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Research report

Prior exposure to repeated immobilization or chronic unpredictable stress protects from some negative sequels of an acute immobilization

Jordi Pastor-Ciurana[a,1], Cristina Rabasa[a,1,2], Juan A. Ortega-Sánchez[a], Maria Sanchís-Ollè[a], Marina Gabriel-Salazar[a], Marta Ginesta[a], Xavier Belda[a], Núria Daviu[a], Roser Nadal[b], Antonio Armario[a,*]

[a] Institut de Neurociències and Red de Trastornos Adictivos (RTA), Unitat de Fisiologia Animal, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra, Spain
[b] Unitat de Psicobiologia, Facultat de Psicologia, Universitat Autònoma de Barcelona, Bellaterra, Spain

HIGHLIGHTS

- Chronic immobilization protects from the effects of an acute immobilization.
- Chronic unpredictable stress partially protects from an acute immobilization.
- There is evidence of cross-adaptation between different stressors.

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ABSTRACT

Exposure to chronic unpredictable stress (CUS) is gaining acceptance as a putative animal model of depression. However, there is evidence that chronic exposure to stress can offer non-specific stress protection from some effects of acute superimposed stressors. We then compared in adult male rats the protection afforded by prior exposure to CUS with the one offered by repeated immobilization on boards (IMO) regarding some of the negative consequences of an acute exposure to IMO. Repeated exposure to IMO protected from the negative consequences of an acute IMO on activity in an open-field, saccharin intake and body weight gain. Active coping during IMO (struggling) was markedly reduced by repeated exposure to the same stressor, but it was not affected by a prior history of CUS, suggesting that our CUS protocol does not appear to impair active coping responses. CUS exposure itself caused a strong reduction of activity in the open-field but appeared to protect from the hypo-activity induced by acute IMO. Moreover, prior CUS offered partial protection from acute IMO-induced reduction of saccharin intake and body weight gain. It can be concluded that a prior history of CUS protects from some of the negative consequences of exposure to a novel severe stressor, suggesting the development of partial cross-adaptation whose precise mechanisms remain to be studied.

1. Introduction

Exposure to purely or predominantly emotional stressors (herein emotional stressors) resulted in a wide range of physiological and behavioural changes. The best characterized physiological changes are the activation of the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adreno-medullary (SAM) axes [1]. The activation of the HPA axis results in the release of ACTH and glucocorticoids (corticosterone in rats), whereas the activation of the latter increases plasma levels of adrenaline and noradrenaline. Physiological response to stress is accompanied by behavioural changes that typically include alterations of normal activity and exploratory behaviour in novel environments, enhanced anxiety, and interference with learning and memory processes [2–4].

Abbreviations: CUS, chronic unpredictable stress; GEE, generalized estimating equations; GENLIN, generalized linear models; HPA, hypothalamic-pituitary-adrenal axis; IMO, immobilization on boards; IMOa, acute immobilization; IMOch, chronic intermittent immobilization; NS, non acutely stressed; SAM, sympathetic-adreno-medullary axis.

* Corresponding author at: Institut de Neurociències and Unitat de Fisiologia Animal, Facultat de Biociències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain. Tel.: +34 935811664; fax: +34 935812390.
E-mail address: antonio.armario@uab.es (A. Armario).

[1] These authors contributed equally to this work.

[2] Present address: Department of Physiology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Medicinaregatan 11, PO Box 432, SE-405 30 Gothenburg, Sweden.

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After daily repeated exposure to the stressor, the impact of an acute session of the same (homotypic) stressor is very often reduced. This has been frequently reported regarding the HPA and the SMA axes [1,5]. As exposure to novel (heterotypic) stressors resulted in normal or enhanced HPA and SMA responses [1,5–7], it is assumed that the reduction of the response caused by daily repeated exposure to the same stressor is due to a lower emotional activation, consequence of the familiarity with the situation.

Less is known about how repeated exposure to a particular stressor affects the behavioural response to an acute challenge with the same stressor. In a series of papers in the 60–70s some authors reported that acute exposure to severe stressors impaired performance of rats in some tasks requiring an important degree of motor activity, but such impairment progressively decreased after repeated exposure to the stressor [8–10]. Quite interestingly, protection offered by chronic stress was not limited to the homotypic stressor, demonstrating cross-adaptation between different stressors. This non-specific adaptation is likely to involve brain noradrenaline function as severe chronic stressors consistently increased noradrenaline synthesis capabilities (i.e. synthesis of tyrosine-hydroxylase and other enzymes, see [7]) and tyrosine supplementation prevented both noradrenergic depletion after severe stressors and behavioural inhibition [11,12].

