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Exploring the Potential Role of Nf-Kb Signaling Cascade in Stress Adaptation

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Abstract

The present study aimed to investigate the role of NF-kB signaling in stress adaptation during exposure of repeated immobilization stress of varying duration in mice. Animals were subjected to two paradigms of immobilization stress i.e., short duration (30 minutes) or long duration (120 minutes). Mice were subjected to homotypic stressor for 5 days to induce stress adaptation. Actophotometer, open field, social interaction and hole board tests were performed to assess stress-associated alteration in behavior; while serum corticosterone levels were measured as a biochemical parameter of stress induction. The levels of p-NF-kB were assessed in stress sensitive prefrontal cortex region. A single episode of short as well as long immobilization stress resulted in changes in behavior, increased plasma corticosterone levels and p-NF-kB levels in prefrontal cortex. In contrast, continuous exposure to short as well as long stress restored behavior, corticosterone and p-NF-kB levels. Treatment with diethyldithiocarbamic acid (DDTC), a selective NF-kB inhibitor, attenuated acute stress associated changes in behavior and corticosterone levels. Moreover, DDTC restored the NF-kB levels in stress subjected mice. It suggests that acute stress may increase the levels of p-NF-kB in the prefrontal cortex may be responsible for the induction of behavioral and biochemical changes. Therefore, NF-kB may serve as an important target in inducing stress adaptation in immobilization stress.

Key Words: Stress • Immobilization • Corticosterone • NF-kB • Behavior

Introduction

Hans Selye, a Canadian endocrinologist and Father of stress, described stress as a complex, but uniform response elicited in mammals during threatened homeostasis [1]. In more modern time, stress is a state of threatened homeostasis, which mobilizes a composite spectrum of adaptive physiological and behavioral response with an aim to restore the challenged body homeostasis [2]. Stress response is usually referred as an orchestrated process, which involves various mechanisms for physiological and metabolic adjustments in the body to cope with the demands of a homeostatic challenge [3]. These changes occur at the psychological (emotional and cognitive), behavioral (fight and flight), and biological level (altered autonomic and neuro-endocrine function). Stress response is mainly influenced by the duration as well as the intensity of stressors [4]. There are two key aspects of the body's response to stress. On the one hand, the body responds to stress by releasing mediators to promote adaptation and cope with a stressor, which is a crucial determinant of health and disease (However, chronic persistent stressors produce pathophysiological changes due to maladaptive signaling[5]. Accordingly, the term allostasis (achieving stability through change) is used, and it refers to an active process by which the body responds to daily events and maintains homeostasis [6]. However, allostatic load/overload either due to too much of stress or from inefficient management of allostasis (not turning off the response when it is no longer needed) leads to pathophysiological changes such as anxiety and depression [7].

It has been well described that a variety of stressors of different duration (short and prolonged) initiate the stress response in the form of activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic adrenomedullary system to increase the release of glucocorticoids and catecholamines in the blood stream, respectively (Figure 1). However, the repeated exposure of stress for few days/ weeks results in the reduction of stress response in terms of the blunted HPA axis response and normalization of behavioral deficits [8]. This blunted response to the stress stimulus during repeated exposure is referred to as 'stress adaptation' and it has been suggested to be a key protective mechanism against repeated stress exposure [9]. Individuals have an inherent capacity to cope with the psychological stress in the form of stress adaptation and there is a reduction in the sensitivity of stress responsive elements, including the HPA axis during repeated stress episodes [10]. It may also be

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characterized in terms of the restoration of behavioral alterations and normalization of neuro-endocrinological changes, in comparison to the initial stress response [11]. The stress adaptive response in an organism, in terms of blunted HPA axis response, is generally observed against stressors which are not inherently harmful [12]. Studies have shown that the repeated exposure of mild stress produce stress adaptation [13]. Furthermore, stress adaptation has also been observed against repeated episodes of severe stress of varying durations [14]. The decline of the initial stress response after repeated exposure to same stressor (homotypic) might result due to the release of endogenous neuropeptides. Indeed, the mechanisms responsible for the unresponsiveness of stress system during repeated exposures of homotypic stress are not known [15].

Figure 1: Stress-induced activation of hypothalamic–pituitary–adrenal axis and sympathetic-adrenal-medullary system.

Diverse animal models, acute as well as chronic, have been created to simulate the stress condition in animals akin to humans [16]. Electric foot shock are more frequently employed stressor by different scientists to evaluate the anti-stress activity of pharmacological interventions [17]. Electric foot shock is a complex stressor, which includes both physical and emotional components [18]. This stress paradigm comprises acute or chronic exposures of foot shocks of varying intensity and duration on the electrified grid floor in an electric foot shock apparatus [19]. Electric foot shock remains the most widely used stimulus for producing the measured amount of discomfort in animals due to its experimental advantage of control over the intensity and duration [20]. A number of studies have reported the development of adaptation in response to electric foot shock stress in terms of neural, endocrine, and behavioral responses in experimental animals [21]. Immobilization is also a frequently applied mixed stressor with both physical and psychological dimensions [22]. The struggling and muscular exertion during the immobilization process constitute the physical component of stress [23]. On the other hand, the limited movement during the immobilized position and exposure in an open area comprises the psychological stress [24]. Immobilization is well tolerated by laboratory rats and mice, and is used in both acute and chronic stress studies extending over weeks or even months. Both foot shock stressor and immobilization stressors are sufficiently intense to activate the stress-responsive system in the body, including HPA axis and the sympathetic nervous system [25].

