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Research report

Activation of CRF/CRFR1 signaling in the basolateral nucleus of the amygdala contributes to chronic forced swim-induced depressive-like behaviors in rats

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ABSTRACT

The basolateral nucleus of the amygdala (BLA) plays a key role in processing stressful events and affective disorders. Previously we have documented that exposure of chronic forced swim (FS) to rats produces a depressive-like behavior and that sensitization of BLA neurons is involved in this process. In the present study, we demonstrated that chronic FS stress (CFSS) could activate corticotrophin-releasing factor (CRF)/CRF receptor type 1 (CRFR1) signaling in the BLA, and blockade of CRF/CRFR1 signaling by intra-BLA injection of NBI27914 (NBI), a selective CRFR1 antagonist, could prevent the CFSS-induced depressive-like behaviors in rats, indicating that activation of CRF/CRFR1 signaling in the BLA is required for CFSS-induced depression. Furthermore, we discovered that exposure of chronic FS to rats could reinforce long-term potentiation (LTP) at the external capsule (EC)-BLA synapse and increase BLA neuronal excitability, and that all these alterations were inhibited by CRFR1 antagonist NBI. Moreover, we found that application of exogenous CRF also may facilitate LTP at the EC-BLA synapse and sensitize BLA neuronal excitability in normal rats via the activation of CRFR1. We conclude that activation of CRF/CRFR1 signaling in the BLA contributes to chronic FS-induced depressive-like behaviors in rats through potentiating synaptic efficiency at the EC-BLA pathway and sensitizing BLA neuronal excitability.

1. Introduction

Chronic stress is a common factor causing stress-related mood disorders [1,2]. The amygdala has an established role in the formation and consolidation of memories for emotional or stressful events [3]. The amygdala consists of several nuclei, among those the lateral and basolateral subdivisions (LA/BLA) form the primary input nuclei while the central subdivision (CeA) forms the primary output nucleus in the amygdala's emotion-related neural circuitry [4]. The LA/BLA receive highly processed sensory information via cortical afferents coursing into the region from the external capsule (EC) [5], and subsequently transmit to the CeA, the output nucleus for major amygdala functions [6], to drive stress-related mood disorders like fear, anxiety and depression [7,8]. While the BLA-CeA pathway is important for relaying highly integrated stress-related information from the BLA to the CeA, the EC inputs via the EC-BLA synapse are proposed to be a principal regulator of BLA principal neuron activity and help modulate the expression of anxiety- and depressive-like behaviors included learned emotional responses [9]. Recently, in an animal model of chronic forced swim (FS) stress-induced depressive-like rats, we have demonstrated that chronic FS stress (CFSS) can sensitize BLA neurons via the EC-BLA pathway and subsequently facilitates synaptic efficiency (e.g. long-term potentiation, LTP) at the BLA-CeA synapse to enhance the output of CeA activities [10], supporting the notion that the BLA is involved in

Abbreviation: ANOVA, analysis of variance; BLA, the basolateral nucleus of the amygdala; CFSS, chronic forced swim stress; CRF, corticotrophin-releasing factor; CRFR1, CRF receptor type 1; EC, the external capsule; ELISA, enzyme-linked immunosorbent assay; *f*EPSPs, field excitatory postsynaptic potentials; FS, forced swim; FST, forced swim test; LTP, long-term potentiation; SPT, sucrose preference test; TST, tail suspension test

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chronic stress-related affective disorders [11,12]. However, the molecular mechanisms underlying chronic FS-induced sensitization of BLA neurons and the depressive-like behaviors in rats are largely unclear.

Corticotrophin-releasing factor (CRF) has been identified as a key neuromodulator responsible for initiating responses to various stressors [13,14]. Altered CRF signaling has been implicated in the regulation of stress-related mood disorders [15,16]. CRF expression has been identified in the BLA [17], where the CRF receptor type 1 (CRFR1) is abundantly expressed and associated with glutamatergic neuronal activity [18,19]. Repeated activation of CRFR1 in the BLA is suggested to be a contributor to stress-induced alterations in affective behavior [20], and the changes of affective behavior are supposed to be associated with a hyperexcitability of the BLA network, thereby blocking the CRF/CRFR1 signaling in the BLA results in impaired stress responses [21]. These findings suggest that the CRF/CRFR1 signaling is likely responsible for stress-induced sensitization of BLA neurons, and therefore contributes to stress-related affective disorders.

