Physiology & Behavior xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Physiology & Behavior

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

A new stress model, a scream sound, alters learning and monoamine levels in rat brain ☆

Lili Hu [a], Juan Yang [a], Tusheng Song [a,*], Ni Hou [a], Yong Liu [b], Xiaoge Zhao [c], Dianzeng Zhang [c], Lumin Wang [a], Tao Wang [d], Chen Huang [a,c,e,**]

[a] Department of Genetics and Molecular Biology, Xi'an Jiaotong University College of Medicine, Xi'an, Shaanxi, China
[b] Research Center for Neuroscience, Xi'an Jiaotong University College of Medicine, Xi'an, Shaanxi, China
[c] Key Laboratory of Environment and Genes Related to Diseases, Xi'an Jiaotong University College of Medicine, Xi'an, Shaanxi, China
[d] Department of Physiology and Pathophysiology, Xi'an Jiaotong University College of Medicine, Xi'an, Shaanxi, China
[e] Cardiovascular Research Center, Xi'an Jiaotong University College of Medicine, Xi'an, Shaanxi, China

HIGHLIGHTS

- Scream sound is a novel stress model and easy to apply.
- Acute scream sound increases learning ability and alters monoamine levels in rat brain.
- Chronic scream sound also increases learning ability and alters the monoamine levels in rat brain.

ARTICLE INFO

Article history:
Received 19 April 2013
Received in revised form 25 August 2013
Accepted 23 September 2013
Available online xxxx

Keywords:
Scream sound stress
Learning
Monoamine
Brain

ABSTRACT

Most existing animal models for stress involve the simultaneous application of physical and psychological stress factors. In the current study, we described and used a novel psychological stress model (scream sound stress). To study the validity of it, we carried out acute and chronic scream sound stress.

First, adult Sprague–Dawley (SD) rats were randomly divided into white noise, stress and background groups. The white noise group and stress group were treated with white noise and scream sound for 4 h in the morning respectively. Compared with white noise and background groups, exposure to acute scream sound increased corticosterone (CORT) level and decreased latency in Morris water maze (MWM) test. The levels of noradrenaline (NE), dopamine (DA), 5-hydroxytryptamine (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were altered in the striatum, hypothalamus and hippocampus of stress rats.

Second, adult SD rats were randomly divided into background and stress groups, which were treated with scream sound for three weeks. Exposure to chronic scream sound suppressed body weight gain, increased corticosterone (CORT) level, influenced the morphology of adrenal gland, improved spleen and thymus indices, and decreased latency in MWM test. NE, DA, DOPAC, HVA and 5-HIAA levels were also altered in the brain of stress rats.

Our results suggested that scream sound, as a novel stressor, facilitated learning ability, as well as altered monoamine levels in the rat brain. Moreover, scream sound is easy to apply and can be applied in more animals at the same time.

Abbreviations: ANOVA, analysis of variance; CORT, corticosterone; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPAC/DA, 3,4-dihydroxyphenylacetic acid/dopamine; DA, dopamine; HPLC-ECD, high performance liquid chromatography with electrochemical detector; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; HPA, hypothalamus pituitary adrenal; NE (NA), norepinephrine, noradrenaline; ZG, zona glomerulosa; ZF, zona fasciculata; ZR, zona reticularis.

* Corresponding author. Tel./fax: +86 29 82655077.
** Correspondence to: C. Huang, Department of Genetics and Molecular Biology, Xi'an Jiaotong University College of Medicine, Xi'an, Shaanxi, China. Tel./fax: +86 29 82655077.
E-mail addresses: tusheng@mail.xjtu.edu.cn (T. Song), hchen@mail.xjtu.edu.cn (C. Huang).

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http://dx.doi.org/10.1016/j.physbeh.2013.09.010

1. Introduction

Stress models, including chronic mild stress [1–3], acute or chronic restraint [4,5], acute or chronic inescapable stress [6], unpredictable chronic stress [7], variable chronic stress [8–10], acute electric footshock [11] and communication box for hours or 7 d [12–14], have been commonly used to explore the relationship between psychological stress and its effects. Chronic mild stress includes food and water deprivation, paired housing, soiled cage, backward tilting of cages and stroboscopic illumination in darkness. Water and food deprivation, isolation, flashing light, forced swimming, restraint and cold are used in variable chronic stress. The communication box is a very good psychological stress model but cruel to rats that are exposed repetitively to electric foot shock. Most of the other existing stress models involve the simultaneous application of both physical and psychological stressors. Moreover, physical stress can influence the effects of psychological stress. Thus, the effects of psychological stress alone are difficult to study. In the current paper, we demonstrated a pure psychological stress model by using scream sound as the stressor.

Acoustic stimulation can be graded from non-stress to stressful levels. Higher noise intensities (90 and 105 dB) significantly increase corticosterone (CORT) level, which is a reliable index of hypothalamo-pituitary-adrenal activation [15]. Several studies have indicated that white-noise exposure of rats at 100 or 90 dB significantly increases CORT [16–19]. However, another study has demonstrated that moderate noise intensities (70 and 80 dB) do not influence CORT level [15]. To avoid audiogenic stress, the sound intensity used in our study was <80 dB.

Psychological stress leads to various changes, including clinical depression, cardiovascular disease, cancer as well as impaired spatial learning and memory [20–22]. Numerous studies have focused on learning and memory ability associated with stress [23–25], but the relationship between the learning and memory ability and the pure psychological stress has not been completely examined.

