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Influence of enrichment on behavioral and neurogenic effects of antidepressants in Wistar rats submitted to repeated forced swim test

Fernanda Possamai, Juliano dos Santos, Thais Walber, Juliana C. Marcon, Tiago Souza dos Santos, Cilene Lino de Oliveira *

Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina—UFSC, Campus Trindade, Florianópolis, 88040-900 SC, Brazil

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ABSTRACT

Repeated forced swimming test (rFST) may detect gradual effects of antidepressants in adult rats. Antidepressants, as enrichment, affected behavior and neurogenesis in rats. However, the influence of enrichment on behavioral and neurogenic effects of antidepressants is unknown. Here, effects of antidepressants on rFST and hippocampal neurogenesis were investigated in rats under enriched conditions. Behaviors of male Wistar rats, housed from weaning in standard (SE) or enriched environment (EE), were registered during rFST. The rFST consisted of 15 min of swimming (pretest) followed by 5 min of swimming in the first (test), seventh (retest 1) and fourteenth (retest 2) days after pretest. One hour before the test, rats received an intraperitoneal injection of saline (1 ml/kg), fluoxetine (2.5 mg/kg) or imipramine (2.5 or 5 mg/kg). These treatments were performed daily until the day of the retest 2. After retest 2, rats were euthanized for the identification of markers for neurogenesis in the hippocampus. Fluoxetine or imipramine decreased immobility in retests 1 and 2, as compared to saline. EE abolished these differences. In EE, fluoxetine or imipramine (5 mg/kg) reduced immobility time in retest 2, as compared to the test. Independent of the housing conditions, fluoxetine and imipramine (5 mg/kg) increased the ratio of immature neurons per progenitor cell in the hippocampus. In summary, antidepressants or enrichment counteracted the high immobility in rFST. Enrichment changed the effects of antidepressants in rFST depending on the type, and the dose of a substance but failed to change neurogenesis in control or antidepressant treated-rats. Effects of antidepressants and enrichment on rFST seemed neurogenesis-independent.

1. Introduction

A process to develop new substances to treat Major Depression requires innovative translational research and more predictive animal models (e.g., Belzung, 2014). Refinement of the current animal models may be a strategy to find innovative ones (e.g., Berton et al., 2012). The use of rat forced swimming test (FST, Porsolt et al., 1978) is frequent in the literature because it is considered uncomplicated, inexpensive, reliable across laboratories, sensitive and relatively selective for detecting substances with potential activity as antidepressants. Several modifications of the FST in rats were tried in order to keep the valuable characteristics of it while overcoming some of its negative aspects (Borsini et al., 1989; Cryan et al., 2005; Dal-Zotto et al., 2000; Detke et al., 1997; Kitamura et al., 2004; Vieira et al., 2008). For example, a modified version of FST in rats (Cryan et al., 2005; Detke et al., 1995, 1997; Lucki, 1997) allowed for detection of substances such as selective serotonin reuptake inhibitors (SSRIs) that were ineffective in the classical protocol developed by Porsolt et al. (1978). In addition, modified FST in rats discriminated between the effects of SSRIs and noradrenaline reuptake inhibitors (NRIs) after subacute (Detke et al., 1995) or chronic treatment (Cryan et al., 2005; Detke et al., 1997). Subsequent modifications in FST provided conditions for the discrimination of antidepressants from substances with psychostimulant properties (such as caffeine, Vieira et al., 2008) or for detecting gradual effects of low doses of antidepressants over time (repeated FST, Mezadri et al., 2011).

Similar to the modified FST (e.g. Detke et al., 1995), the repeated FST (Mezadri et al., 2011) consisted of placing the rat into a tank filled with water for 15-min on the first experimental day (pretest) followed by a subsequent 5-min of forced swimming session 24 h later (test). In addition, the test was then repeated on the seventh (retest 1) and fourteenth (retest 2) days after the pretest (Mezadri et al., 2011). In these sessions, rats adopted a typical posture of immobility (floating in the water, making only minimal movement necessary to keep the head above water)

Abbreviations: DCX, doublecortin; DCX-ir, Doublecortin immunoreactive cell; EE, enriched environment; FST, forced swimming test; Ki-67-ir, Ki-67 immunoreactive nucleus; NRIs, noradrenaline reuptake inhibitors; NSRIs, non-selective inhibitors of monoamine reuptake; PND, postnatal day; rFST, repeated Forced swimming test; SE, standard environment; SSRIs, selective serotonin reuptake inhibitors.

