Physiology & Behavior 140 (2015) 79–88

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Immobility behavior during the forced swim test correlates with BNDF levels in the frontal cortex, but not with cognitive impairments

Milene Borsoi [a], Camila Boque Antonio [b], Alice Fialho Viana [b], Patrícia Nardin [c], Carlos-Alberto Gonçalves [a,c], Stela Maris Kuze Rates [a,b,*]

[a] Graduate Studies Program in Neurosciences, Federal University of Rio Grande do Sul, 500 Sarmento Leite Street, ZIP code 90046-900 Porto Alegre, RS, Brazil
[b] Graduate Studies Program in Pharmaceutical Sciences, Federal University of Rio Grande do Sul, 2752 Ipiranga Avenue, ZIP code 90610-000 Porto Alegre, RS, Brazil
[c] Graduate Studies Program in Biochemistry, Federal University of Rio Grande do Sul, 2600 Ramiro Barcelos Street, ZIP code 90035-003 Porto Alegre, RS, Brazil

HIGHLIGHTS

- FST impairs both short and long-term memories in the object location test in rats.
- FST reduces prepulse inhibition and acoustic startle response in rats.
- BDNF levels in the hippocampus and frontal cortex are not altered by FST.
- Behavioral impairments induced by FST are not correlated with immobility behavior.
- Frontal cortical BDNF levels negatively correlates with immobility behavior.

ARTICLE INFO

Article history:
Received 2 July 2014
Received in revised form 3 December 2014
Accepted 4 December 2014
Available online 10 December 2014

Keywords:
Forced swimming stress
Prepulse inhibition
Acoustic startle response
Object location test
Novel object recognition
Brain-derived neurotrophic factor

ABSTRACT

The forced swim test (FST) is widely used to evaluate the antidepressant-like activity of compounds and is sensitive to stimuli that cause depression-like behaviors in rodents. The immobility behavior observed during the test has been considered to represent behavioral despair. In addition, some studies suggest that the FST impairs rats' performance on cognitive tests, but these findings have rarely been explored. Thus, we investigated the effects of the FST on behavioral tests related to neuropsychiatric diseases that involve different cognitive components: novel object recognition (NOR), the object location test (OLT) and prepulse inhibition (PPI). Brain-derived neurotrophic factor (BDNF) levels in the frontal cortex and hippocampus were evaluated. The rats were forced to swim twice (15-min session followed by a 5-min session 24 h later) and underwent cognitive tests 24 h after the last swimming exposure. The FST impaired the rats' performance on the OLT and reduced the PPI and acoustic startle responses, whereas the NOR was not affected. The cognitive impairments were not correlated with an immobility behavior profile, but a significant negative correlation between the frontal BDNF levels and immobility behavior was identified. These findings suggest a protective role of BDNF against behavioral despair and demonstrate a deleterious effect of the FST on spatial memory and pre-attentive processes, which point to the FST as a tool to induce cognitive impairments analogous to those observed in depression and in other neuropsychiatric disorders.

1. Introduction

Exposure to stress is known to be a risk factor for the development of several neuropsychiatric disorders [1]. The study of cognitive alterations and neurobiological consequences of stress in animal models has been considered a valid strategy to understand the vulnerability of cognitive processes to stressful events [2,3]. Cognitive impairments, which involve compromised working memory and learning and symptoms associated with cortical and hippocampal deficits, have been described in different neuropsychiatric diseases, such as schizophrenia, bipolar disorder and major depression [4,5]. Brain-derived neurotrophic factor (BDNF) has been strongly implicated in depression neurobiology and the cognitive impairments observed in depressed patients [6]. Of note, BDNF levels can be significantly impacted by exposure to stress [3,7].

The forced swim test (FST), developed by Porsolt in 1977, is a well-established stress rodent model used to predict the clinical efficacy of antidepressant drugs [8–10]. In this test, rats are exposed to a 15-min pretest session, followed by a 5-min test session 24 h later. The duration of the immobility behavior greatly increases during the second

* Corresponding author at: Laboratory of Experimental Psychopharmacology, College of Pharmacy, Federal University of Rio Grande do Sul, 2752 Ipiranga Avenue, ZIP code 90610000 Porto Alegre, RS, Brazil.
E-mail address: stela.rates@ufrgs.br (S.M.K. Rates).

http://dx.doi.org/10.1016/j.physbeh.2014.12.024
0031-9384/

swimming exposure compared with the first exposure, and this behavior is interpreted as behavioral despair or a state similar to learned helplessness [9–11]. In addition, various studies have demonstrated that exposure to different FST protocols impairs rats' performance in the Morris water and radial mazes [12–14], which suggests spatial memory impairments. The FST also has been related to alterations in synaptic plasticity [15,16].

More recently, some authors have proposed that the enhanced immobility developed during repeated FST may reflect a lack of initiative or apathy, which is similar to the affective flattening behavior present in schizophrenia [17,18]. Emotional states in psychiatric disorders have been studied by the application of startle modulation [19], which includes the measurement of the acoustic startle response (ASR) and prepulse-inhibition (PPI) as the most used procedures [20]. Impaired PPI is observed in animal models of schizophrenia [21], as well as interventions used to induce depressive-like symptoms in rodents [22–24]. However, to our knowledge, the effect of the FST on the PPI response has not been examined.