Prefrontal cortex has been implicated in the regulation of HPA and autonomic functions [26]. Like the hippocampus, the medial prefrontal cortex expresses large numbers of glucocorticoid receptor positive neurons [27]. The lesions of the anterior cingulate and prelimbic divisions of the medial prefrontal cortex has been shown to enhance ACTH and corticosterone secretion and PVN c-fos mRNA induction following restraint stress, suggesting the inhibitory role of mPFC in regulation of HPA-axis [28]. However, during chronic stress exposure, elevated glucocorticoids have been documented to play an important role in prefrontal-dysfunction [29]. During chronic stress exposure, structural and functional degeneration in multiple PFC sub-regions such as anterior cingulate, prelimbic, and infralimbic cortices has been observed [30]. A significant loss of dendrite and spines in the pyramidal cells occurs following repeated stress exposure . Evidence suggests that in contrast to loss of dendrites in mPFC, chronic stress results increase in dendritic growth in the amygdala, which in turn initiates the imbalance in the amygdala and PFC functions . Moreover, reduced functional connectivity of the PFC and impaired PFC regulation of amygdala has also been observed in subjects with stress related disorders . Thus, it may be suggested that stress-induced PFC degeneration may contribute to the development of anxiety related disorders.

Nuclear factor-kappa B (NF-kB) is a transcription factor and it has an essential role in key physiological processes . Indeed, it is a stress-regulated key transcription factor belonging to the Rel family and plays an important role in inflammatory, innate immune responses, cell cycle and cell survival. The five different members compose the NF-κB family, including p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2). The activated NF-κB subunits collectively form the homo-or hetero-dimerized transcription factor complexes. The most widely studied form of NF-κB is a heterodimer of the p50 and p65 subunits and is a potent activator of gene transcription . Importantly, c-Rel expression in CNS plays a critical role in anti-apoptosis and reduces the age-related behaviors. NF-kB exists in the cytoplasm in an inactive form by virtue of its association with a class of inhibitory proteins called IkBs. In most of the cells, IkBa is phosphorylated and proteolytically degraded, which leads to activation of NF-kB . Studies have indicated that stress may activate NF-kB signaling in different brain regions . Koo et al has demonstrated that stress inhibits neurogenesis in hippocampus to produce depression and treatment with NF-kB inhibitor attenuated the harmful effects of stress . Cohen et al has also demonstrated increased expressions of NF-κB in hippocampus brain region in stress-induced PTSD rats and administration of pyrrolidine dithiocarbamate (NF-kB inhibitor) was shown to attenuate the PTSD-associated behavioral response in rats . Stress-induces activation of NF-kB in humans and transgenic mice subjected to psychological or immobilization stress, respectively . Our own study has also described that inhibition of NF-kB significantly attenuated the restraint stress-induced behavioral responses . In another study, benzyl butyl phthalate (industrial plasticizer) treated animals have shown to disrupt the normal learning and social behavior. However, no changes were observed in NF-kB levels in benzyl butyl phthalate treated animals .Based on above evidences, the present study aimed to investigate the key role of NF-kB in stress as well as stress adaptive response in immobilization stress-subjected animals of variable duration.

Material and methods

To execute and achieve the aim and objectives of this study following materials and methods were employed.

Animals and Drugs

Swiss albino mice (25 ± 5 g) were fed on the normal laboratory feed and were kept in the animal house. Institutional Animal Ethics Committee (IAEC) approved the experimental protocol and experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. CPCSEA/107/1999). Animals were housed in the departmental animal house and were exposed to natural cycles of 12 h light and dark and were fed on the standard laboratory feed and water.

Acclimatization of Animals

Before initiating the actual experimentation, mice were acclimatized for five minutes on the behavioral test apparatus for three days. Such acclimatization of animals to the test apparatus potentially avoids the confounding effects due to the novelty of the test apparatus and thus, reduces the variation in the experimental data . The animals were also habituated to blood withdrawal procedure by subjecting the animals to a tail vein nick procedure under resting conditions.

Experimental stress and adaptation

The animals were subjected to immobilization stress by immobilizing mice in a prone position with their limbs stretched on a board using an adhesive tape. The animals were immobilized either for 30 minutes (short duration stress) or 120 minutes (long duration stress) . The movement of the head was also restricted by keeping the head in a metal loop coiled around the neck . Mice were exposed to immobilization stress (homotypic) for five days to induce the development of adaptive behavior.

Behavioral Parameters

Ten minutes after the stress protocol, the series of behavioral tests were performed in animals with the sequence of the actophotometer, hole board, open field, and social interaction tests with a time gap of 5 minutes between the successive behavioral tests. The behavioral test equipments were cleaned after each test with alcohol and water. The behaviors in the hole board, open field and social interaction were video-recorded and then analyzed.

