Original Research Article

Effect of voluntary running on expression of myokines in brains of rats with depression

Sami A Algaidi[1], Magda A Eldomiaty[1,2] (iD),
Yasser M Elbastwisy[1,3], Shaima M Almasry[1,3],
Maha K Desouky[1,4] and Ahmed M Elnaggar[5]

Abstract

This study aimed to demonstrate the histopathology and immunoexpression of exercise-derived myokines in dentate gyrus (DG), medial prefrontal cortex (mPFC) and cerebellum of depressed Wistar rats during depression and after practising voluntary running. Depression was developed by forced swimming for 2 weeks. Voluntary running was performed by voluntary running for 3 weeks. Brain sections were processed and immunostained to detect brain-derived neurotrophic factor (BDNF), macrophage migration inhibitory factor (MIF), vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6). ImageJ software was used to measure the optical density (OD). BDNF was expressed in neurons in DG, mPFC and granular and Purkinje cells in cerebellum. MIF was expressed in neurons of sub-granular zone in DG, mPFC and Purkinje cells. VEGF was expressed in many neurons in DG, mPFC and Purkinje cells. IL-6 was expressed in some neurons in DG, in neuropil of mPFC and in Purkinje cells. In depression, the OD of studied myokines significantly decreased in all examined areas. After voluntary running, the OD of myokines significantly increased in all areas. This study defines the immunohistochemical expression of myokines in brain areas in depression and after voluntary running and reveals the involvement of the mPFC and cerebellum in the pathophysiology of depression.

Introduction

The beneficial effects of physical exercise on the indicators of depression have been previously shown.[1,2] Variable levels of cytokines and growth factors are released in response to exercise and are termed myokines. Conflicting outcomes have been reported by studies investigating the correlation between these exercise-derived factors and depressive behaviours in rodents.[3–6]

In patients with major depression, brain-derived neurotrophic factor (BDNF) is positively related to the volume and function of the hippocampus,[7] and BDNF deficiency is associated with significant neurological defects in the frontal cortex.[8]

Macrophage migration inhibitory factor (MIF) is thought to play a role in the neuro-immune

[1]Department of Anatomy, Faculty of Medicine, Taibah University, Almadinah Almunawarah, Saudi Arabia
[2]Department of Anatomy, Faculty of Medicine, Tanta University, Tanta, Egypt
[3]Department of Anatomy, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[4]Department of Anatomy, Faculty of Medicine, Minia University, Minia, Egypt
[5]Good Samaritan Hospital, Dayton, OH, USA

Corresponding author:
Magda A Eldomiaty, Department of Anatomy, Faculty of Medicine, Taibah University, PO Box 30001, Almadinah Almunawarah 41411, Saudi Arabia.
Email: Dr_majda@hotmail.com

interaction, which may be linked to depression.[9] The levels of vascular endothelial growth factor (VEGF) have been recently shown to be related to neurogenesis in depression cases.[10] In addition, interleukin-6 (IL-6) has recently been found to play a significant role in mediating the psychological and neurobiological manifestations of depression.[11]

The dentate gyrus (DG) in the hippocampal formation has been implicated in cognitive and mood disorders due to the reduction in adult hippocampal neurogenesis.[12] The medial prefrontal cortex (mPFC) has connections with hippocampal structures associated with long-term memory and central autonomic structures associated with the expression of emotions.[13,14] The cerebellum has strong connections with brain regions responsible for emotions and is, thus, believed to play an important role in the pathophysiology of depression.[15,16] Recent studies investigating the effect of physical exercise on the morphological changes in the cerebellum[17] and whether physical exercise promotes angiogenesis and the functional recovery of cells after brain injuries have yielded inconsistent results.[18,19]

While the levels of myokines could be linked to depression and influence the plasticity and survival of neurons,[20] no studies have used immunohistochemistry to investigate the expression of these myokines in brain areas vulnerable to depression. This study aims to investigate the following: (a) the expression of BDNF, MIF, VEGF and IL-6 in the DG, mPFC and cerebellum using a rat model of depression and (b) the effect of voluntary running on the expression of these myokines in these areas of the brain.

Materials and methods

This experimental case–control study was conducted at King Fahd Centre for Medical Research, King Abdul-Aziz University, Jeddah, Saudi Arabia, and the research laboratory of the College of Medicine, Taibah University, Almadinah, Saudi Arabia.

All animal handling and experimental procedures were conducted according to the EC Directive 86/609/EEC for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm) and the guidelines of the National Committee of Bio-Ethics in Saudi Arabia (https://doi.org/10.1111/dewb.12114). Ethical approval was obtained from the ethics committee of King Abdul-Aziz University, Jeddah, Saudi Arabia.