In this study, we aimed to clarify the molecular mechanisms underlying chronic FS-induced sensitization of BLA neurons and the depressive-like behaviors in rats. We mainly focus on the roles of BLA CRF/CRFR1 signaling in LTP at the EC-BLA synapse and excitability of BLA neurons. We provide valid evidence showing that chronic FS stress may activate CRF/CRFR1 signaling in the BLA, which subsequently leads to the reinforcement of LTP at the EC-BLA synapse and sensitizes BLA neurons, thereby mediating chronic FS-induced depressive-like behavior in rats. We conclude that activation of CRF/CRFR1 signaling in the BLA contributes to chronic FS-induced depressive-like behaviors in rats through potentiating synaptic efficiency at the EC-BLA pathway and sensitizing BLA neuronal excitability.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats, 8–10 weeks of age at the beginning of the experiment, were provided by the Department of Experimental Animals Sciences, Peking University Health Science Center. The rats were housed in separated cages with free access to food and water, and maintained in a temperature (20–22 °C), humidity (50–55%) and illumination (12: 12-h light: dark cycle) controlled vivarium. All procedures were approved by the Animal Care and Use Committee of Peking University.

2.2. Chronic forced swim stress (CFSS) procedure

The forced swim (FS) test is a well-established stress model used to predict the clinical efficacy of antidepressants [22]. It is now proved that chronic FS can be used for inducing cognitive impairments analogous to those observed in depression [23,24]. The behavioral and biochemical characteristics of animals in a state of learned helplessness produced by a period of inescapable swimming during the FS test have led some investigators to believe this condition itself provides a useful animal model of depression [22,25,26]. Forced swim provokes neurochemical and endocrine alterations and is used as a stressor by itself [27,28]. In this study, chronic FS was chosen as a stressor, and carried out according to the procedure as previously described [10,29]. Briefly, the rats were placed in a glass cylinder (45 cm high, 20 cm in diameter) filled with water (22–24 °C) up to a height of 30 cm. The FS procedure was conducted to rats individually once per day in 15-min sessions, continued for 14 consecutive days. According to the methods described elsewhere [30–32], control rats were subjected to a sham swimming (sham FS) sessions by allowing them to wade in the cylinder that contained only 2–4 cm of warm water at 24–26 °C. Here we used sham FS animals rather than naïve animals as control for the reason to exclude the factor of habituation for rats to the water [32,33]. Rats were allowed to dry in a warm environment (30–33 °C) after swimming. The water was changed and the container was thoroughly cleaned for each rat.

2.3. Behavioral studies

2.3.1. Measurement of body weight

Rats were weighed on the day before CFSS (day 0) and then were repeatedly weighed on days 5, 10, and 15 post-CFSS, respectively, to calculate the body weight gains during days 1–5, days 5–10 and days 10–15 post-CFSS. A positive number suggests weight increase, and a negative number indicates body weight decrease [34].

2.3.2. Sucrose preference test (SPT)

Sucrose preference test (SPT) was performed as previously described [35]. Briefly, rats were habituated to 1% sucrose solution for 48 h, and then were deprived of water for 12 h, followed by the SPT, in which each rat had free access to two bottles that contained 1% sucrose or tap water for 1 h. The position of the two bottles was varied 0.5 h in the test. In the end, the consumption was measured, and sucrose preference (%) was calculated. The SPT was carried out on the day before CFSS (day 0) and the day 24 h after last exposure of rats to CFSS (day 15).

2.3.3. Forced swim test (FST)

The forced swim test (FST), also known as the “behavioral despair” test, was developed in 1978 by Porsolt et al. [36] as a rodent model for predicting the clinical efficacy of antidepressant drugs. It is also one of the most commonly used tests to assess depressive-like behavior in animal models [37]. The modified FST was performed from a method described earlier [10,22]. In brief, twenty-four hours after the last exposure to CFSS, rats were forced to swim for 6 min as described in aforementioned methods and behaviors were monitored by video camera for subsequent analysis. 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. The time of staying immobile was recorded by an expert observer blind to the experimental conditions. The duration of immobility was recorded in the last 4 min of the 6-min testing period. This is due to the fact that most animals are very active at the beginning of the FST, and the potential effects of the treatment can be obscured during the first two minutes [33].

2.3.4. Tail suspension test (TST)

The tail suspension test (TST) also is widely used to assess depressive-like behavior in rodents and was developed by Steru et al. [38]. The test is based on the fact that animals subjected to short term, inescapable stress of being suspended by their tail will develop an immobile suspended by their tail will develop an immobile posture [39]. The rat TST was performed according to a previous publication [40]. Briefly, using the apparatus consisted of a wooden box painted gray (54 × 30 × 52 cm) with a hook in the centre of the ceiling, the rats were suspended 50 cm above the floor with adhesive tape placed approximately 1 cm from the tip of the tail. The test was videotaped and the amount of time the rats spend immobile is measured during a 6-min period of test. Immobility was defined as the absence of any limb or body movements, with the exception of those required for respiration, when the mouse hung passively and completely motionless. During the test, the rats were separated from each other to prevent visual and acoustic associations. The observers were blind to the treatment groups.

2.3.5. Assessment of locomotor function

Inclined-plate test was used for the assessment of locomotor function. Rats were placed crosswise to the long axis of an inclined plate. The initial angle of the inclined plate was 50°. The angle was adjusted in 5° increments. The maximum angle of the plate was determined on which the rat maintained its body position for 5 s without falling [41].