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Research report

Unpredictable chronic mild stress exerts anxiogenic-like effects and activates neurons in the dorsal and caudal region and in the lateral wings of the dorsal raphe nucleus

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HIGHLIGHTS

- Unpredictable chronic mild stress is anxiogenic.
- Chronic stress increases *Fos* immunoreactivity in the caudal dorsal raphe.
- Chronic stress activates serotonergic cells in the dorsal region of the dorsal raphe.
- Chronic stress activates serotonergic cells in the lateral wings of the dorsal raphe.

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ABSTRACT

In previous studies, we verified that exposure to unpredictable chronic mild stress (UCMS) facilitates avoidance responses in the elevated T-maze (ETM) and increased *Fos*-immunoreactivity in different brain structures involved in the regulation of anxiety, including the dorsal raphe (DR). Since, it has been shown that the DR is composed of distinct subpopulations of serotonergic and non-serotonergic neurons, the present study investigated the pattern of activation of these different subnuclei of the region in response to this stress protocol. Male Wistar rats were either unstressed or exposed to the UCMS procedure for two weeks and, subsequently, analyzed for *Fos*-immunoreactivity (*Fos*-ir) in serotonergic cells of the DR. To verify if the anxiogenic effects observed in the ETM could be generalized to other anxiety models, a group of animals was also tested in the light/dark transition test after UCMS exposure. Results showed that the UCMS procedure decreased the number of transitions and increased the number of stretched attend postures in the model, an anxiogenic effect. UCMS exposure also increased *Fos*-ir and the number of double-labeled neurons in the mid-rostral subdivision of the dorsal part of the DR and in the mid-caudal region of the lateral wings. In the caudal region of the DR there was a significant increase in the number of *Fos*-ir. No significant effects were found in the other DR subnuclei. These results corroborate the idea that neurons of specific subnuclei of the DR regulate anxiety responses and are differently activated by chronic stress exposure.

1. Introduction

Stress was first defined by Hans Selye as the organism's nonspecific reaction to any change that requires a physiological, behavioral and/or an emotional response [1]. The factors that cause the stress response are called "stressors". Although responses to short-term

Abbreviations: UCMS, unpredictable chronic mild stress; ETM, elevated T-maze; DR, dorsal raphe; *Fos*-ir, *Fos* immunoreactivity; 5-HT, serotonin; SAPs, stretched attend postures; trpOH-ir, tryptophan hydroxylase immunoreactivity; *Fos*-/TrpOH-ir, *Fos*-/tryptophan hydroxylase immunoreactivity; DRD, dorsal subnucleus of the dorsal raphe; DRV, ventral subnucleus of the dorsal raphe; lwDR, lateral wings of the dorsal raphe; DRC, caudal subnucleus of the dorsal raphe; DRI, interfascicular subnucleus of the dorsal raphe.

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or acute stress are adaptive, if the stressor is persistent or intense, the stress response might be associated with a greater risk for pathologies, including psychiatric disorders, such as depression and anxiety [2].

To better study the consequences of stress and its relationship with these so-called stress-related disorders, animal models have been developed. The unpredictable chronic mild stress (UCMS) protocol is an animal model that presents good face validity and that has been broadly used in the past years to investigate both the physiological and the behavioral consequences of chronic stress [3–6]. Briefly, in this test, rodents are exposed to a variety of relatively mild stressors (i.e., restriction, inversion of the light–dark cycles, water/food deprivation, damp sawdust) intermittently, in general for two to four weeks [3–6]. Behavioral and neurobiological alterations caused by the UCMS protocol in animals are usually measured during and/or immediately after stress exposure.