In this context, the aim of this study was to investigate the effects of the FST on three distinct cognitive tests: novel object recognition (NOR), the object location test (OLT) and prepulse inhibition (PPI). In addition, we measured the BDNF levels in the prefrontal cortex and the hippocampus of rats subjected to the FST.

2. Material and methods

2.1. Animals and experimental design

Adult male Wistar rats (250–300 g) purchased from the Fundação Estadual de Produção e Pesquisa em Saúde — RS, Brazil (FEPPS) colony were used. The animals were housed in standard plastic cages (5 rats per cage) on a shelf with a controlled ventilation system. The animals were maintained on a 12-h light/dark cycle (lights on 7:00 a.m.) at a constant temperature (23° ± 1 °C) with free access to a standard rodent diet (Nuvilab®) and tap water. All animals were allowed to acclimate to the housing conditions for at least one week prior to beginning the experiments. The animals were allowed to adapt to the room laboratory conditions 1 h before the tests, which were performed in a room under a controlled temperature (22 ± 1 °C). The experiments were conducted between 11:00 a.m. and 3:00 p.m. All efforts were made to minimize the number of animals used and their suffering. All procedures were previously approved by the Animal Care Local Ethical Committee (CEUA-UFRGS; project's approval number 20406) and were performed according to the European Communities Council Directive of November 24, 1986 (86/609/EEC).

The behavioral tasks (novel object recognition, object location test and prepulse inhibition) and the BDNF quantification were performed using different groups of animals and were initiated 24 h after the second swimming exposure. Prepulse inhibition was performed before and after the FST separated by a 24-h interval. Naïve animals not previously exposed to the forced swim test (non-stressed) were used as the control.

2.2. Forced swim test

The forced swim test (FST) was performed in an acrylic box with four sections of 30 cm × 30 cm × 40 cm. The external walls and the cover were transparent; however, the inside sections were dark, which enabled the isolation of each of the four quadrants. The rats were subjected to two swimming sessions in water at 22 ± 1 °C and a height of 30 cm. The first session had a 15 min duration. The rats were exposed to another swimming session for 5 min 24 h later. The immobility time was measured in both sessions by an expert observer and expressed in seconds. The rats were considered immobile when they ceased struggling and remained floating motionless in the water, with only movements necessary to maintain their heads above water. At the end of each swimming session, the rats were removed from the water and gently dried.

2.3. Novel object recognition test

The novel object recognition (NOR) test is a simple behavioral assay of memory that primarily relies on a rodent's innate exploratory behavior in the absence of externally applied rules or reinforcement [25]. This task comprised three phases: habituation, training and test; each session had a 5 minute duration and was performed in an open field apparatus (80 cm diameter; 12 quadrants). The habituation phase was performed 24 h after the second swimming session. The rats were placed in the center of the apparatus and allowed to freely explore the open-field arena in the absence of objects. The number of crossings and rearings were counted by a single observer.

Twenty-four hours after the habituation phase, the animals underwent the training phase: the rat was returned to the apparatus that contained two identical sample objects (A + A), which were placed at a distance of 10 cm from the wall. Ninety minutes or 24 h after the training phase, the rats returned to the apparatus to test short-term (STM) or long-term (LTM) memory, respectively. Different animals were used to access STM and LTM. In the test session, the rat was returned to the open-field arena that contained two objects; one object was identical to the training session and the other object was novel (A + B). The recognition index in each session was calculated as follows: time exploring the familiar or novel object /time exploring both objects. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on objects was not considered exploratory behavior. The apparatus and the objects were thoroughly cleaned with 50% ethanol between the trials to ensure the absence of olfactory cues. All sessions were performed between 11:00 a.m. and 15:00 p.m.

2.4. Object location test

The object location test (OLT) is based on the rat's innate tendency to spontaneously explore objects in a novel place and is used for the assessment of spatial memory [26]. The test comprised three phases: habituation, training and test; each phase had a duration of 5 min. The habituation and training phases were performed as described for NOR (Section 2.3). The STM and LTM test phases were performed in different animals. In the test phase, the two training objects used during the training phase were presented to the animals, but one object was situated in a different spatial location. The recognition index in each session was calculated as follows: time exploring the object in a familiar or novel position /time exploring both objects. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the objects was not considered exploratory behavior. The apparatus and the objects were thoroughly cleaned with 50% ethanol between the trials to ensure the absence of olfactory cues. All sessions were performed between 11:00 a.m. and 3:00 p.m.

2.5. Prepulse inhibition

The prepulse inhibition test (PPI) measures the attenuation of the startle reflex amplitude in response to a sudden intense startling stimulus (pulse) when this intense stimulus is shortly preceded by a weaker, non-startling sensory stimulus (prepulse) [27]. The PPI evaluation was performed twice (before and after the FST), and the tests were separated by a 24 h interval. The first PPI session was conducted to verify the basal response to the test and to acclimate the animals to the procedure. In addition to the PPI response, the magnitude of the acoustic startle response (ASR), which represents the reflex response to the pulse alone, was also evaluated in both PPI sessions. The PPI and ASR evaluations were performed in a startle chamber (Insigth®, São Paulo, Brazil), in which a loudspeaker produced a continuous background noise (60 dB), as well as the acoustic startle pulse and the prepulse described