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Correlation of ERα/ERβ expression with dendritic and behavioural changes in CUMS mice

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HIGHLIGHTS

- CUMS increases the ratio of ERα/ERβ mRNA and protein expression.
- CUMS exposure decreases dendritic arborization but increases spine density.
- The increase in spine density is restricted to initial distance of dendrite length.

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ABSTRACT

In response to chronic stress, oestrogen receptor (ER)α acts as an anxiogenic agent as opposed to ERβ which predominantly acts as an anxiolytic agent. These properties of ER play an important role in mediating anxiety- and depression-like behaviour and physiological responses. However, the precise underlying mechanism remains unclear. In particular, not much is known about the expression of ERα and ERβ in the stress-sensitive brain regions such as the prefrontal cortex, hippocampus and amygdala. Using a rodent model of chronic unpredictable mild stress (CUMS), we report that two weeks of CUMS in young male mice (10 ± 2 weeks) induces noteworthy changes in the ratio of ERα/ERβ in the prefrontal cortex and hippocampus. While we observed a significant ($P < 0.05$) increase in ERα mRNA and protein expression levels, the expression of ERβ in the prefrontal cortex, hippocampus and amygdala was significantly reduced. This increase in ERα expression with concomitant decrease in ERβ expression was associated with increased anxiety- and depression-like behaviour as observed in elevated plus maze test, open field test, forced swim test and sucrose preference test. In addition to these behavioural changes, we report the decrease of dendritic complexity with concomitant increase in spine density in the medial prefrontal cortex, dorsohippocampal CA3 region and basolateral complex of amygdala (BLA). Taken together, these results suggest that the CUMS-induced increase in the ratio of ERα/ERβ causes dendritic remodeling, which in turn might be responsible for increase in anxiety- and depression-like behaviour in young male mice.

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1. Introduction

Exposure to chronic stress acts as a risk factor as well as a precipitating agent for mood and anxiety disorders, and chronic stress in early life manifests itself in common mental disorders in adults [1,2]. Several studies focus on the manifestation of stressful experiences in young, but there are only a few reports on the immediate consequences of early-life stress. Chronic stress activates neuroendocrine adaptive responses to cope with consequent crisis. However, due to prolonged or chronic stress, these neuroendocrine responses turn maladaptive and contribute to a phenomenon known as allostatic load [3]. These stressful events act as predisposing factors in the development of psychiatric disorders and anxiety- and depression-associated behavioural alterations in susceptible individuals [3,4].

Initially developed as a model to screen antidepressant drugs, chronic unpredictable mild stress (CUMS) is increasingly used as a means to investigate behavioural, neurochemical and structural changes underlying anxiety and depression [5,6]. CUMS acts as a suitable model with strong predictive validity as evidenced by decreased intake of sucrose, increase in anxiety- and depression-like behaviour, and reversal of these abnormalities by antidepressant drugs [7,8].

Abbreviations: ANOVA, analysis of variance; BLA, basolateral complex of amygdala; bw, body weight; CA3, cornu ammonis area 3; CUMS, chronic unpredictable mild stress; DAPI, 4,6-diamidino-2-phenylindole; dNTP, deoxynucleoside triphosphate; DPX, dibutylphathalate xylene; ECL, enhanced chemiluminiscence; EPM, elevated plus maze; ER, oestrogen receptor; FITC, fluorescein isothiocyanate; FST, forced swim test; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HRP, horseradish peroxidase; IDV, integrated density value; LTP, long-term potentiation; OFT, open field test; PBS, phosphate-buffered saline; PVDF, polyvinylidene fluoride; RT-PCR, reverse transcriptase polymerase chain reaction; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SEM, standard error of the mean; SERM, selective oestrogen receptor modulators.

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Among the neuroendocrine changes accompanying chronic stress, the interaction between gonadal hormones (estradiol, progesterone) and stress hormones (glucocorticoids) plays a pivotal role in the alteration of behaviour and complexity of neuronal circuitry. Estradiol has a fundamental role in this process as it competes against the allostatic load [9,10]. Earlier studies have established that humans and rodents with low level of estradiol exhibit symptoms of anxiety, depression and cognitive dysfunction, whereas estradiol replacement therapy ameliorates these psychological conditions [11–13]. Estradiol is also known to influence the two interconnected stress-sensitive limbic regions, the prefrontal cortex and hippocampus, by altering dendritic morphology and spine density in neurons [14,15]. To transduce these modulations, estradiol binds with similar affinity to the two types of oestrogen receptor (ER) in the brain, ERα and ERβ [16,17]. ERα and ERβ are key mediators of anxiety- and depression-modulating functions of oestrogen as ERα and ERβ null mutant mice show abnormalities in rearing behaviour, stretching rate, measures of latency and are associated with reduced threshold for synaptic plasticity in amygdala [18,19]. Activation of ERα and ERβ is found to have an antagonistic effect on fear and anxiety behaviours with the former capable of upregulation and the latter suppression of these behavioural aspects [12]. Moreover, selective oestrogen receptor modulators (SERM) are known to counteract anxiety- and depression-like behaviour in CUMS mice [20,21].

