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Modulations on Neurogenesis Due to Chronic Unpredictable Stress in Adult Male Albino Rats: Overactivation of Neural Stem Cells and Microglia

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Abstract

Background: Chronic unpredictable stress could alter the synaptic regulation, morphological changes of neurons, and adult neurogenesis. The severity of these changes varies in proportional to the duration and intensity of stress.

Aim of Study: This study aimed to investigate the deleterious effect of the chronic unpredictable stress on the brain's cognitive function, focusing on the expected modulations on the adult neurogenesis in the hippocampus.

Material and Methods: Twenty-four adult male albino rats were divided equally into two groups. Control group (stayed safe with free access to food and water), and chronic unpredictable stress group exposed to different stressors at different times for 25 days. Barnes Maze assessed cognitive performance (spatial learning and memory performance). We evaluated serum corticosterone serum level, oxidative stress, and the antioxidant capacity in the hippocampus. The hippocampal neural cell proliferation was assessed using basic fibroblast growth factor2 (bFGF2), histological and immunohistochemical examinations using an ionized calcium-binding adaptor (Iba-1) for microglial activity and doublecortin (DCX) as an indicator of hippocampal adult neurogenesis.

Results: The chronic unpredictable stress group showed a significant decline (p<0.05) in cognitive performance. Also, it exacerbated the oxidative stress in the hippocampus in parallel with high corticosterone serum levels. Increased expression of bFGF2 and DCX in chronic unpredictable stress revealed the tendency of the neural stem cells towards proliferation.

Conclusion: The deleterious effect of the unpredictable chronic stress on the hippocampus is partly due to the over activation of the neural stem cells and microglia, leading to an incomplete process of neurogenesis proved by the deteriorated behavioral tests.

Key Words: *bFGF2 – Stress – Hippocampal – Microglia – Neurogenesis.*

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Introduction

STRESS is defined as a mismatch/disruption between the person and its environment [1]. The repeated stress elicits continuous inflammation all over the body leading to many health problems, like diabetes and heart disease [2]. Chronic stress increases the risk of developing neuropsychiatric illnesses such as depression, schizophrenia, and drug use relapse [3]. Unpredictability, lack of information, and loss of control produce multiple stress responses. These responses are emotional disturbances, endocrinological disorders, and pathological changes in brain areas involving mainly cognition and memory formation [4]. In particular, stress leads to structural and functional changes affecting synaptic plasticity, neuronal morphology, and adult neurogenesis in the hippocampus. The severity of these changes varies in proportion to the duration and intensity of stress [5]. Stress hormones, mainly cortisol, adrenaline, and noradrenaline, lead to deleterious neuronal effects by crossing the blood-brain barrier during chronic stress [6]. The high level of cortisol is a major trigger of the inflammatory cycle in the hippocampus [7]. This part of the brain is a cornerstone for declarative (episodic) memory in humans and spatial memory in rats. It aids in merging information to form the memory space and eases information in both types of memory [8]. Chronic stress enhances the generation of the reactive oxygen species by corticosteroids whose receptors are highly expressed on microglia [9]. This effect leads to morphological and behavioral changes in microglia and loss of the oxidative/antioxidant balance [10]. Deleterious modifications were linked to inducing morphological changes in the hippocampal cells as a decrease in the dendritic spines, synaptic

pruning, and reduction in the volume of the hippocampus [11]. Stress also affects factors regulating adult neurogenesis in the hippocampus [12]. Adult neurogenesis is the process of developing functional neural cells from the neural stem cells during adulthood. It is the only process by which neural cells can be generated. The main function of neurogenesis remains in its strong contribution to the learning and memory process [13]. In the hippocampus, neurogenesis starts by dividing neural progenitor cells in the subgranular zone [14]. Many factors have been proven to regulate neurogenesis; one crucial factor is bFGF2 [8]. It is becoming essential to study the different mechanisms responsible for chronic unpredictable stress on adult neurogenesis. Studies demonstrated different and controversial results regarding neural stem cell behavior in response to exposure to chronic stress [12,15]. We aimed by our study to unravel some mechanisms related to the abnormal neurogenesis resulting from the exposure to chronic unpredictable stress. We explained that although we may observe increased hippocampal neurogenesis accompanying stress, the enhanced neural growth may not continue to be mature neuronal cells. We proved our expectations by demonstrating the changes in the cognitive, functional, and structural aspects accompanying the chronic unpredictable stress. We also demonstrated the microglial contribution to the abnormalities detected histologically while the rats were exposed to the chronic unpredictable stress.

Material and Methods

Animals 24 adult male albino rats aged four months and weighed 170-200 grams. The rats were brought and housed in the animal house under standardized conditions, away from any stressful stimuli with 12 hours a day/light cycle, $25 \pm 1^{o}C$ temperature and humidity controlled environment with free access to food and water available ad libitum for 1 week acclimatization before being subjected to any stress. Any animals with overt signs of illness were removed from the study. The protocol of this study was approved and conducted according to the ethical guidelines by the Research Ethics Committee, Faculty of Medicine, Suez Canal University, Egypt, protocol no (#3004).

Experimental design the rats were randomly divided into two groups. Control group (Group I): Rats of this group were kept with standardized conditions, away from any stress, and had free access to food and water ad libitum. Chronic unpredictable stress group (Group II): Rats of this group were subjected to different stressors at different times to prevent predictability and adaptation. The schedule (Table 1) was modified from (16, 17) and applied to the animals in group II. On day 26th, all rats in both groups were submitted to the behavioral test (Barnes maze test) 24 hours after the last stressor (the exposure to bright light) and lasted for four days, including the test day.