Actophotometer test

The locomotors activity has been considered as an index of alertness and was assessed in the actophotometer. There has been a inverted-U-shape relationship between stress and alertness. The digital actophotometer was made up of opaque metal with the square arena (30 cm×30 cm) with six in-built photo-sensors and four digital counters. The movement of animals interrupts the beam of light falling on the photocell and a count is recorded digitally. Therefore, the number of counts is directly related to the movement of animals inside the actophotometer chamber. Accordingly, the number of counts were used to indicate the locomotor activity. The animals were placed in the actophotometer for ten minutes and their activity was assessed in terms of count per 10 minutes .

Open field test

The open field test was employed to assess the stress-related behavior in rodents on the basis of changes in the exploration, general locomotor activity, and spontaneous activity . A standard open field arena was made up of square shaped plywood with opaque walls and size of 44 X 44 X 30 cm3 (height). Each mouse was exposed to open field trial for 10 minutes in a dim light room. A typical standard open field test is generally conducted under bright light to generate a high level of anxiety and the number of entries along with the time spent in the central arena are used as standard anxiety parameters for assessing novelty-based anxiety . However, in the present study, the open field testing was performed under dim light conditions in which the purpose was not to induce novelty-based anxiety, but was to assess the changes in explorative behavior in response stress exposure. The novelty-based anxiety paradigm may not be employed for retesting the state of anxiety as animals tend to familiarize with the apparatus and therefore, novelty-induced anxiety tend to decrease with retesting. In order to overcome this limitation, in the present investigation, the open field testing was performed under dim light conditions, in which the purpose of employing the open field was not to induce novelty-based anxiety . Rather, it nearly served as a neutral chamber and was employed to assess the changes in explorative behavior in response to stress. Mice were placed in the center of the open field and the number of line crossings and rearings were noted .

Hole board test

The test is based on the assumption that the number of head dippings and rearings in the familiarized environment (employed in the present study) represent the explorative behavior of animal. The animals were assessed for number of head dips and rearings. The low number of head dipping reflects the high anxiety state level, and the number of rearing represents the exploration in the novel surrounding. The mouse was placed in the center of the hole-board and was allowed to explore the apparatus for 10 minutes.

Social interaction test

The social interaction test was used to assess stress-related behavioral changes in which the behavior of a mouse with its social partner was observed during a specified test period. The social behavior test was carried out in the same box in which open field test was performed. The main principle of this test is based on the free choice by an experimental mouse (test mouse) to spend time with an unfamiliar mouse (test partner) during the experimental sessions. Rodents are actively prosocial and demonstrate apparent altruistic behaviour to fellow animals . During a 10 minutes test, each experimental mouse was allowed to interact with a marked partner mouse, which was socially housed and not subjected to any stressor. The behavior of a test mouse with test partner such as close proximity; facing and sniffing the partner; contact interaction (physical contact) and climbing over was considered as a social behavior. The remaining time interval was considered as a non-social behavior, which included actively turning away, keeping the partner at a

distance with forepaws in an upright posture, freezing, self-grooming and remaining alone.

Serum corticosterone levels

The blood sample was taken by a tail vein nick procedure, which consisted of gently placing animal in a restrainer. A 22G needle was inserted perpendicular to the vessel in a quick puncture type motion and free flowing blood drops were collected in the tube. This method is extensively used in various laboratories and the hormone levels are maintained very close to the resting levels, provided that animals had previous experience with the procedure. Thereafter, the serum was isolated by centrifugation and samples were assayed for corticosterone using a corticosterone enzyme Immunoassay Kit (K014-H1, Arbor assay).

Procedure of sample preparation

Serum samples were treated with the supplied dissociation reagent to get the total corticosterone in the serum. The dissociation reagent was allowed to warm completely to room temperature before use. 5 µL of dissociation reagent was added into 1 mL eppendorf tubes and 5 µL of serum was added to the dissociation reagent in the tube. It was vortexed gently and incubated at room temperature for 5 minutes. Then, total 10 µL solution of sample was diluted with 490 µL of diluted assay buffer.

Reagents

Corticosterone standard, DetectX corticosterone antibody, DetectX corticosterone conjugate, assay buffer, dissociation reagent, wash buffer concentrate, 3,3',5,5'-tetramethylbenzidine (TMB) as a chromogenic substrate, and stop solution.

Procedure

50 µL of samples or standards were added into wells in the plate. Then, 75 µL of assay buffer was added into the non-specific binding (NSB) wells, which served as blank. In the next step, 25 µL of the DetectX® corticosterone conjugate was added to each well using a micropipette. Afterwards, 25 µL of the DetectX® corticosterone antibody was added to each well, except the NSB wells using a micropipette. The sides of the plate were gently tapped to ensure adequate mixing of the reagents and the plate was shaken at room temperature for 1 hour. The plate was aspirated and well were washed 4 times with 300 µL wash buffer. 100 µL of the TMB substrate was added to each well, using a micropipette. Afterwards, the plate was incubated at room temperature for 30 minutes without shaking. Then, 50 µL of the stop solution was added to each well, using a micropipette. The optical density was read at 450 nm. The standard plot was prepared between the range of 10-100 ng/ml. The ELISA kit is based on competitive ELISA, therefore, there is an inverse relationship between corticosterone concentration and optical density.

