitters, and more specifically, deficiencies in norepinephrine (NE) and serotonin (5-hidroxitriptamina; 5-HT). The Diagnostic and Statistical Manual of Mental Disorders<sup>1</sup> describes the emotional symptoms of depression as sadness, lack of interest, guiltiness, and suicidal thoughts. Although this definition emphasizes the presence of emotional symptoms, physical symptoms are also recognized as a key component of the depressive syndrome. These physical symptoms include a lack of energy, sleep disorders, pain, headaches, changes in appetite, gastrointestinal disorders, and changes in psychomotor function. Depressed patients visit doctors and hospitals more frequently than the non-depressed population<sup>3</sup> and have more limited activity levels<sup>4</sup>. Because the emotional symptoms of depression are more easily recognized, the physical symptoms are often underestimated, which makes a proper diagnosis and prognosis more difficult<sup>5</sup>. Antidepressant drugs that potently inhibit 5-HT/NE reuptake, such as duloxetine (Cloridrato de (+)-(S)-

N-metil-y-(1-naftanelinoxi)-2-tiofenopropanamine, formula: C<sub>18</sub>H<sub>19</sub>NOSHCl<sup>5</sup>, have been developed to treat major depression, especially in patients with pain and urinary incontinence. Duloxetine weakly inhibits the reuptake of dopamine and does not have a significant affinity for muscarinic, histamine, beta-adrenergic, dopaminergic, serotonergic, or opioid receptors. Duloxetine Cloridrate is not chemically related to other selective inhibitors of serotonin and NA reuptake, tricyclics, tetracyclics, or other available drugs prescribed for the treatment of major depression disorder<sup>5</sup>. In animals, duloxetine lowers brain 5-HT levels via chloroamphetamine and hypothalamic NE and epinephrine depletion<sup>6</sup>. Using in vitro and in vivo analyses, Kasamo *et al.*<sup>7</sup> showed that duloxetine is a double inhibitor of 5-HT and NE reuptake. Moreover, the results of various experiments indicate that duloxetine has preferential effects on the 5-HT transporter. Antidepressants that inhibit the reuptake of 5-HT and NE, such as duloxetine, have fewer reported side effects than tricyclic antidepressants, and thus are ideal for the treatment of chronic pain associated with depression<sup>8</sup>. Iyengar *et al.*<sup>9</sup> recently found duloxetine to be more effective than gabapentine, an antiepileptic drug, in the treatment of neuropathic pain. In addition, Rénéric<sup>10</sup> demonstrated that fluoxetine, or compounds with mixed activity for inhibiting NE and 5-HT reuptake, has effects similar to those of desipramine alone and may have serotonergic antidepressant behavioral effects due to the selective reuptake of NE inhibitors. The forced swimming test (FST) is a behavioral task used in rodents that predicts the efficacy of antidepressant treatments in humans. In this task, the rodents are placed in a tank of deep water for an extended period of time, and the length of time the rodent remains immobile, making only those movements necessary to keep its head above water, is measured<sup>11</sup>. This immobilization represents a state of desperation in the rodents, which is a symptom seen in depression. Antidepressant drug treatment reduces this immobility time, and thus results in increased activity, which is reflective of the efficacy of the antidepressant drug<sup>11</sup>. This test is relatively selective for antidepressant drugs, as few other psychoactive drugs elicit effects in the FST. One study, in which fluoxetine was administered with either desipramine or bupropion (to enhance either both 5-HT and NE or dopamine transmission) indicated that these drug combinations could simultaneously increase two distinctive active behaviors in the FST. However, the overall behavioral pattern of the duloxetine versus imipramine alone was not tested yet.

The purpose of the present study was to compare the behavioral effects of the antidepressant drugs duloxetine and amitriptyline on the antidepressive behaviors of Wistar rats submitted to the FST.

## Methods

This is an experimental study, prospective essay, placebo controlled. Fifteen male Wistar rats, weighing between 200 and 300 g, were housed in groups of five in polycarbonate cages. They were maintained on a 12-h light-dark cycle in a temperature-controlled (22°C) colony room and had free access to food and water. The experiments were performed according to the Guide for the care and use of laboratory animals, and the Ethics Committee for Experiments on Animals approved all procedures.

## Drug treatment

The rats were divided into three groups of five: Amitriptyline group, Duloxetine group, and Control group. All injections were given intraperitoneally at 24, 5, and 1 h before the SFT. The Duloxetine HCl (Lilly Co.) group received 40 mg/kg duloxetine, dissolved in sterile water 1 mL/kg. The amitriptyline group received 2.5 mg/kg amitriptyline. The Control group received 0.25 ml of saline solution.