Table (1): Chronic unpredictable stress model applied to adult rats.

Time	Stressor
Day 1	Inverse light-dark cycle
Day 2 (9:00 AM - 9:00 AM)	Water deprivation for 24 hours
Day 3 (12:00 PM - 12:00 PM)	Food deprivation for 24 hours
Day 4 (4:00 PM - 4:00 PM)	Hot environment and overcrowding for 24 hours
Day 5 (at 7:00 PM)	Immobilization for 15 minutes (rats located in
Day 6 (at 9:00 AM)	Cold swimming for 1 hour
Day 7 (10:00 AM - 10:00AM)	Isolation for 24 hours
Day 8 (12:00 PM - 12:00 PM)	Water and food deprivation for 24 hours
Day 9 (at 2:00 PM)	Tail pinch for 1 minute
Day 10	Inverse light-dark cycle
Day 11 (at 4:00 PM)	Immobilization for 30 minutes
Day 12 (at 9:00 AM)	Cold swimming for 2 hours
Day 13 (11:00 AM - 11:00 PM)	Hot environment and overcrowding for 24 hours
Day 14 (7:00 AM - 7:00 AM)	Isolation for 24 hours
Day 15 (at 1:00 PM)	Tail pinch for 2 minutes
Day 16 (10:00 AM - 10:00 AM)	Water and food deprivation for 24 hours
Day 17 (12:00 PM - 12:00 PM)	Hot environment and overcrowding for 24 hours
Day 18 (at 3:00 PM)	Immobilization for 1 hour
Day 19 (8:00 AM - 8:00 AM)	Exposure to bright light for 24 hours
Day 20 (At 5:00 PM)	Tail pinch for 2 minutes
Day 21 (8:00 PM - 11:00 PM)	Cold swimming for 3 hours
Day 22 (7:00AM - 7:00 AM)	Hot environment and overcrowding for 24 hours
Day 23 (8:00 AM - 8: 00 AM)	Isolation for 24 hours
Day 24 (1:00 PM - 3:00 PM)	Immobilization for 2 hours
Day 25 (6:00 PM - 6:00 PM)	Exposure to bright light for 24 hours

Behavioral assessment of the rats by Barnes maze the rats were in their standard home cages in the test room about 30 minutes before beginning the first trial for habituation. The maze was put directly centered below lights with its escape cage (29cm x 19cm x 13cm) under the designated escape hole, and other holes were plugged to prevent falling out. The tester's chair was approximately 122cm from the nearest edge of the maze top and remained in the same location throughout testing. The habituation day: The rat was left for 3min on the maze after removing the plastic box after 1 0sec, and the overhead light was turned on. If it failed

to find the escape cage within 3 minutes, it was gently guided to the correct location in the escape cage in which the rat stayed for 2 minutes, and then the rat was removed from the escape cage and returned to the home cage. The maze surface and escape cage were cleansed regularly with 70% ethanol and left dry after each trial. Each rat was tested for two trials/day with an inter-trial interval of approximately 30min. All these steps were repeated until all rats were tested for three consecutive days. The probe (test) day: We started the test day following a 24-h delay from the training day, in which the escape box was removed, and all the cues remained in the same place. The rat was tested for remembering what had been previously learned. Acquisition in the training phase typically was assessed by time latency obtained from each rat to reach the targeted hole.

Sample collection and processing: After the test, the rats were anesthetized by diethyl ether, and blood samples were collected by the orbital sinus blood sample method From the serum, assess the corticosterone level Then, rats were sacrificed by decapitation. Their brains rapidly removed and bisected along the midline: The hippocampus was bisected from the left hemibrain, a part stored in –20ºC for ELISA assessment of oxidant and total antioxidant and the other part stored in –80ºC for polymerase chain reaction (PCR). The right hemibrain was fixed in 10% neutral-buffered formalin and processed for paraffin embedding. Sections from these blocks were cut in the parasagittal plane at 5 µm for histological staining and immunostaining.

Serum corticosterone level measured by ELISA: The concentration of corticosterone serum level in both groups was determined by ELISA kits (CS-E07014r, Cusabio Co., Wuhan, China) to evaluate its response to the stress level (Wynne-Edwards et al., 2013).

Assessment of hippocampal tissue malondialdehyde level by ELISA: The hippocampal tissue homogenate was used to measure the antioxidant level of malondialdehyde (MDA). The MDA level was determined by using ELISA [Catalog No.:MBS268427, MyBioSourceCo, San Diego, CA, USA] **[18]**.

Hippocampal tissue total antioxidant (TAC) concentration by colorimetric method (TAC) in the hippocampal tissue was measured using a colorimetric method (Fraga et al., 2014).

Hippocampus tissue basic fibroblast growth factor 2(bFGF2) expression by RT-PCR: (bFGF2) was assessed to determine CUBS effect on the hippocampus cells. Hippocampus tissue was homogenized, and a total RNA isolation system was used (Promega, Madison, WI, USA) to extract RNA. Ultraviolet spectrophotometer quantification was done to measure RNA concentration and purity.