Brain sample preparation

After sacrificing the mice, the prefrontal cortex was isolated and was homogenized in 1 ml of lysis buffer (137 mM NaCl, 20mM Tris-HCL, pH 8.0, 1% NP-4, 10% glycerol and protease inhibitor). Thereafter, the homogenized mixture was centrifuged for 20 minutes at 3000 rpm and the supernatant was removed. The supernatants were employed for ELISA-based estimation of NF-kB levels using microplate reader and the procedure was conducted in accordance with the instructions of the manufacturer.

Reagents

Mouse NF-kB biotin conjugated detection antibody, standard, streptavidin: HRP conjugate, wash buffer, standard diluent, substrate A, substrate B, stop solution.

Procedure

The kit uses a double-antibody sandwich ELISA to assay the levels of mouse NF-kB in the samples. A sample containing NF-kB was added to wells pre-coated with monoclonal NF-kB antibody. After incubation, secondary antibodies labeled with biotin against NF-kB were added followed by streptavidin- HRP to form immune complex. An unbound immune complex was removed by washing step. This was followed by addition of chromogenic solutions A and B, which led to development of blue color. Thereafter, stop solution was added to stop the reaction. The concentration of NF-kB was directly proportional to the color development.

All reagents were kept at room temperature prior to use. 50 µl of standards or samples were pipetted into the different wells. 10 µl of biotin conjugate was added into each sample well, excluding the blank. Thereafter, 50 µl of streptavidin-HRP conjugate was added into sample and standards wells, excluding the blank well. The plate was incubated for 1 hour at 37 °C in the incubator. Then, the plate was aspirated and washed 4 times with 1x wash buffer. Then, substrate A (50 µl) and substrate B (50 µl) were added to each well, including blank. The contents were gently mixed, incubated for 10 min at 37 ℃ in dark. 50 µl of stop solution was added in the wells. The color was turned from blue to yellow in color and the absorbance was read at 450 nm within 15 minutes after adding the stop solution. The standard plot was prepared between the range of 0.75-12 ng/ml. The concentration of NF-kB (ng/ml) was converted to % age, considering the value of non-stress control as 100 % and values in other groups was also expressed in % age.

Experimental Protocol

Thirteen groups, each comprising of seven Swiss albino mice, were employed in the present study. Due to ethical concerns, five animals from main representative groups, including I, II, III, V, VI, VII, VIII and IX were sacrificed and prefrontal cortex was isolated to estimate the p-NF-kB levels.

Group I: Non-stress

The mice were not subjected to any stressor and different behavior tests were performed in these non stressed animals. The corticosterone levels in the serum and p-NF-kB in the prefrontal cortex were also measured.

Group II: Acute short immobilization stress

Mice were subjected to a single episode of immobilization stress for 30 minutes. Thereafter, behavioral and biochemical tests were performed in acute stress subjected mice.

Group III: Stress adaption in short immobilization

Mice were subjected to five episodes (once daily) of 30 minutes immobilization for 5 days. Thereafter, behavioral and biochemical tests were performed in stress adapted mice.

Group IV: Acute long immobilization stress

Mice were subjected to long immobilization stress of 120 minutes to produce acute stress. Thereafter, behavioral and biochemical tests were performed in acute stress subjected mice.

Group V: Stress adaptation in long immobilization stress

Mice were subjected to five episodes (once daily) of immobilization of 120 minutes for 5 days. Thereafter, behavioral and biochemical tests were performed in stress adapted mice.

Groups VI: Diethyldithiocarbamic acid (150 mg/kg i.p.) in acute short immobilization stress

Diethyldithiocarbamic acid (150 mg/kg i.p.) was given 30 minutes before subjecting to short immobilization stress. Thereafter, behavioral and biochemical tests were performed in acute stress subjected mice.

Groups VII: Diethyldithiocarbamic acid (150 mg/kg i.p.) in acute long immobilization stress

Diethyldithiocarbamic acid (150 mg/kg i.p.) was given 30 minutes before subjecting to long immobilization stress. Thereafter, behavioral and biochemical tests were performed in acute stress subjected mice.

Group VIII: Vehicle in short immobilization stress control

The vehicle of diethyldithiocarbamic acid i.e., DMSO (4%dissolved in normal saline) was given 30 minutes before subjecting to acute short immobilization stress. Thereafter, behavioral tests and corticosterone levels were measured in acute stress subjected mice.

Group IX: Vehicle in long immobilization stress control

The vehicle of diethyldithiocarbamic acid DMSO (4%) was given 30 minutes before subjecting to acute long immobilization stress. Thereafter, behavioral tests and corticosterone levels were measured in acute stress subjected mice.

Group X: Diethyldithiocarbamic acid (150 mg/kg i.p.) per se

Diethyldithiocarbamic acid was given in normal (no stress) mice. Thereafter, behavioral tests and corticosterone levels were measured in these mice.