## Forced swimming test

The procedure used has been previously described by Porsolt *et al.*<sup>11</sup>; Porsolt *et al.*<sup>12</sup>; Porsolt<sup>13</sup>, Detke<sup>14</sup>, and Rénéric<sup>10</sup>. The animals were forced to swim inside a cylinder filled with water, without the possibility of escaping. The resulting anxiety produces vigorous swimming activity and attempts at escaping by diving or climbing the walls of the cylinder. When the animals ceased all movements except those necessary for survival (keeping the head above the water), the behavior was considered to be immobile. This was classified as induced depression. On the first day (pre-session test) the rats were forced to swim for 15 min in an acrylic cylinder (29 cm diameter x 50 cm tall), containing 25°C water 40 cm deep. The water was changed after every swimming test to eliminate urine, excrement, and fur. After the swimming session, the rats were removed from the cylinder, dried with towels, and placed under a light bulb (32°C) for 15-30 min. They then received the first dose of their respective drug and were returned to their home cage. Twenty-four h later, the rats were tested under the same conditions for 5 min (test session). They received their second and third doses of their respective drugs at 5 and 1 h prior to the test session. Immobility was measured (sec) as well as the number of times they stopped swimming (number of stops). Immobility referred to the absence of movement, with the body inclined forward, passively floating, and the paws immobile. The pretest and test sessions were videotaped (Panasonic video color camera and recorder) for scoring the movements and behavior of the animals.

## Statistical analysis

Behavioral scores of the duloxetine, amitriptyline and control groups were compared using a one-way ANOVA and multiple comparisons were analyzed by the Tukey test. Nonparametric analyses were compared with the Kruskal-Wallis test.  $p < 0.05$  was considered statistically significant.

## Results

As shown in Table 1, there was no significant difference in weight between the Duloxetine, Amitriptyline and Control groups ( $p=0.8$ ). Duloxetine group made less number of stops than Amitriptyline and Control groups ( $p<0.01$ ). There was no difference between the Amitriptyline and Control groups in immobility and number of stops ( $p=0.08$ ).

The analysis of the immobility time showed significant statistic difference between the Control group and the Duloxetine group ( $p<0.05$ ), which was also observed between the Duloxetine group and the Amitriptyline group ( $p<0.05$ ).

TABLE 1 - Drug effects on weight and FST behavior

Groups	Duloxetine	Amitriptyline	Control	p
Weight (g)	232.8 ±20.12	245.2 ±28.40	263.8 ±11.41	0.8A
Number of stops	7.4 ±1.51*	18.4 ±6.54	19.4 ±8.56	<0.01B
Immovable time 5 min (seconds)	88.2 ±62.67 <sup>+</sup>	18.7 ±15.09	19.5 ±14.68	<0.05B

Mean ± standard deviation; A One-way ANOVA; B Kruskal-Wallis test.

## Discussion

In the present study, we used the FST to compare the effects of duloxetine and amitriptyline on antidepressant behaviors, including immobility. Antidepressant drugs, as well as electroconvulsive shocks and REM sleep deprivation, have been shown to reduce immobility. Porsolt *et al.*<sup>12</sup> found that such immobility is reflective of a low-mood state in the rats, which is sensitive to antidepressant treatment. The FST technique, described in detail by Porsolt *et al.*<sup>13</sup>, has been widely used to evaluate depressive behavior in rats, dogs<sup>15</sup>, and in children separated from their parents<sup>16</sup>. According to Harlow *et al.*<sup>17</sup>, young monkeys separated from their mothers demonstrate two very distinctive behavioral stages. The first stage consists of high levels of calmness, then screams and protests; in the second stage the monkeys cease all activity, clearly showing need, sadness, and depression. Similar behaviors were observed in the control group in the present study. Rats given Duloxetine treatment displayed significantly less stops than the control rats. Thus, the drug made them more active and less depressed which was also observed by Rénérac and Lucki<sup>10</sup>. However, the immobility of animals in the Duloxetine group, despite getting more agitated, the length of stops was higher than in the Amitriptyline and Control groups, this fact, described herein, was not found in the correlated literature. Similar studies using other antidepressant drugs with different pharmacodynamics, described by Dableh *et al.*<sup>18</sup> and by Mendes da Silva *et al.*<sup>19</sup>, reported an effect that contradicts the results of this study, once it deals with a new antidepressant drug with pharmacodynamics that is different from the drugs tested by the aforementioned authors, what allows us to believe there might be a new meaning to the findings in the present study to be elucidated in further studies.

We did find a difference between the duloxetine and amitriptyline groups in the number of stops. This finding may indicate that duloxetine is a stronger antidepressant drug than amitriptyline.

Although the amitriptyline group displayed a slightly lower number of stops than the control group, the difference was not significant. It is also likely that experimental variations or the smaller sample size contributed to the lack of an effect of amitriptyline on immobility, as there was a non-significant trend.

## Conclusion

Duloxetine (40mg/kg) is more effective than amitriptyline in reducing forced swimming test induced depressive behaviors.

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#### **Correspondence:**

Prof. Honório S. Menezes  
Instituto de Cardiologia do Rio Grande do Sul/FUC  
Unidade de Pesquisa  
Av. Princesa Isabel, 370  
90620-001 Porto Alegre – RS Brazil  
Phone/Fax: (55 51)3219-2802  
[hsmenezes@computech.com.br](mailto:hsmenezes@computech.com.br)

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