Histopathological examination H&E staining: Imaged x 10 and x 100 for histopathological changes in the hippocampus in the form of shrunken cells, cytoplasmic and nuclear changes. The mean thickness of both the cornu ammonis & dentate gyrus in H&E stained sections was done using Pro Plus software in the histology department, faculty of Medicine, Suez Canal University. Five high power fields (HPF)/section, and three sections/animal were examined and imaged.

Immunohistochemistry for Ionized calcium-binding adaptor molecule (Iba-1) and Doublecortin (DCX): Five µm paraffin-embedded sections from the right hemi-brain were mounted on positively charged slides. Sections were put in an incubator for 10 minutes at 60 °C for proper adhesion of tissue sections on slides. Sections were deparaffined in two subsequent xylene baths, 10 minutes each, then rehydrated through descending concentrations of ethanol till 70%, each of ten seconds, then slides were placed in distilled water. Slides were incubated overnight with mouse anti- Iba-1 [CATALOG # sc-32725, Santa Cruz Biotechnology, Dallas, TX, USA] in concentration 200 µg/ml within the hippocampus [brown coloration of the cell membrane and cytoplasm of the microglia] or to doublecortin immunostaining [# sc-271390, Santa Cruz Biotechnology, Dallas, TX, USA] in concentration 1:400, within the dentate gyrus; a marker for immature neurons (indicated by brown coloration) as evidence of chronic stress on the hippocampal adult neurogenesis. Slides were imaged at magnification power x40 by a PC-driven digital camera on a light microscope [Leica, Model: DM 1000].

Statistical analysis: All data were subjected to statistical assessment using Graph Pad Prism software (version 5.01, San Diego, USA). The difference between both groups was assessed by unpaired *t*-test analysis. The obtained data were presented as means and standard error of the mean (SEM). Significance was designated at a probability value of $p<0.05$.

Results

Unpredictable chronic stress causing memory deficit in adult male rats: The latency measured by Barnes maze to reach the escape hole (Fig. 1-A) increased in group II compared to group I (23.11 ±2.214 vs. 11.33±1.344 sec; $p<0.05$), respectively,

indicating a shortfall in spatial search learning strategy and revealing memory deficits in stressed animals.

Unpredictable chronic stress causing an increase in serum corticosterone level: We assessed the effect of stress on corticosterone level (Fig. 1-B) in stressed (group II) and control (group I) animals. Chronic unpredictable stress significantly increased serum corticosterone level in group II compared to group I; (114.5 ±4.25 vs. 104.8± 1.185 pg/ml; $p<0.05$).

Unpredictable chronic stress causing oxidative stress in hippocampus: The impact of stress on TAC (Fig. 2-A) and MDA (Fig. 2-B) levels were measured in the hippocampus of the stressed and unstressed animals. In group II, stress decreased the TAC level significantly compared to group I (73.99± 13.76 vs. 120.3± 11.56U/mg; $p<0.05$), respectively. In contrast, stress increased MDA level in group II compared to group I (1.4 ±0.108 vs. 1.125±0.025 U/mg; $p<0.05$), respectively. Increased hippocampus reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde and causing toxic stress in cells.

Unpredictable chronic stress enhances hippocampus bFGF2 expression: Basic fibroblast growth factor 2 (bFGF2), a growth factor and signaling protein encoded by the FGF2 gene, regulate cell survival activities, especially the hippocampus neural stem cell proliferation. Stressed Group II showed a significantly increased b FGF2 expression almost 7-folds compared to control (Group I) (6.951 ±1.79 vs. 1.0±0.001; $p<0.05$) respectively (Fig. 2-C).

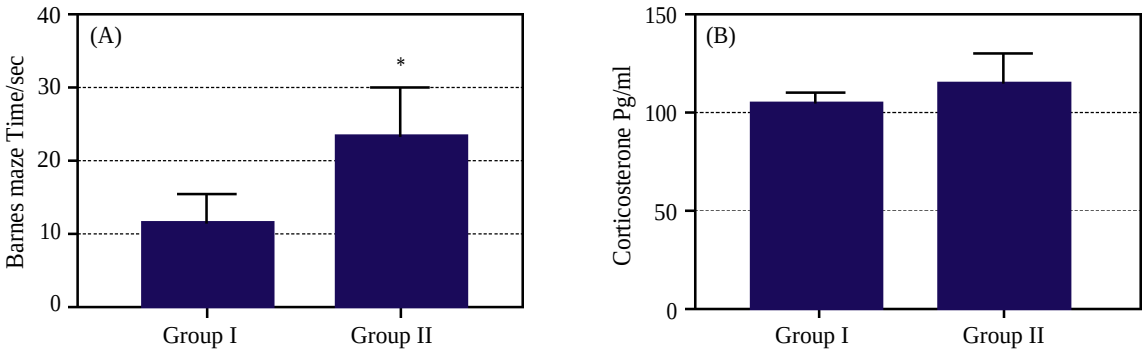


Fig. (1): Effect of chronic unpredictable stress on Barnes maze and corticosterone level in blood. (A): Barnes maze test results for control non-stressed adult rats (Group I) and adult rats exposed to unpredictable stress (Group II). (B): Serum blood corticosterone measurements level in both groups. Values are mean ± SEM. (n=10), analyzed by *t*-test. X $p<0.05$; compared with control group I.