Statistical Analysis

The results were represented in mean ± standard deviation (S.D.). One way ANOVA was used to compare multiple groups and Tukey's multiple comparison test was used to compare different experimental groups. The p value<0.05 was used to find the statistical significance.

Results

Effect of acute and repeated exposure of immobilization stress on the behavioral parameters

A single exposure of short (30 minutes) as well as long duration immobilization stress (120 minutes) also led to significant decrease in behavioral activities including the locomotor activity in actophotometer (Figure 2), number of head dips and rearing in the hole board (Figures 3 and 4), total motor activity i.e. rearings and line crossings in the open field (Figures 5 and 6) and time of following in social interaction test (Table 1) on day 1 as compared to non-stress control. The behavioral alterations were significantly higher in prolonged stress as compared to short immobilization stress subjected mice. However, acute stress-induced behavioral alteration were significantly restored on repeated exposure of immobilization stress for five days, in short as well in prolonged immobilization stress subjected mice.

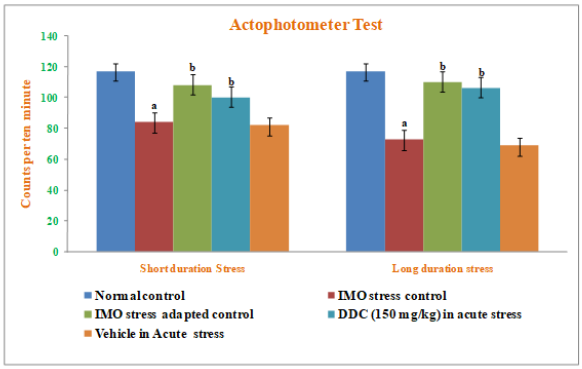

Figure 2: Locomotor activity in terms of counts in 10 minute time interval in the actophotometer test. a= p<0.05 as compared to normal; b= p<0.05 as compared to stress.

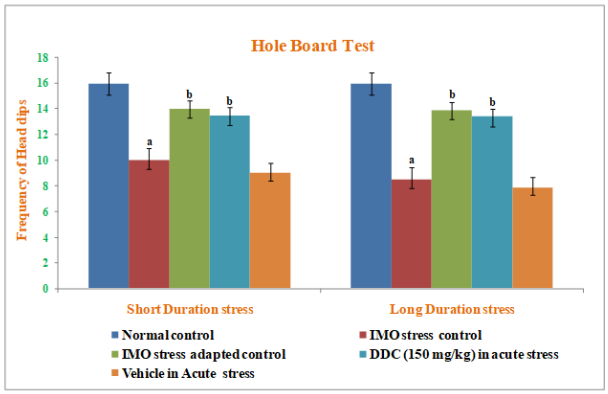

Figure 3: Frequency of head dips in the hole board test a= p<0.05 as compared to normal; b= p<0.05 as compared to stress.

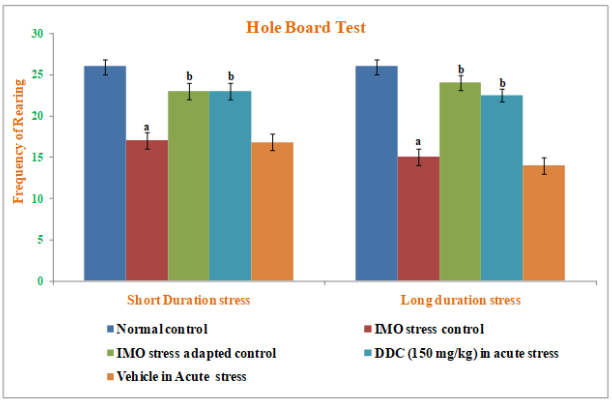

Figure 4: Frequency of Rearing in the hole board test a= p<0.05 as compared to normal; b= p<0.05 as compared to stress.

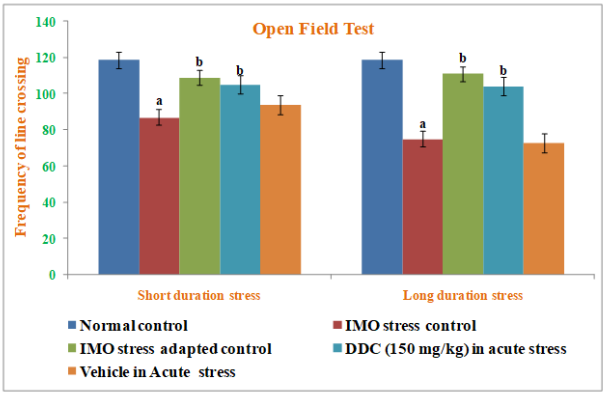

Figure 5: The frequency of line crossing in the open field test. a= p<0.05 as compared to normal; b= p<0.05 as compared to stress.