* Corresponding author at: Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Campus Universitário Trindade, 88049-900 Florianopolis, SC, Brazil. Tel.: +55 48 3721 4689; fax: +55 48 3721 9672.
E-mail address: cilene@ccb.ufsc.br (C. Lino de Oliveira).

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after vigorous struggle and alternated it with climbing and swimming movements (Lino-de-Oliveira et al., 2005; Mezadri et al., 2011; Porsolt et al., 1978). Repetition shortened latency to immobility and increased endurance of immobility in the retests as compared to the test (Mezadri et al., 2011). Antidepressant treatment counteracted the effects of repetition (Gutiérrez-García and Contreras, 2009; Mezadri et al., 2011). Since the pharmacological treatment began 1 h prior the test, and was repeated daily until retest 2, there was three different opportunities for the evaluation of drug effect in the same group of rats (Gutiérrez-García and Contreras, 2009; Mezadri et al., 2011). Therefore, the within-subject analysis allowed for using a smaller number of rats compared to the standard protocols because it might detect the effects of short and long-term treatments in a single group of rats. In addition, repeated FST provided an opportunity to compare the onset of action for different treatments.

Despite the potential of repeated FST to detect new antidepressants, the mechanisms underlying antidepressant action in this test remain elusive. In other behavioral tests, hippocampal neurogenesis seemed a requirement to the effects of antidepressants (Santarelli et al., 2003). In addition, antidepressants increased hippocampal neurogenesis after long-term treatment (e.g. Keilhoff et al., 2006; Malberg et al., 2000; Pinnock et al., 2009). Therefore, the present work investigated the correlation between behavior in repeated FST and markers of proliferation and immature neurons in the hippocampus of adult rats. Hippocampal neurogenesis, as well as behavior of adult rats were regulated by changes in the environment (Bjørnebekk et al., 2006, 2008; Brenes et al., 2008; Brenes-Saenz et al., 2006; Gutiérrez-García and Contreras, 2009; Simpson et al., 2012). For that reason, in the present work pharmacological treatments were performed in rats housed in standard or enriched environment. Previously (Gutiérrez-García and Contreras, 2009; Mezadri et al., 2011), the low doses of fluoxetine or imipramine selected for this study reduced immobility in repeated FST. Factorial analysis suggested that active behaviors of rats in repeated FST could discriminate between distinct classes of antidepressants (Mezadri et al., 2011). Therefore, active behaviors were also scored in the present study.

2. Method

2.1. Animals

All rats (n = 80) used in this study were supplied by the central vivarium facilities of the Federal University of Santa Catarina, and all procedures were previously approved by the local Committee for Ethics in Animal Research (CEUA—UFSC, 158/CEUA/PRPE/2011). Male Wistar rats, all 21 days old (post-weaning, postnatal day 21, PND21) were housed 4-per-cage, under standard conditions of temperature (21 ± 1 °C), on reversed 12 h–12 h light–dark cycle (lights on at 6 p.m.) and with ad libitum access to food (Nuvital®) and water during all experimental period. Reversed light–dark cycle allows for experimentation in rat's active period (e.g. Prager et al., 2011). Behavioral experiments (injections and swimming sessions) were performed between 8 a.m. and noon (i.e. during the dark phase of the reversed light–dark cycle). During the experimental period, rats were housed in standard (SE, cages with 41 cm length × 34 cm wide × and 16 cm height) or in enriched (EE) environments. EE consisted of two different cages of plexiglass (the large one with 55.5 cm length × 36.5 cm wide × 40.3 cm height and a small one with 45.7 cm length × 28 cm wide × 32.6 cm height) connected by a PVC tube (100 mm diameter). Cages were lined with sawdust and contained several toys such as plastic tubing, small balls, clappers, rope, ramps and toilet paper tubes to shred. The food and water devices were kept in a box and the toys in another.