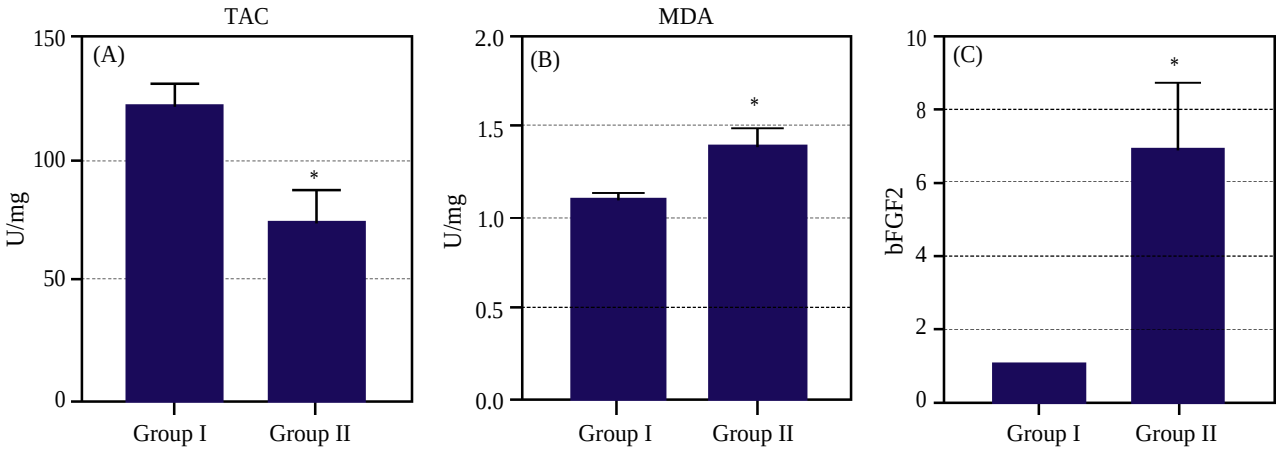


Fig. (2): Effect of chronic unpredictable stress on TAC, MDA and bFGF2 level in hippocampal tissues. (A): Total antioxidant capacity (TAC); (B): Malondialdehyde (MDA); (C): b-Fibroblast Growth Factor 2 (bFGF2) in hippocampal tissue of control (Group I) and stress (Group II) animals. Values are mean ± SEM. (n=10), analyzed by *t*-test. X $p<0.05$; Compared with control group I.

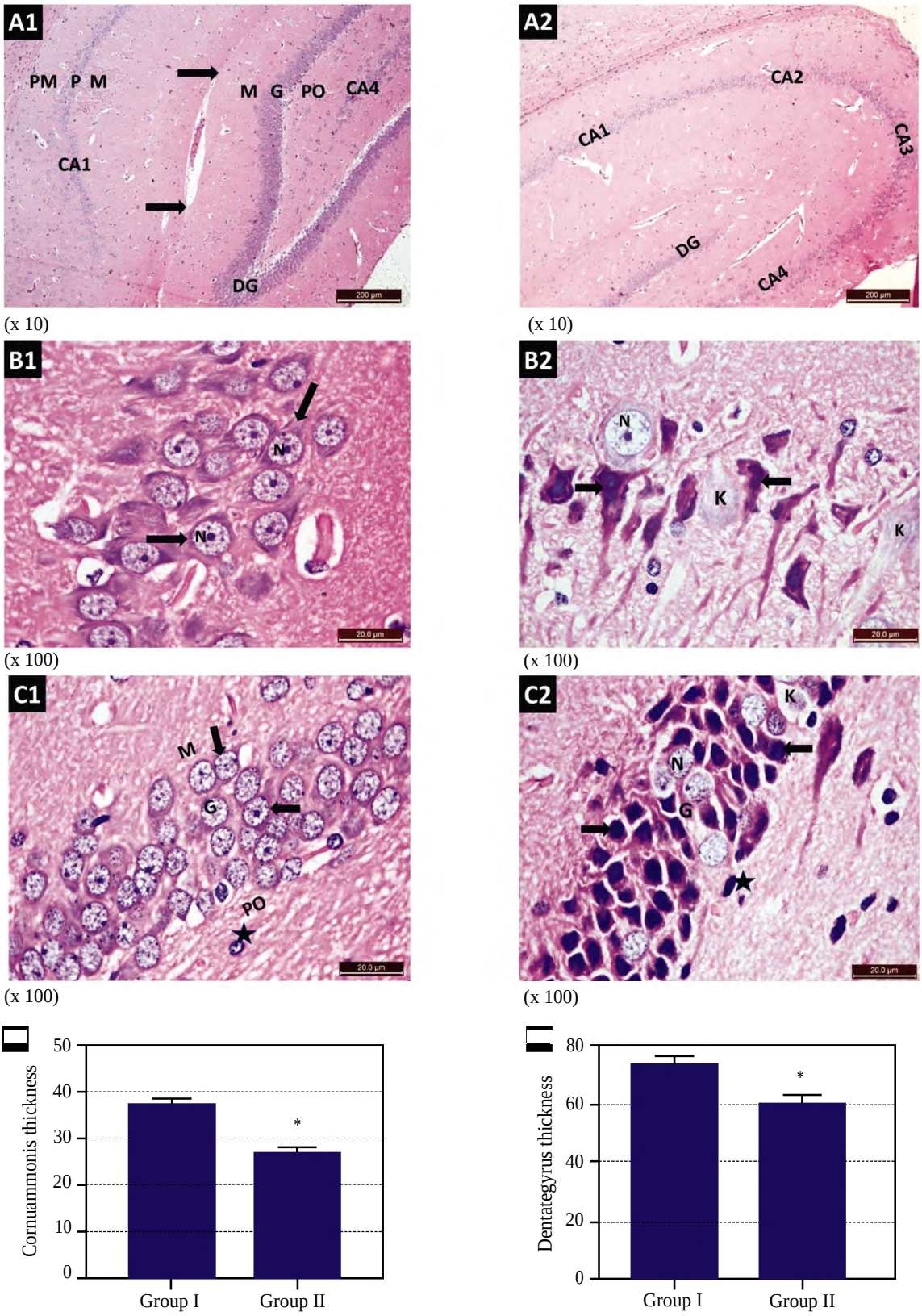


Fig. (3): (H&E x 10 & x 100).