The possibility of non-specific cross-adaptation is particularly important regarding the consequences of exposure to chronic unpredictable stress (CUS), also known as chronic variable or chronic mild stress. Exposure to CUS, a model developed by Katz et al. (1981) [13] and later developed by Willner and colleagues (see [14]), has been considered as a putative animal model of depression causing for instance reduced activity in novel environments, anxiety, anhedonia (mainly evaluated by reduced consumption of sucrose), and the development of passive coping strategies in the forced swim test [14,15]. However, there is also evidence that under certain conditions CUS not only did not induce anxiety, but can even reduce it [16–20]. Moreover, some recent studies in rats suggest that activation of the HPA axis and brain c-fos expression in response to a novel stressor may be reduced in animals by prior exposure to CUS [21,22], although results regarding the HPA axis are not consistent [21–24].

From all the above considerations we hypothesized that a prior history of chronic experience with unpredictable stressful situations may confer partial protection from the detrimental consequences of an acute severe stressor such as immobilization on boards (IMO). Then, in the present work we compared the protection offered by a prior history of chronic IMO stress and by a CUS procedure that did not include IMO as stressor, regarding the negative consequences of an acute session of IMO. This comparison can shed lights on the possible dual consequences (detrimental, protective) of a prior history of stress on the response to novel encountered stressful situations.

2. Methods

2.1. Animals

Fifty-three male Sprague–Dawley rats obtained from the breeding centre of the Universitat Autònoma de Barcelona were used. Rats were 2-month-old at the beginning of the experiment. Animals were housed individually under standard conditions of temperature (22 ± 1 °C) in a 12 h light/dark schedule (lights on at 7:00) with ad libitum access to food and water. Rats were allowed at least 1 week to acclimate themselves to the animal room before starting the experiment. Animals were handled at least 3 times on different days for approximately 2 min. The experimental procedures were always done in the morning, with exception of CUS (Fig. 1). This work has been carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and approved by the Ethical Committee for Animal Experimentation of the Universitat Autònoma de Barcelona and by the Generalitat de Catalunya.

2.2. Experimental procedures

Animals were assigned to three experimental groups: (i) controls (n = 18), undisturbed from day 1 to 9, (ii) chronic intermittent IMO (IMOch, n = 18), daily exposed to 1 h of IMO from day 1 to 9, and (iii) chronic unpredictable stress (CUS, n = 17), animals exposed to the CUS paradigm for 9 days (Fig. 1). On day 10, 9 animals from the two former groups and 8 for the last group were exposed to 1 h IMO (IMOa) and the others remained undisturbed, with no additional exposure to stress (NS). On day 11 (24 h after the last IMO) all animals were exposed to an open-field for 15 min. After that, three animals from each NS group were sacrificed for other purposes. In the remaining animals, food intake and body weight were daily measured for 4 days. Saccharin intake was only measured in those animals exposed to the acute IMO on day 10 because preliminary results indicated that neither chronic IMO nor CUS affected significantly saccharin intake measured at the end of the chronic stress period. These latter values could then be used as a baseline to study the impact of the acute IMO.

The CUS consisted of the exposure to 3 different stressors (restraint, footshock and forced swim) following the schedule indicated in Fig. 1. Animals were always transported to the stress room in their home-cage. For restraint, animals were placed during 30, 60 or 90 min into cylindrical PVC tubes measuring 6 cm diameter and 21.5 cm length. The rear top of the apparatus was closed by a cork letting the tail to protrude from the tube. Several holes (0.5 cm in diameter) in the walls of the cylinder provided fresh air. For the footshock, rats received repeatedly a 6 s shock (1.5 mA) each min for 30, 60 or 90 min. Rats were put into individual clear Plexiglas® boxes (19.7 cm × 11.8 cm × 20.0 cm) provided with a metal removable grid floor of 15 stainless steel rods (0.4 cm diameter and spaced 0.9 cm centre to centre) connected to a shocker that delivered scrambled AC current (Cibertec, Madrid, Spain). Shock chambers were carefully cleaned with ethanol (5%, v/v) before introducing the animals. For forced swim, animals were allocated in transparent cylindrical plastic tanks (height = 40 cm, internal diameter = 19 cm) containing water (25 °C) to a level of 24 cm where they remained for 20 min [25]. Afterwards, they were withdrawn from water and kindly dried with a towel before being returned to their home-cages. After 1 h of rest, they underwent 10 additional min of forced swim. Water was always changed before introducing the animals into the tanks. All the CUS procedures were done in a room with white walls illuminated by a white fluorescent light.

The chronic IMO procedure consisted of immobilizing the animals for 1 h by taping their four limbs to metal mounts attached to a board [26]. Head movements were restricted with two plastic pieces (7 cm × 6 cm) and the body was subjected to the board by means of a piece of plastic cloth (10 cm-wide) attached with Velcro®, which surrounded all the trunk. Animals remained immobilized in a room provided with white fluorescent light.

2.3. Behavioural assessment

2.3.1. Activity/exploration

The open-field was a rectangular grey plastic box opened at the top (56 cm × 36.5 cm × 31 cm) with dim illumination provided by a white 25 W bulb placed 1.20 m above the centre of the surface of the box. Animals were placed in a corner of the open-field facing the wall. The box was cleaned between animals with ethanol solution