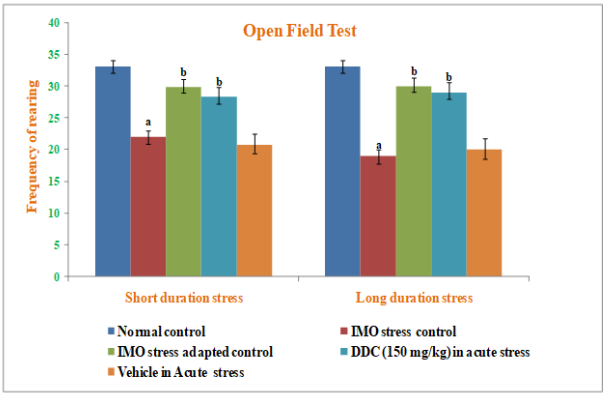

Figure 6: The frequency of rearing in the open field test. a= p<0.05 as compared to normal; b= p<0.05 as compared to stress.

S. No	Experimental Groups	Social Interaction Tests	
		Following time (s)	Avoidance time (s)
	Non-stress	528.0±11.8	72.0 ± 11.8
	Acute short IMO stress	110.0±17.3a	490.0±17.3a
	Short IMO Stress adapted	495.0±9.5b	105.0±9.5b
	Diethyldithiocarbamic acid (150 mg/kg) in acute short IMO stress	499.5± 16.6b	100.5±16.6b
	Acute long IMO stress	89.5±16.3a'	510.5±16.3a'
	Long IMO Stress adapted	520.4 ± 9.9b'	79.6± 9.9b'
	Diethyldithiocarbamic acid (150 mg/kg) in acute long IMO stress	493.5± 16.3b'	106.5±16.3b'
	DMSO (vehicle) in acute short IMO stress	107.5± 8.2	492.5±8.2
	DMSO (vehicle) in acute long IMO stress	98.5± 9.8	501.5±9.8

Table 1: The time of following and the time of avoidance in social interaction test. a= p<0.05 as compared to normal of day 1; b= p<0.05 as compared to acute stress.

Effect of acute and repeated immobilization stress on the serum corticosterone levels

Acute stress exposure to short (30 minutes) and long duration immobilization stress (120 minutes) also produced a significant increase in the serum corticosterone levels as compared to non-stress control mice. However, following repeated exposure of short and prolonged immobilization stress, the serum corticosterone levels were restored on the 5th day and the levels were comparable with the levels of corticosterone on the 5th day of non-stress control (Figures 7 and 8).

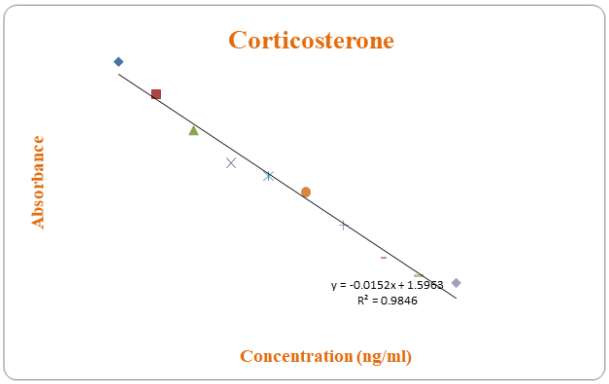

Figure 7: Standard plot of corticosterone.

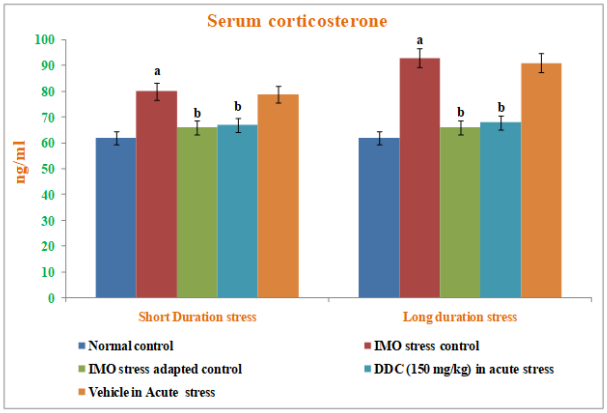

Figure 8: The changes in serum corticosterone levels. a= p<0.05 as compared to normal; b= p<0.05 as compared to stress.

Effect of acute and repeated short/long immobilization stress-induced changes in p-NF-kB expression levels

A single exposure of short or prolonged immobilization stress significantly increased the p-NF-kB levels in the prefrontal cortex in comparison to non-stressed mice. Further, on repeated exposure of immobilization stress for five days, the levels of p-NF-kB were significantly restored in the prefrontal cortex as compared to acute stress subjected mice (Figure 9, 10 and 11).

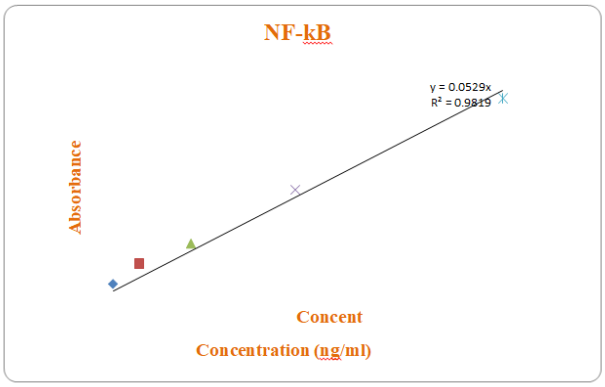

Figure 9: Standard plot of NF-kB using ELISA.