Photomicrographs of sections in the hippocampus of A1. Group I shows part of (CA1) with the polymorphic (PM), the pyramidal (P), and the molecular (M) layers. The hippocampal sulcus (arrow) separates the CA1 region from the dentate gyrus. Dentate gyrus with its three layers; the stratum moleculare (M), granulosum (G), and the polymorphic (PO) lying deep to the hippocampal sulcus (arrow). CA4 projects into the concavity of the dentate gyrus. A2. Group I shows the C-shaped cornu (CA) and the dentate gyrus (DG) part. B1. Higher magnification of CA1 of group I showing the pyramidal cells (arrow) with large cell body and basophilic cytoplasm. The nuclei (N) are large, spherical, and centrally located with prominent nucleoli. B2. Group II shows pyramidal cell bodies in the CA1 area shrunken and irregular in shape, with eosinophilic cytoplasm. Most of the nuclei are dark, pyknotic (arrow), and karyolytic (K). C1. Higher magnification of DG of group I showing the granule cells (G) of the dentate gyrus with large rounded nuclei and prominent nucleoli (arrow) and glial cells (*) with small dark nuclei and scanty cytoplasm in the polymorphic layer (PO). C2. Group II shows granule neurons in the dentate gyrus (G) have shrunken and eosinophilic cytoplasm (arrow). The nuclei are pyknotic and karyolytic (K) nuclei, but few appear vesicular (N). Glial cells (*) show dense nuclei. D 1. CA thickness in group I and group II. D2. Dentate gyrus thickness in group I and group II. * p<0.05 in group II compared to group I.

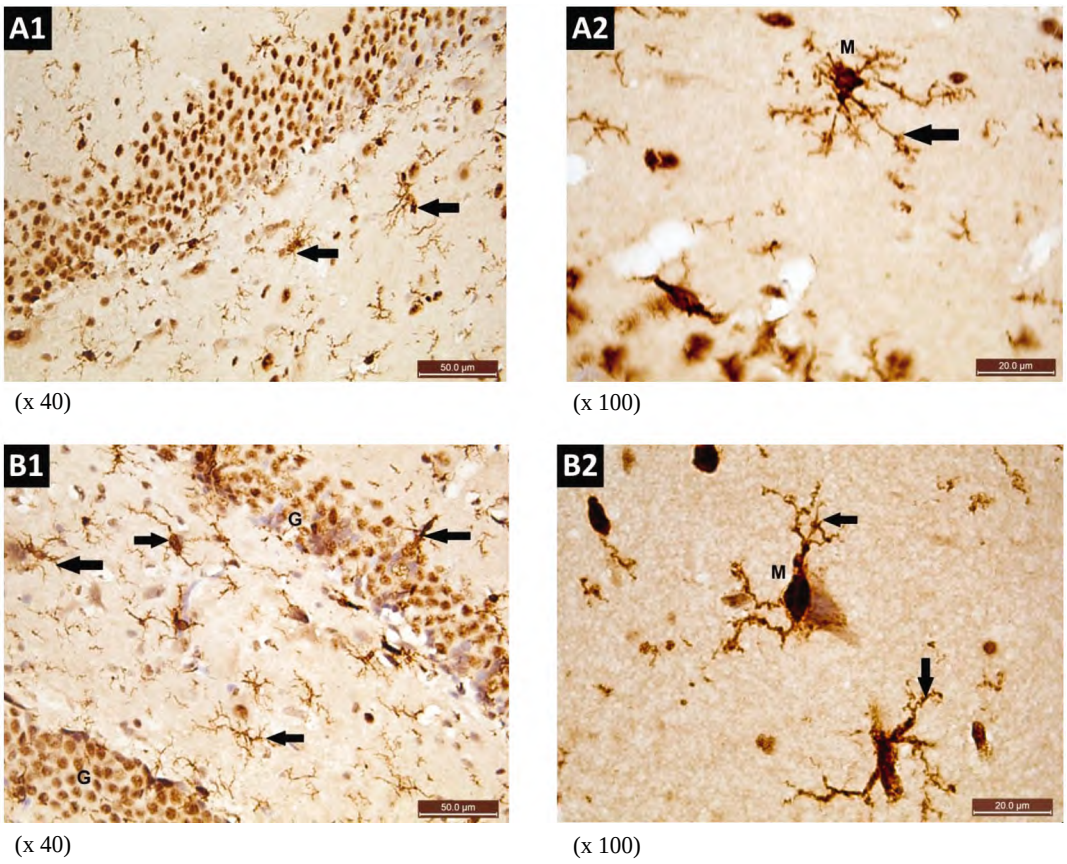


Fig. (4): Anti-iba-1 (x 40 & x 100).
Photomicrographs of sections in the hippocampus of A1&A2. Group I shows Iba-1 immunoreactive microglia (M), positive brown, stellate shaped with dense cell body and numerous delicate branching processes penetrating the hippocampus neuropil (arrow). B1&B2. Group II shows parts of the dentate gyrus (G) with an apparent increase in the positive brown Iba-1 immunoreactive microglia (M) with densely stained, larger, curved cell body with multiple processes (arrow). (C): Optical density of Iba-1 stained sections in group I and group II. * p<0.05 in group II compared to group I.