Despite these advances, there is limited knowledge about the physiological levels of ER and its role in modulating neuronal complexity in the male brain during chronic stress. Substantial progress has been made in elucidating the role of estradiol and ER in cellular and morphological changes in the prefrontal cortex, hippocampus and amygdala of the female brain after repeated stress. Studies have demonstrated that ERα and ERβ present in these regions modulate the synaptic function, even in the absence of circulating gonadal steroids in response to estradiol synthesized within neurons [22–24].

In the current study, we sought to investigate whether CUMS in young males causes any change in the expression of ER and its propensity to result in subsequent alterations in the dendritic morphology. Our data suggest that CUMS in young males increases the ratio of ERα/ERβ expression and decreases the dendritic complexity, which together are associated with an increase in the anxiety- and depression-like behaviour.

2. Methods

2.1. Animals

Young (10 ± 2 weeks, n = 54) inbred male Swiss albino mice weighing 25–30 g, maintained in the animal house of Department of Zoology, Banaras Hindu University, India, were used. Nine mice were housed per cage in a temperature-, humidity-, and light-controlled (12:12 h light/dark cycle) animal room, and provided with food and water ad libitum. Mice were divided into control and experimental groups (n = 27 each). All animal experiments were carried out in accordance with the principles and procedures outlined in the guidelines of animal ethical committee of Banaras Hindu University.

2.2. CUMS procedure

The CUMS procedure employed was a modification of published reports [5,25,26]. Experimental mice underwent CUMS exposure for 14 consecutive days as outlined in Fig. 1 and stressors were given in a random order to ensure unpredictability. Mice were subjected each day to at least one stressor that was randomly chosen from 9 different stressors. Briefly, the regimen consisted of 30 min restraint stress, 12 h rat saw dust, 12 h social isolation, 8 h intermittent light exposure, 4 h cage tilt, 12 h wet bedding, overnight light exposure, 5 min predator sense and 12 h without bedding. For restraint stress, mice were placed in clear plastic restraint bags with opening in one corner allowing free respiration but restricting any movement. The predator sense experiment was carried out by placing experimental mice with a Sprague–Dawley rat individually and care was taken to avoid any physical contact. Intermittent light exposure was given for 8 h which consisted of successive light and dark intervals of 2 h duration. For social isolation, mice were individually housed in separate cages overnight and regrouped the following morning. Alterations in the mice bedding included replacing it with rat saw dust, wet bedding by pouring 200 ml of water and no bedding. After each stressor, mice were allowed to rest for 2 h and then placed in clean cages with fresh bedding. Control mice did not receive any kind of stressor and were housed in normal conditions. After 2 weeks of CUMS exposure, mice were subjected to different behavioural tests such as elevated plus maze (EPM), open field test (OFT), forced swim test (FST) and sucrose preference test.

2.3. Behavioural tests

After the two weeks CUMS paradigm, all behavioural tests were conducted in the light phase between 8:00 AM and 12:00 PM. Mice were brought to the testing room approximately 30 min before the test, temporarily placed in a cage after each test and then regrouped to their original cage after each test. An interval of at least 24 h was maintained between successive behavioural tests. All tests were recorded with a video camera for off-line analysis.

2.3.1. Elevated plus-maze

Twenty four hours after the last stress, control and CUMS mice were subjected to EPM test [27]. The apparatus was elevated 50 cm from the ground and consisted of two open arms (30 × 5 cm, surrounded by 0.25 cm high wall) and two closed arms (30 × 5 cm, surrounded by opaque 15 cm high wall) emerging from a central platform (5 × 5 cm). Mice were placed at the centre of the maze facing an open

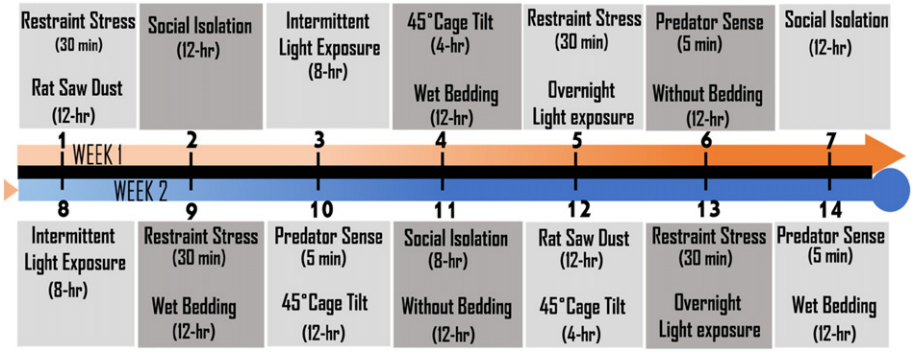

Fig. 1. Schematic representation of the CUMS protocol. Mice were subjected to nine different stressors and maximum of two stressors were given every day for two weeks (Week 1: orange; Week 2: blue). Duration of individual stressors is mentioned in the brackets below the type of stressor.

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