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Individual differences in the forced swimming test and neurochemical kinetics in the rat brain

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HIGHLIGHTS

- Time course of depression-related factors was studied in rats with low/high despair.
- BDNF variations across time were different in animals with low and high immobility.
- Monoamine changes after FST outlasted from hours to at least a day.

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ABSTRACT

Individual differences in the forced swimming test (FST) could be associated with differential temporal dynamics of gene expression and neurotransmitter activity. We tested juvenile male rats in the FST and classified the animals into those with low and high immobility according to the amount of immobility time recorded in FST. These groups and a control group which did not undergo the FST were sacrificed either 1, 6 or 24 h after the test. We analyzed the expression of the CRF, CRFR1, BDNF and TrkB in the prefrontal cortex, hippocampus and nucleus accumbens as well as norepinephrine, dopamine, serotonin, glutamate, GABA and glutamine in the hippocampus and nucleus accumbens. Animals with low immobility showed significant reductions of BDNF expression across time points in both the prefrontal cortex and the nucleus accumbens when compared with non-swim control. Moreover, rats with high immobility only showed a significant decrease of BDNF expression in the prefrontal cortex 6 h after the FST. Regarding neurotransmitters, only accumbal dopamine turnover and hippocampal glutamate content showed an effect of individual differences (i.e. animals with low and high immobility), whereas nearly all parameters showed significant differences across time points. Correlational analyses suggest that immobility in the FST, probably reflecting despair, is related to prefrontal cortical BDNF and to the kinetics observed in several other neurochemical parameters. Taken together, our results suggest that individual differences observed in depression-like behavior can be associated not only with changes in the concentrations of key neurochemical factors but also with differential time courses of such factors.

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1. Introduction

Behavioral research based on individual differences (i.e. classification of individuals according to systematic variations of specific behaviors) has shown to be a very informative approach to understand brain function and mood disorders [1–4]. The individual phenotype of an organism is influenced, to a greater or lesser extent, by a number of interacting factors such as endocrine status, genetic variation and environmental effects among others (reviewed in [5]). Thus, the study of individual differences in behavioral traits allows the identification of relevant information concerning such influencing factors [6]. The individual differences approach has been used, for example, to study critical elements underlying the development of anxiety- and depression-like disorders [7–11].

The development of depression is associated with previous stress exposures and involves altered function of many brain regions and physiological and molecular pathways (reviewed in [12]). Several limbic structures play a central role in the development of the disease, with the hippocampus (HPC) and the nucleus accumbens (NAc) being widely studied so far [13–15]. Likewise, the prefrontal cortex (PFC) has also been shown to play an important role [16,17]. In addition, depression is associated with alterations in a growing number of molecular regulators such as the corticotropin-releasing factor (CRF) and the corticotropin-releasing factor receptor 1 (CRFR1)

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[18,19]. Furthermore, the brain-derived neurotrophic factor (BDNF) and its receptor tyrosine-related kinase B (TrkB) were shown to have a relevant modulatory action. Changes in their expression profiles have been considered as vulnerability factors for disease development and/or antidepressant activity [20,21]. On the other hand, alterations in monoamine neurotransmission have been repeatedly associated with depression (for a meta-analysis see [22]).

We have previously found that individual differences in the duration of immobility in the forced swimming test (FST), a validated model of behavioral despair [23], are associated with differential expression of CRFR1 and monoaminergic neurotransmission one week after the exposure to the FST [24]. Nevertheless, such findings offered no insights regarding the dynamics shortly after the aversive experience. Therefore, we studied the temporal dynamics of gene expression and monoamine and amino acid neurotransmission in order to determine if individual differences in FST behavior are associated with the dynamics of these factors across a period of 24 h. We found that, in comparison with a non-swim control group, prefrontal and accumbal BDNF levels differentially decreased across the first 24 h, suggesting that high or low despair responses are related to further downstream neurobiological events that could be associated with the development of depression. Thus, it should be noticed that not only differences in levels but also in the time course dynamics of BDNF could play an important role in the stress response.

2. Materials and methods

2.1. Animals

Three groups of thirty-four (experimental groups) and one group of ten (control group) outbred male Sprague–Dawley rats (*Rattus norvegicus*) four weeks old were used in this study (provided by LEBi Laboratories, University of Costa Rica). The use of juvenile prepubertal animals was based on our previous findings showing that juvenile rats are more vulnerable to the swim stress [24], which would increase the possibility of finding factors with differential time course dynamics between animals with low and high immobility. Each group of animals was studied separately. Rats were individually marked and housed in groups (5 animals per cage) in standard polycarbonate home cages (37.5 × 22 × 18 cm; with woodchip bedding) with ad libitum access to food and water, under 12:12 h light–dark schedule (lights on at 06:00 until 18:00 h) at a room temperature of 25.5 °C ± 1.20 °C and 78–87% relative humidity. A one-week acclimatization period was used prior to the behavioral tests. Afterwards, each group (except the non-tested control one) was subjected to two behavioral tests: the open field test (OFT) and the FST (see below). Experimental procedures were done in accordance to the guidelines of the Costa Rican Ministry of Science and Technology for the Care and Use of Laboratory Animals and were approved by the Institutional Committee for Animal Care and Use of the University of Costa Rica.