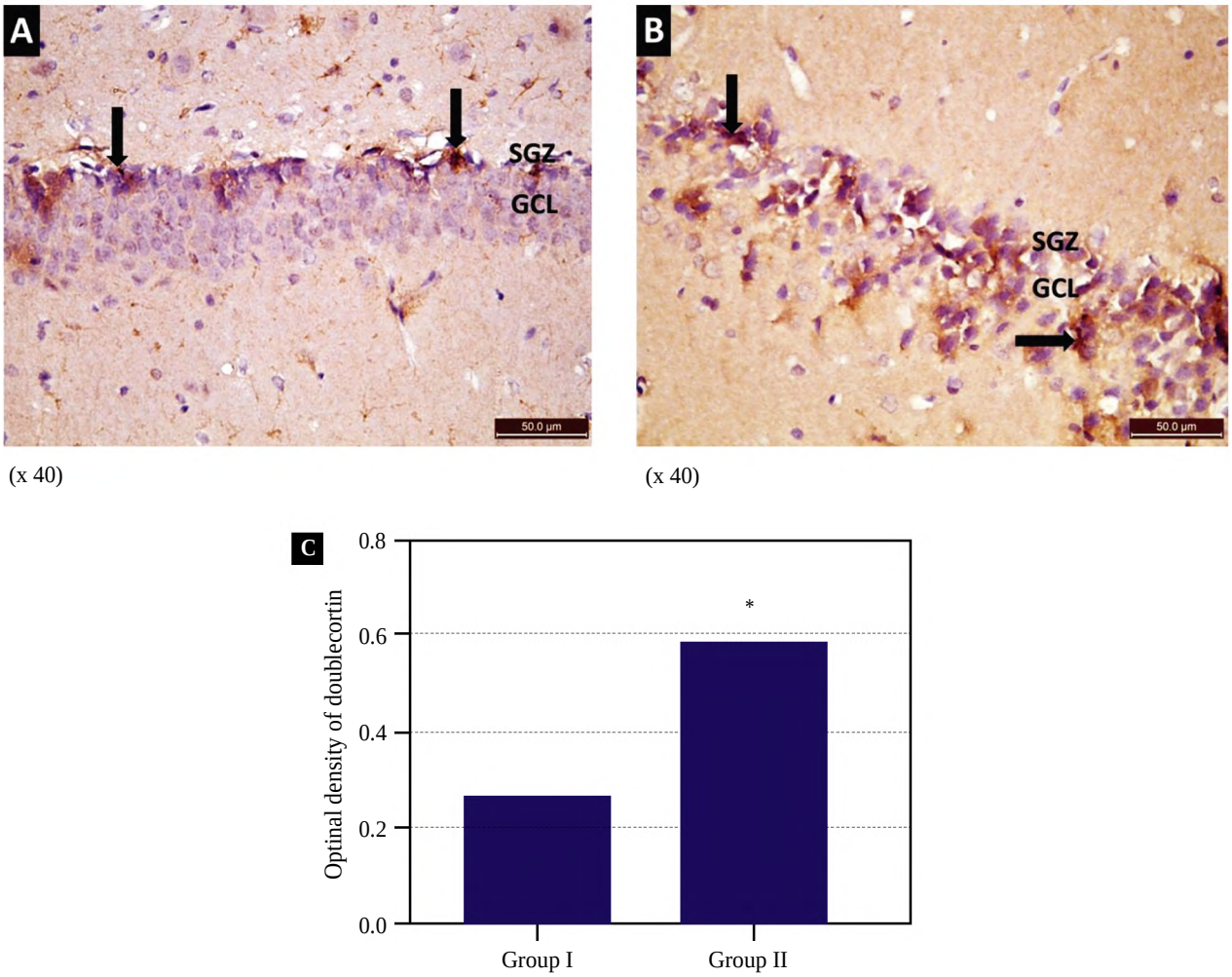
Effect of chronic stress on CA1 and Dentate gyrus areas of rats:

Hematoxylin and Eosin stained sections of the hippocampus of group I showed normal laminar organization. The cornu ammonis consists of a pyramidal cell layer sandwiched between polymorphic and molecular layers (Figs. 3-A1&A2). The pyramidal cell layer had pyramidal cells with large cell bodies, processes, and basophilic cytoplasm. The nuclei were large, spherical, and centrally located with large prominent nucleoli (Fig. 3-B 1). Sections from group II, CA1 area, showed irregular pyramidal cell bodies, shrunken with eosinophilic cytoplasm. Most of the nuclei were shrunken, dark, pyknotic, or even karyolytic (Fig. 3-B2). The quantitative assessment showed a significant decrease in the mean thickness of the stratum pyramidalis in group II compared to group I (Fig. 3-D 1). While the dentate gyrus of group I extend deeply from the hippocampal sulcus: Molecular, granule, and polymorphic layers (Fig. 3-A1). The granule cells aligned in columns, tightly packed together in about six layers forming the granule cell layer. They are small neurons, rounded, deeply basophilic cytoplasm, had large vesicular nuclei (Fig. 3-C1). Although, group II showed shrunken and irregularly arranged granule cells of the dentate gyrus. They had eosinophilic cytoplasm and pyknotic nuclei.