Through the use in particular of animal models, it has been previously shown that one of the main neurochemical systems that has been associated with stress and stress-related disorders is the serotonergic system that arises in the dorsal raphe nucleus (DR) [7–8]. Interestingly, previous evidence indicates that the DR is not a homogenous structure, but a region composed of distinct subpopulations of serotonergic and non-serotonergic neurons, both morphologically and functionally distinct [9]. It has been suggested, for instance, that these different subnuclei are distinctly activated by stress exposure and that mainly the mid-rostrocaudal and caudal parts of the DR regulate anxiety-related responses, while serotonin (5-HT) neurons of the lateral wings of the DR inhibit panic-related responses [10]. In agreement with this hypothesis, it has also been previously shown that the mid-rostrocaudal and caudal parts of the DR provide 5-HT innervation to different forebrain structures, such as the medial prefrontal cortex, the bed nucleus of the stria terminalis, the central amygdala, the dorsal hypothalamus and the periaqueductal gray [11–13], and receive afferent inputs from several brain structures implicated in the control of anxiety and anxiety-related behaviors, including the infralimbic and prelimbic cortices, the lateral habenula, the bed nucleus of the stria terminalis and the central amygdala [14–15]. On the other hand, the lateral wings of the DR send projections to brain structures that control fight/flight responses, such and the dorsolateral periaqueductal gray and the lateral hypothalamus [12–13] and receive afferents from regions associated with autonomic control, i.e., the lateral parabrachial nucleus and the nucleus of the solitary tract [15–16], and from glossopharyngeal and vagal nerves [17].

Although evidence shows that exposure to the UCMS model induces anxiety and depression-like behavior [18–20], until recently it was unclear which types of anxiety-related responses this type of chronic stress model affected. In an attempt to better elucidate the behavioral and neurobiological alterations induced by UCMS exposure, in a previous study [21] we investigated the consequences of this procedure in two distinct behavioral responses generated by the elevated T-maze model (ETM) of anxiety [22–26]. The ETM allows the measurement of avoidance and escape responses, which have been respectively associated with generalized anxiety and panic disorder [22–26]. Our results showed that avoidance responses were facilitated by UCMS exposure, an anxiogenic-like effect [21]. On the other hand, escape measurements were not altered, suggesting a lack of a panicogenic-like effect [21]. Furthermore, exposure to the UCMS procedure increased *Fos* immunoreactivity (*Fos*-ir) in different brain structures involved in the modulation of anxiety, including the DR. Unfortunately, however, in this previous study the different subnuclei of the DR were not separately analyzed. Also, we did not verify if the increases in *Fos*-ir in the DR were related to the activation of serotonergic neurons, even though the therapeutic effects of 5-HT-acting compounds on reverting the behavioral and neurobiological effects of UCMS exposure have been well described in the literature [27].

Taking the above results into account, the purpose of the present study was to better analyze the pattern of activation of the DR in response to the UCMS procedure. For that, male Wistar rats were either unstressed or exposed to the UCMS procedure for two weeks and, subsequently, analyzed for *Fos*-ir in serotonergic neurons of the DR. It is important to point out that although repeated stress reduces *Fos*-ir in some brain structures (e.g., the septum, the lateral hypothalamus and the central amygdala), others regions, such as the raphe nuclei, continue to present increased *Fos* [28]. To further verify the anxiogenic effects of this 2-week UCMS exposure, a different group of animals was tested in the light/dark transition model. As the avoidance responses of the ETM, the measurements obtained in the light/dark transition model have also been associated with generalized anxiety [29].

2. Materials and methods

2.1. Animals

Forty male Wistar rats, weighing 250–300 g (CEDEME, Federal University of São Paulo) were housed in groups of 4–6 per cage (50 cm × 60 cm × 22 cm). Room temperature was maintained at 22 ± 1 °C with lights on from 0700 to 1900 h. Food and water were freely available throughout the experiments. UCMS animals were housed under the same conditions except during the periods they were exposed to some of the mild stressors (i.e., food and water restriction/deprivation, inversion of the light/dark cycle). The study was approved by the Ethical Committee for Animal Research of the Federal University of São Paulo, under the number 121725/2013, and was performed in compliance with the recommendations of the Brazilian Society for Neuroscience and Behavior, which are based on the conditions stated by the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996).

2.2. Apparatus

2.2.1. Light/dark transition model

The apparatus used was a box made of acrylic and divided by a doorway (100 mm × 100 mm), which allowed animals to cross into two chambers of different measures: one painted black (210 × 350 × 410 mm), not illuminated, and one painted white and illuminated (210 × 450 × 410 mm).

2.2.2. Open field

An open field, composed of a round arena (60 × 60 cm), with the floor divided into 12 parts, and walls 50 cm high, was used to evaluate locomotor activity.

Luminosity at the center of the lit compartment of the light/dark transition model and of the open field was 60 Lux. After each experimental session, the apparatus were cleaned with a 10% ethanol solution.

2.3. Stress procedure

The animals were organized into two groups with 20 individuals each: control and UCMS.

2.3.1. Control

Unstressed animals remained for 15 days in the laboratory, under standard housing conditions.