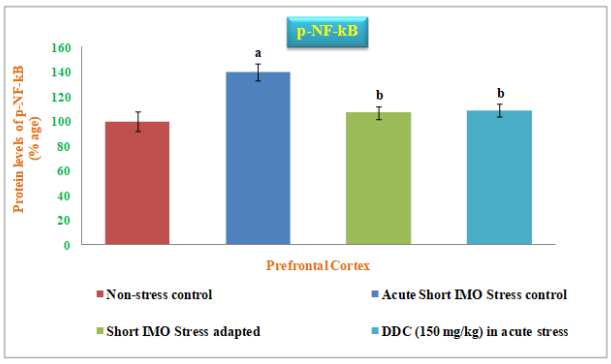

Figure 10: Assessment of the p-NF-kB levels in the prefrontal cortex in short duration immobilization stress. a= p<0.05 as compared to normal; b= p<0.05 as compared to stress.

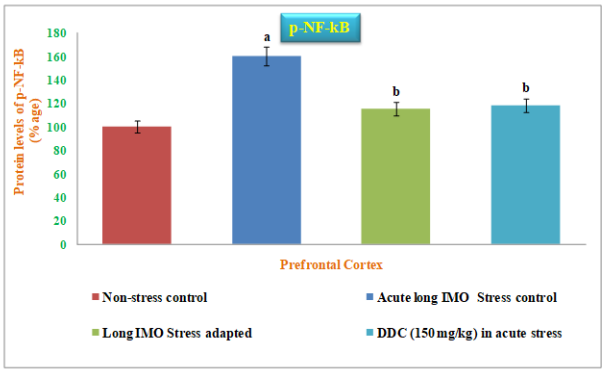

Figure 11: Assessment of the p-NF-kB levels in the prefrontal cortex in long duration immobilization stress. a= p<0.05 as compared to normal; b= p<0.05 as compared to stress.

Effect of diethyldithiocarbamic acid and repeated stress exposure on short and long immobilization stress-induced behavioral and biochemical alterations

A single administration of diethyldithiocarbamic acid (150 mg/kg) significantly restored acute short and long immobilization stress-induced decrease in locomotor activity (Figure 2), number of rearings and head dips in the hole board (Figures 3 and 4); total motor activity i.e. rearings and line crossings in the open field (Figures 5 and 6) and social behavior on the first day of stress exposure (Table 1). In addition, similar treatment also restored corticosterone levels in acute short and long stress-subjected mice (Figure 8). Administration of vehicle did not modulate short or prolonged immobilization stress-induced changes in behavior and serum corticosterone levels in a significant manner. Per se administration of diethyldithiocarbamic acid (150 mg/kg) in non stressed mice did not modulate behavior and serum corticosterone levels in a significant manner.

Discussion

In the present study, acute exposure to short (30 minutes) and prolonged (120 minutes) immobilization stress produced the significant behavioral and biochemical alterations on the first day as compared to non-stress control mice. There was a significant decrease in locomotor activity; spontaneous and orientation-investigating activities and alteration of social behavior. Moreover, acute exposure to immobilization of varying duration also produced the significant increase in the serum corticosterone levels as compared to non-stress control mice. Corticosterone is a biochemical parameter of HPA axis activation and is very widely employed as a stress parameter . These alterations in response to 120 min immobilization were significantly higher as compared to 30 min immobilization stress. Immobilization is also one of the most frequently applied stressors. In the immobilization stress, the struggling and muscular exertion during the immobilization process constitute the physical component of stress. On the other hand, the limited movement during the immobilized position and exposure in an open area comprises the psychological stress . Different studies have reported the development of behavioral and biochemical alterations in response to immobilization stress in experimental animals and the animals exhibit the behavioral deficits of varying

degree depending on the duration of stress . However, repeated exposure to short as well as prolonged immobilization stress for five days resulted in the restoration of behavioral changes, indicating the development of stress adaptation. Furthermore, in repeated stress (short as well as prolonged) subjected animals, the serum corticosterone levels were comparable to non-stressed animals on 5th day, again suggesting the development of stress adaptation. The observed adaptive responses on repeated exposure to immobilization stress are consistent with the earlier reports demonstrating the development stress adaptation following repeated stress exposure .

Adaptation is a behavioral and physiological response with an aim to return the organism to a basal state following the stress exposure. The body responds to stress by releasing mediators that promote adaptation and cope with a stressor, and these mediators are the crucial determinants of health and disease. To explore the role of NF-kB signaling in acute stress and stress adaptation, the levels of NF-kB were measured in the prefrontal cortex. The prefrontal cortex is a highly stress responsive brain region and has been implicated in stress related changes in motor behavior, behavioral flexibility, regulation of affect, working memory, decision making and planning . There have been number of studies of rodents and human research indicating the important role of prefrontal cortex in stress adaptive processes . It has been demonstrated that prefrontal cortex is well positioned to act as a coordinator of autonomic and neuroendocrine responses, supporting the energetic mobilization needed for behavioral adaptation . Moreover, there are number of evidence suggesting that stress-induced deficits in the rodent prefrontal cortex region of brain are similar to that of stress related disorder in humans including depression and anxiety . In stress related disorders including anxiety and depression, significant changes have been observed in this specific brain region in humans including reduction of grey matter volume in the left subgenual cingulate cortex and hyper and hypoactivation depending upon the patients . The dysregulated activity of mPFC prevents appropriate response to stress, inability to effectively coordinate these response and, may lead to neuropsychiatric disorders.