Few granule cells appeared near normal with large vesicular nuclei (Fig. 3-C2). The quantitative assessment showed a significant decrease in the mean thickness of the stratum granulosum in group II compared to group I (Fig. 3-D2).

Iba-1 immunostaining sections of the hippocampus of the group I showed microglia dispersed among the different layers of the hippocampus, appeared as stellate cells, had dark brown cytoplasmic granules with numerous delicate branching processes penetrating the hippocampus neuropil (Fig. 4-A1& A2). Group II showed microglia with a marked increase in the Iba-1 staining of the soma and microglia processes compared to group I. This group's primary microglia processes have numerous branches and different directions; changes in the directions of processes form angles (Fig. 4-B 1 &B2). The quantitative assessment showed a significant increase in the mean optical density of Iba-1 immunore active microglia in group II compared to group I (Fig. 4-C).

Double cortin immune staining sections of the dentate gyrus (subgranular zone) of the group I showed small neurons, intense brown granules in the cytoplasm, and the nuclei were negative (Fig.-3-A). Sections from group II showed a marked increase in double cortin stained soma and processes of the neurons compared to group I (Fig. 5-B). The quantitative assessment showed a significant increase in the mean optical density of double cortin in group II compared to group I (Fig. 5-C).



(x 40) (x 40)

Fig. (5): (Anti-DCX x 40).

Photomicrographs of sections in the hippocampus of (A): Group I showing the subgranular zone (SGZ) of dentate gyrus with positive brown immunoreactive neurons (arrow). (B): Group II shows positive brown neurons (arrow) in the subgranular zone (SGZ) and the granule cell layer (GCL) of the dentate gyrus. (C): Optical density of doublecortin stained sections in group I and group II.* $p<0.05$ in group II compared to group I.

Discussion

The finding regarding the escape latency of the Barnes maze was similar to [19,20] who recorded memory impairment in rats exposed to chronic stress. Also, this result was compatible with [21,22] who explained the memory impairment recorded in behavioral tests to impairment in long term potentiation in place cells of CA1 owing to a decrease in brain-derived neurotrophic factor and cyclic adenosine triphosphate (cAMP) response element protein, which regulates long term potentiation. This memory impairment in chronically stressed rats may be attributed to dysfunction in hypothalamic-pituitary-adrenal axis, imbalance of neurotrophic factors, and decrease in AMPA receptors, mainly in temporoammonic synapses of CA1, which is essential in memory consolidation [23]. Thus, the chronic unpredictable stress initiated variable degenerative pathways leading to impairment in memory formation. The high serum corticosterone level in group II compared to group I reveal the impact of chronic unpredictable stress. Some studies reported that the variability in the exposure to different stressor types and time prevented the adaptation, which avoids the increase in serum corticosterone level [24,25]. On the other hand, many others reported that chronic unpredictable stress stimulates the medial amygdaloid nucleus, which releases the corticotropin-releasing hormone storage from the paraventricular nucleus through the bed nucleus of stria terminalis, exciting the secretion of adrenocorticotropic hormone, and then corticosterone from the adrenal gland. Thus, chronic unpredictable stress was a major inducer to keep the serum corticosterone at a high level [26-28]. There was a significant increase in hippocampal tissue MDA and decrease in TAC in group II compared to group I. These findings were similar to [18,29] who noticed an increase in MDA and decrease in superoxide dismutase, glutathione reductase and catalase in the rats' brains exposed to chronic stress. This result was also consistent with Tagliari [30] who found that rats exposed to unpredictable stress showed inhibition in complex VI, one of the respiratory chain complexes, which reduces oxygen molecules into water, leading to an increase in oxygen-free radicals. Also, high corticosterone levels resulted in oxidative balance impairment and consequently potentiated the oxidative damage to fat [31]. The increase in corticosterone level upon exposure to chronic stress enhances the glutamate release and activation of NMDA receptors resulting in neurotoxicity inducing microglia activation [32]. Therefore, the high corticosterone level initiates oxidative stress in the hippocampus. In the current study, the intensity of Iba-1 staining microglia in the hippocampus of group II was high compared to group I. This finding was per [33] who showed an increase in Iba-1 stained microglia in rats exposed to psychosocial stressors. Also, this study was compatible with [34] who showed over-activation of microglia stained with Iba-1 in the hippocampus of rats exposed to repeated social defeat. The oxidative stress stimulates different signaling pathways to activate microglia [35]. Moreover, the activated microglia produces more reactive oxygen species and exacerbates neurotoxicity and oxidative stress [36]. Also, Chronic stress down-regulates CD200 expression, a protein marker expressed on neurons, binds with its receptor on the glial cells, mainly microglia preventing the activation of Iba-1 and keeping the microglia in a quiescent state [37]. The over activation of microglia contributes to memory recall decline [38]. Thus, oxidative stress with high corticosterone levels enhances the activation of microglia.