The aim of present experiments is to study the validity of this psychological stress model (scream sound). We determined the body weight, CORT level, learning and memory ability, adrenal morphology and monoamine level in the brain of scream sound-exposed rats; and we concluded that scream sound exposure caused suppressions in body weight gain, increases in CORT level and learning ability, and alterations in adrenal morphology and monoamine level, suggesting that scream sound might be a new stress model.

2. Materials and methods

2.1. Animals

Adult male and female Sprague–Dawley rats weighing 246 g to 270 g at the start of the experiments were obtained from the Medical Experimental Animal Centre of Shaanxi Province, China. All of the rats were housed in cages where four rats were placed in each standard plexiglass cage (30 cm × 47 cm × 15 cm) with sawdust, maintained at 23 ± 2 °C on 12 h light/dark cycles (light was on from 06:00 h to 18:00 h) and given standard rat diet (Laboratory Animal Centre, Xi'an Jiaotong University, China) as well as water ad libitum. All tests were performed during the light phase of the cycle. Body weight was measured after the adaptation period. The experimental protocol was approved by the Institutional Animal Care Committee of Xi'an Jiaotong University.

2.2. Production of scream sound

One, two and three male rats produced scream sound at frequencies of 1.3 kHz to 3.2 kHz, 0.9 kHz to 4 kHz and 0.5 kHz to 3.8 kHz when they were exposed to electric foot shock for 49, 28 and 41 s, respectively. By contrast, one, two and three female rats produced scream sound at frequencies of 1.6 kHz to 10.3 kHz, 1.0 kHz to 3.3 kHz and 3.0 kHz to 3.8 kHz when they were exposed to electric foot shock for 66, 28 and 102 s, respectively. The scream sound was simultaneously recorded in a professional recording room (Xi'an Yin Zhi Xuan). Digital audio production systems, including pro tools HD audio workstation, Yamaha digital mixer, GENELEC (Genelec) monitors, Neumann U87 AI condenser microphone and NEVE1 were used to record the scream sound.

2.3. Acute stress

2.3.1. Experimental design

After the adaptation period for 7 d, the male rats for acute stress were randomly divided into three groups (25 rats in each group), the white noise group, the stress group and the background group. The rats in the white noise group and stressed group were treated with white noise and scream sound for 4 h in the morning respectively (7:00 a.m.–11:00 a.m.). White noise was produced by a white noise generator and amplified by an amplifier (40 W). A loudspeaker (Panda CD-100 CD player) was placed 50 cm above the animal cages. The intensity of the sound (45 dB to 75 dB) was measured using a sound level meter (SL-5800). The rats in the background group were exposed to background noise. The level of the background noise produced by the ventilation system inside the room and eating or fighting of the rats was 40 dB to 45 dB [26].

2.3.2. Serum CORT concentration

Before the rats were treated with white noise and scream sound, their blood was collected at 7:00 a.m. The rats were sacrificed by decapitation immediately 0 min, 30 min and 1 h after the stress treatment, their blood was collected within 11:00 a.m. to 12:00 a.m. The background group rats were sacrificed at the same four points mentioned above. The serum was left to stand at room temperature for 1 h, centrifuged at 3000 rpm for 15 min and stored at −80 °C for subsequent analysis. The CORT concentration was determined using radioimmunoassay (cort (HY-10063) RIA KIT, Beijing Sino-uk institute of Biological Technology).

2.3.3. Morris water maze (MWM) test

The rats of background, white noise and stress groups were tested using a Morris water maze (MWM) after the acute stress period immediately. The MWM setting (diameter = 150 cm; wall height = 50 cm) was used in combination with a platform that has a diameter of 8 cm [27]. The MWM comprised a circular pool filled with water at 23 ± 1 °C. The escape platform was placed at a fixed position in the center of one quadrant and hidden 1 cm beneath the water surface. The acquisition, or training phase, consisted of five training days, starting at four different positions in a random order. Eight trials were performed per day, with four trials in the morning and four trials in the afternoon. If an animal did not reach the platform within 120 s, then it was placed on the platform where it had to remain for 15 s. The water maze was surrounded by a number of fixed clues. In addition, the experimental room was kept invariable [27–29].

A retention trial was performed five days after the completion of the acquisition phase. At 6 d, the platform was removed from the maze. Spatial activity was expressed as the number of times the rats crossed the previous platform location.

2.3.4. Measurement of monoamines and their metabolites

The rats were sacrificed immediately after the acute stress period, and their brains were rapidly removed under frozen condition. The frontal cortex, hippocampus, hypothalamus and striatum were separated, weighed, frozen in liquid nitrogen and stored at −80 °C [12,30,31]. The monoamines and their metabolites in the tissue were directly assayed using high-performance liquid chromatography with coulometric electrochemical detection (ESA, USA) following the Neuroscience Application Guide on Monoamines and Acid Metabolites Analysis for Tissue Samples by Bruce Bailey. The samples were ultrasonicated on ice in a solution in which every 50 mg of wet weight was added to

Please cite this article as: Hu L, et al, A new stress model, a scream sound, alters learning and monoamine levels in rat brain, Physiol Behav (2013), http://dx.doi.org/10.1016/j.physbeh.2013.09.010