A single episode of short and long immobilization produced an increase in p-NF-kB levels in the prefrontal cortex in comparison to non stressed animals. NF-kB is a widely expressed transcription factor in the central nervous system, including the prefrontal cortex region . Exposure to acute stress has been documented to trigger the signaling cascade involving the activation and potentiation of NF-kB in the brain . Furthermore, studies have shown that acute stress exposure may activate the NF-kB dependent signaling to induce stress-associated deleterious effects . A recent study has also documented an increase in NF-kB expression in the prefrontal cortex in the chronic unpredictable mild stress-induced depression in mice . Different type of stress exposures including social isolation, immobilization, electric foot shock stress has been shown to regulate the NF-kB transcriptional factor. Increased NF-kB levels have been observed in chronic isolation stress-induced anxiety and depression-like behavior in the hippocampus region of rats . Further, increased NF-kB levels have also been observed in the LPS-induced depression-like behaviour . Rejitha et al has also demonstrated the up-regulation of NF-kB in alcohol-induced neurotoxicity in brain . In contrast, decreased NF-kB levels were found in the mild stress (constant light for one day) subjected animals in the hamster pineal gland . In another study, increase in NF-kB levels has been reported in the epidermal tissue of mice in repeated restraint stress subjected mice .

In the present study, on repeated exposure of electric foot shocks, the normalization of p-NF-kB levels in the medial prefrontal cortex was observed. It probably suggests that acute stress-induced up-regulation of NF-kB dependent signaling may contribute in the development of the stress response and the normalization of this signaling cascade during repeated stress exposure may possibly contribute in the development of stress adaptation. Furthermore, administration of diethyldithiocarbamic acid, a selective NF-kB inhibitor, attenuated acute foot shock stress-induced behavioral and biochemical alterations again suggesting that the up-regulation of NF-kB may contribute in stress induction and its down-regulation may contribute in attenuating stress associated deleterious effects (Figures 12 and 13).

Above evidences suggest that NF-kB is the key regulator in the number of neurological disorders. Our previously published studies indicate that in stress adaptation process, there is a key role of angiotensin II in short duration immobilization stress and opioids in long duration immobilization stress . Nevertheless, the results of present study suggest that involvement of NF-kB signaling system irrespective of duration of immobilization stress. Therefore, it is possible to suggest that the upstream mediators may be different (i.e. angiotensin II and opioids) in inducing stress adaptation; however, the downstream signaling may be same and NF-kB may serve as an important target in inducing stress adaptation.

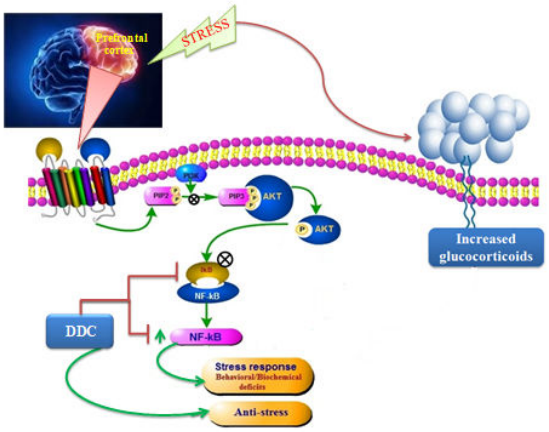

Figure 12: Representation of signaling cascade involving p-NF-kB levels during stress induction.

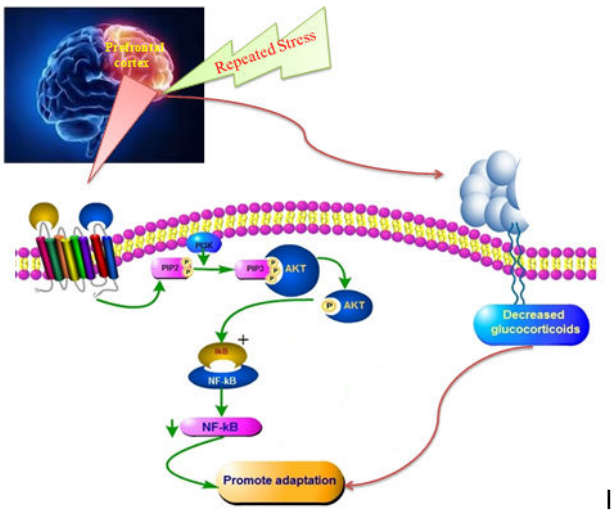

Figure 13: Representation of signaling cascade involving p-NF-kB levels during stress adaptation.

Conclusion

The up-regulation of NF-kB signaling in the prefrontal cortex may contribute in inducing behavior and biochemical changes. The restoration of NF-kB signaling may be associated with development of stress adaptation in experimental stress.

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