There was a significant increase in bFGF2 expression in hippocampal tissue of group II compared to group I in the current study. This result was consistent with [39], who found that high corticosterone level increased bFGF2 expression and considered this as a defense mechanism against the drastic effect of chronic stress. This finding was also similar to [40] who observed an increase in bFGF2 m RNA is highly expressed in CA1, CA2, and dentate gyrus in rats exposed to repeated tail shock. bFGF2 also induces the production of reactive oxygen species, which also explains the high oxidative stress in group II [41]. However, a study found a decrease in bFGF2 expression in rats exposed to chronic stress. This decrease may refer to the possibility of developing a psychological disorder in which neurotrophic factors are significantly reduced [42]. In another study, the rats exposed to social defeat showed downregulation of bFGF2 owing to the difference in genetic susceptibility to stress [43]. Thus, Group II showed a significant increase in neural stem cell proliferation expressed as an increase of doublecortin staining neurons intensity compared to group I. This finding was compatible with [44] who recorded an increase in doublecortin staining neurons in rats exposed to chronic social defeat. He also stated that the time window is critical for neurogenesis showing that neural stem cell proliferation and survival of neurons decrease immediately after the last stressor.In contrast, there was an increase in neural stem cell proliferation and neuronal survival 24 hours after the end of the last stressor. However, doublecortin staining neurons intensity decreased in rats exposed to chronic unpredictable stress [45]. Also noticed a decrease in doublecortin staining neurons in rats sacrificed immediately after the final stress [46,47].

This controversy may refer to stress susceptibility related to individual variations, including disturbance in genetic, epigenetic, and biochemical mechanisms regulating the end of stress responses **[48,49]**. There was a significant decrease in dentate gyrus thickness in group II compared to group I. This finding is confirmed by the microscopic picture of the dentate gyrus in group II showing shrunken dentate gyrus with darkly stained granule cells and cytoplasmic vacuolations.

This result agreed with **[46]** who noticed a decrease in the granular cell layer in rats exposed to chronic stress due to changes in angiogenic factors. This result was similar to **[50]** who also noticed a decrease in the survival rate of neurons despite an increase in cell proliferation, leading to a reduction in hippocampus volume in rats exposed to chronic stress **[51]**. Reported the decrease in hippocampus volume due to excessive increase in autophagy, another programmed cell death, in response to chronic stress and high corticosterone serum level. The oxidative stress promotes cell cycle reentry leading to duplication of the genome without cell division, causing aneuploidy; this mechanism leads to apoptosis, cell death, and failure in the developmental program of neurons **[52]**. Thus, oxidative stress and high corticosterone serum levels lead to shrinkage in dentate gyrus thickness. In the present study, group II showed a significant decrease in cornu ammonis thickness compared to group I. This finding is also supported by a microscopic picture of cornu ammonis, which offers many necrotic pyramidal cells with shrunken soma and pyknotic nuclei. Chronic unpredictable stress with increased oxidative stress leads to neuronal death and shrinkage in cornu ammonis thickness. This result agreed with **[53]** who noticed a decrease in the volume of the CA3 pyramidal cell layer and its somal volume in rats exposed to chronic stress. The volume of pyramidal neurons in CA3 declined, and dendrites of CA1 and CA3 shrank in rats exposed to chronic stress **[54]**. High ROS production caused significant damage in CA1 neurons due to increased activated microglia **[36]**. The increase in oxidative stress occurred in chronic stress leads to the death of neurons in the hippocampal CA3 region with pyknotic nuclei **[55]**.

Conclusion:

The chronic unpredictable stress maintains a high corticosterone level that impairs the oxidative state, which activates microglia and neural stem cell proliferation and production of bFGF2, leading to more impairment in oxidative state and neuronal death in the hippocampus accompanied by deterioration of memory.

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التحويرات على تكوين الخلايا العصبية بسبب الإجهاد المزمن الذى لا يمكن التنبؤ به فى ذكور الجرذان البيضاء البالغة: الإفراط فى تنشيط الخلايا الجذعية العصبية

الإجهاد العصبية، وتكوين الخلايا العصبية للبالغين. تختلف شدة هذه التغييرات بما يتناسب مع مدة وشدة الإجهاد.

هدفت هذه الدراسة إلى التحقيق فى التأثير الضار للضغط المزمن الذى لا يمكن التنبؤ به على الوظيفة الإدراكية للدماغ وتكوين الخلايا العصبية للبالغين فى الحصين. تم تقسيم أربعة وعشرين ذكور الجرذان بالتساوى إلى مجموعتين. المجموعة الضابطة (بقيت آمنة مع حرية الوصول إلى الطعام والماء)، ومجموعة الإجهاد المزمن غير المتوقعة التى تتعرض لضغوط مختلفة فى أوقات مختلفة لمدة ٢٥ يوماً. قام بارنز مايز بتقييم الأداء المعرفى (التعلم المكانى وأداء الذاكرة). قمنا بتقييم مستوى مصل الكورتيكوستيرون فى الدم، والإجهاد التأكسدى، والقدرة المضادة للأكسدة فى الحصين. تم تقييم تكاثر الخلايا العصبية فى الحصين باستخدام عامل نمو الخلايا الليفية. أظهرت مجموعة الإجهاد المزمن انخفاضاً فى الأداء المعرفى وتفاقم الإجهاد التأكسدى فى الحصين بالتوازى مع ارتفاع مستويات مصل الكورتيكوستيرون وعامل النمو ذو دلالة إحصائية.