2.2. Drugs and injections

Fluoxetine (Sigma-Aldrich Inc., St. Louis, USA; dose 2.5 mg/kg) and imipramine (Sigma-Aldrich Inc., St. Louis, USA; dose 2.5 or 5 mg/kg) were dissolved in 0.9% NaCl solution (injected in the control group as well) and administered intraperitoneally (IP, 1 ml/kg). These substances and doses were selected based on the literature (Gutiérrez-García and Contreras, 2009; Mezadri et al., 2011).

2.3. Experimental design and forced swimming procedures

On the first two days in the laboratory (from PND21 to PND23), all rats were kept in SE for adaptation to laboratory conditions. From PND23 on, half of the rats (n = 40) was randomly assigned to the EE and transferred to the EE cages. Both groups, SE and EE, were maintained in their particular environments for 40 days, and after this time, were submitted to the repeated FST (Mezadri et al., 2011, Experimental Design at Fig. S1). On the 41st day of differential lodging, the rats (PND64) were exposed to the pretest session of forced swimming (15 min, in the first experimental day) and then randomly assigned to a particular group of pharmacological treatment: fluoxetine, imipramine 2.5 mg/kg, imipramine 5 mg/kg or saline. Twenty-four hours later, rats were treated with an IP injection of the selected treatment and, 1 h later, presented to the test session (5 min, second experimental day). In the following 13 days, rats were kept in their particular environmental condition and treated daily with an IP injection according to their experimental group initially assigned. The test session (5 min) was repeated on the 7th day (retest 1) and the 14th day (retest 2). In these days, pharmacological treatment was completed 1 h before the sessions of swimming. After retest 2, rats (PND 78) were anesthetized and perfused transcardially. The repeated FST consisted of individually placing the rats into a cylindrical tank (50 cm height × 25 cm diameter) containing clean water at 25 °C (25 cm deep). These conditions of the test were already described previously (Detke et al., 1995; Lino-de-Oliveira et al., 2005; Mezadri et al., 2011). After each session rats were taken out of the water and allowed to dry under a lamp (40 W, 10 min) before being returned to their home cages. All test sessions were recorded by a webcam (Logitech QuickCam) positioned 70 cm above the tank, to enable posterior evaluation. Behavioral categories scored were immobility, swimming, climbing and diving (definitions in Table S1). An experimenter blind to the treatment performed the behavioral analyses. The parameters evaluated for each category were: 1—latency (time elapsed between placing the animal in the tank and the first bout of each behavior observed), 2—frequency (number of bouts), and 3—duration (summary of the time spent in all bouts). The behavioral parameters were scored by the software Ethowatcher® (developed by the Laboratory of Comparative Neurophysiology of the Federal University of Santa Catarina, freely available on www.ethowatcher.ufsc.br, IEB-UFSC, Crispim-Junior et al., 2012).

2.4. Immunohistochemistry procedures and cellular quantification

After the retest 2, rats were anesthetized (Urethane 35%) and perfused transcardially with a sucrose solution (9.25% in 0.02 M phosphate buffer (PB), pH 7.2, with 0.3 ml of heparin, at 37 °C), followed by 4% paraformaldehyde in PB. The brains were removed, blocked and post-fixed for 4 h in the same fixative, transferred to a 0.1 M phosphate-buffered saline solution (PBS, pH 7.2) and then cut on a vibratome at 40 μm (250 μm apart throughout hippocampus). Sections were stored in a cryoprotectant at −20 °C, until required for the immunohistochemical reactions to detect Ki-67 (Ki-67-ir) or doublecortin (DCX-ir) (according to Schiavon et al., 2010). Briefly, all washings and incubations steps were performed under free-floating (gentle shaking) and room temperature (RT), unless otherwise stated. Washing steps (5 min each) consisted of three changes of 0.1 M PBS plus 0.25% Triton X-100 (PBST) between incubations. Endogenous peroxidase was blocked by incubation (30 min) with 100% methanol plus 0.3% H2O2 solution. Unspecific sites were blocked by incubation (90 min) in a solution containing 1% bovine serum albumin (BSA) in PBST followed by an overnight incubation (4 °C) with the primary antibody [rabbit anti-