Rats were sacrificed 1 h (experimental group 1), 6 h (experimental group 2) and 24 h (experimental group 3) after FST exposure (see below). Rats from the control group (non-subjected to the behavioral tests) were sacrificed simultaneously with the experimental group 1. All experimental and control groups were managed identically during the study in order to avoid management-derived variation. For each experimental group, animals were classified post-hoc as animals with low (lower third) or high (upper third) immobility depending on the duration in the FST test session. Animals showing medium scores for this parameter were not included in the study. Then, high, low and control animals were compared regarding neurochemistry and gene expression profiles in different brain areas in order to determine if differences in the duration of immobility are associated with neurochemical profiles at different time points after the FST exposure.

2.2. Behavioral tests

The OFT was carried out on post natal day (PND) 29. Animals were subjected to this test in order to replicate previously published experiments [24]. Behavioral testing was conducted between 8:00 and 11:00 in the morning. One hour before each test animals were placed in a red light room adjacent to the testing room for context habituation. The testing room was illuminated with one 25 W red bulb located 130 cm above the floor. The open field arena consisted of a black, square, wooden chamber (55 cm × 55 cm × 40 cm). Single animals were placed in the center of the arena during a 10 minute session. A trained observer manually scored behavioral parameters using the Etholog 2.25 software [25]. In addition, the software Any-maze™ 4.3 (Stoelting Co., USA) was used to measure the total distance traveled (m). The following variables were studied: locomotion represented by the number of squares crossed with the four paws in the center and the periphery (i.e., crossings), total distance traveled, average speed, time (in seconds) spent in the center, rearing time (posture sustained with hind paws on the floor) and total grooming time (including washing or mouthing of forelimbs, hind paws, face, body and genitals). In the FST rats were individually placed into plastic cylinders (45 cm height, 31 cm diameter) containing water (25 °C ± 0.5 °C) to a depth of 30 cm (the animals' hind paws and tail did not touch the cylinder's bottom). After each session, rats were removed from water, dried with a towel, and placed in a warmed enclosure, and the cylinders were cleaned and refilled. The FST consisted of two sessions on two consecutive days: a 15 minute pre-test and a 5 minute test (at PNDs 31 and 32 respectively). Behavior was videotaped and scored by a trained observer by means of the Etholog 2.25 software. The analyzed parameters were: duration of immobility, swimming and climbing. It is worth noting that only the first 5 min was scored in the pre-test in order to properly compare them with the 5 min of the test session.

2.3. Gene expression

Animals were sacrificed by decapitation without anesthetics 1, 6 and 24 h after the second FST. Brains were quickly dissected on ice and three different areas were dissected out: HPC, PFC and NAc (40.13 ± 0.06 mg, 13.70 ± 0.06 mg and 8.50 ± 0.03 mg, respectively). In the case of PFC the two hemispheres were pooled, whereas the HPC and the NAc sample collection followed a right-and-left alternating method. The remaining hemispheres were used for neurochemical analysis (see below). Tissue samples were collected in a tube with 300 μL TRIzol (Invitrogen, USA), homogenized by 20 s of sonication using an ultrasonic dismembrator (Fisher, USA), immediately frozen and stored at −70 °C. The extraction of total RNA was carried out according to the manufacturer's instructions. Briefly, samples were thawed, incubated for 5 min at 25 °C and mixed with 100 μL of chloroform. A centrifugation step of 15 min at 12,000 *g* and 4 °C separated the mixture into three phases. The aqueous phase (containing the RNA) was transferred to a fresh tube and the RNA was precipitated with 250 μL of isopropyl alcohol and centrifugation at 12,000 *g*. The RNA pellet was washed with 75% ethanol and centrifuged at 7500 *g* for 5 min at 4 °C. Finally, the pellet was dissolved in RNase-free water. RNA samples were immediately quantified by means of a NanoDrop spectrophotometer (Thermo Scientific, USA) and stored at −70 °C. The integrity of total RNA was assessed by electrophoresis on 1.5% (w/v) agarose gels. Samples were treated with DNase I (Fermentas, USA) in order to avoid genomic DNA contamination. cDNA synthesis was carried out by RevertAid First Strand cDNA Synthesis Kit (Fermentas, USA) according to the manufacturer's specifications, but adapted to a final volume of 10 μL. In brief, for each sample, 500 μg of total RNA was mixed with 2 μL of 5× reaction buffer, 0.25 μg of oligo $(dT)_{18}$ primer, 1 mM dNTPs, 10 U of RNase inhibitor and 100 U of RevertAid M-MuLV Reverse Transcriptase. Reactions were incubated for 60 min at 42 °C, followed by 5 min at 70 °C. Samples were diluted 1:10 and stored at −20 °C.

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