Experimental animals

A total of 60 male Wistar rats aged 8–10 weeks weighing 200–250 g were obtained from the Experimental Animal Centre of the King Fahd Centre for Medical Research, Jeddah, Saudi Arabia. The rats were housed for 7 days to allow acclimatisation to the laboratory conditions and were observed for any abnormal behaviour in their ordinary movement and feeding behaviour. Only those rats that showed apparently normal movements and normal feeding behaviour were included in the study.

Before the experiment, all rats were exposed to a 6-min forced swimming test (FST), and only those animals that showed extreme behaviour during the test were selected for the experiment.[21] The selected animals (45 rats) were singly housed in well-ventilated cages with ad libitum access to food and water. The environment in the skill laboratory was controlled at a 12 h/12 h light/dark cycle (the lights were turned on at 7:00 a.m.), temperature of 22°C–24°C and humidity of 50%–60%.[22]

The 45 selected rats were randomly divided into the following three groups each comprising 15 rats:

- *Control group*. These rats were maintained on a standard chow diet for 5 weeks, including the weekends (35 days), screened using the 6-min FST to automatically record their locomotor activity and immediately sacrificed.
- *Depression group*. These rats were subjected to the forced swimming (FS) protocol for 2 weeks, including the weekends (14 days), housed for an additional 3 weeks without exercise, screened using the 6-min FST to record their locomotor activity and immediately sacrificed.
- *Depression/exercise group*. These rats were subjected to the FS protocol for 2 weeks, including the weekends (14 days), allowed to perform voluntary wheel running for 3 weeks, screened using the 6-min FST to record their locomotor activity and immediately sacrificed.

Figure 1. Voluntary rat running wheel (Lafayette Instrument Company, Inc., North Lafayette, IN, USA). The figure shows a rat while accessing the wheel voluntarily.

Development of the animal model of depression

This model was developed using a protocol described by Eldomiaty et al.[23] The rats were individually subjected to FS inside a vertical glass cylinder (height: 60 cm; diameter: 22 cm) containing water (height: 45 cm) maintained at 23°C–25°C with no possibility of escape. After 15 min of swimming, the rats were removed and allowed to dry for 15 min in a heated enclosure (32°C) before being returned to their home cages. This procedure was repeated once daily for 2 weeks, including the weekends (14 times). This procedure was a modification of other protocols that also used FS to develop animal models of depression.[24–26]

Voluntary wheel running

After developing depression (2 weeks of FS), the rats in the depression/exercise group were moved to cages equipped with rat running wheels prepared for voluntary running and activity wheel counters (Lafayette Instrument Company, Inc., North Lafayette, IN, USA) (Figure 1). The rats were allowed to run voluntarily for 3 weeks (21 days). The wheels were inspected every other day to ensure that they were working properly.[27]

## Assessment of locomotor parameters in rats from different groups during the FST

To confirm the development of the depressive state after the FS and/or its relief after voluntary running, we performed the 6-min FST with automatic tracing using computer software (Ethovision XT version 8.0; Noldus Information Technology, Wageningen, The Netherlands) to determine the distance moved by the rat in centimetres and the immobility duration (time spent passively floating) in seconds.[24,28–30] All rats were subjected to this test immediately before sacrifice between 8:00 and 10:00 a.m.[31]

Light microscopy structural study of different brain regions

At the specified time of sacrifice, the brain was freshly dissected, and half of the brain was coronally sectioned and fixed in buffered formalin for the histological examination of the DG, mPFC and cerebellum.[32] The brain tissue sections were stained with haematoxylin and eosin (H&E) and examined under an Olympus BX 36 Bright field Automated microscope. The images were digitised in a 2448×1920-pixel matrix using a DP27 colour digital video camera to determine the structural changes in different areas.

Immunohistochemical staining and evaluation of immunoexpression

Serial paraffin sections were stained using primary polyclonal anti-rat antibodies against selected myokines (Uscn Life Science Business Co., Ltd, Wuhan; polyclonal Rat BDNF PAA011Ra01, Rat MIF PAA698Ra01, Rat VEGF PAA143Ra71, Rat IL-6 PAA079Ra71). The staining procedure was performed using an immunoperoxidase technique with a Bench-Mark instrument (recommended by Ventana Company, Oro Valley, AZ, USA). The paraffin sections were deparaffinised, rehydrated, treated with 3% H_2O_2 for 5 min and washed with phosphate-buffered saline (PBS) for 15 min. The sections were blocked with 1.5% normal goat serum in PBS and then incubated for 45 min at room temperature with the primary antibody. The sections were subsequently incubated with a second-stage biotinylated antibody at room temperature. After rinsing with PBS, the reaction products
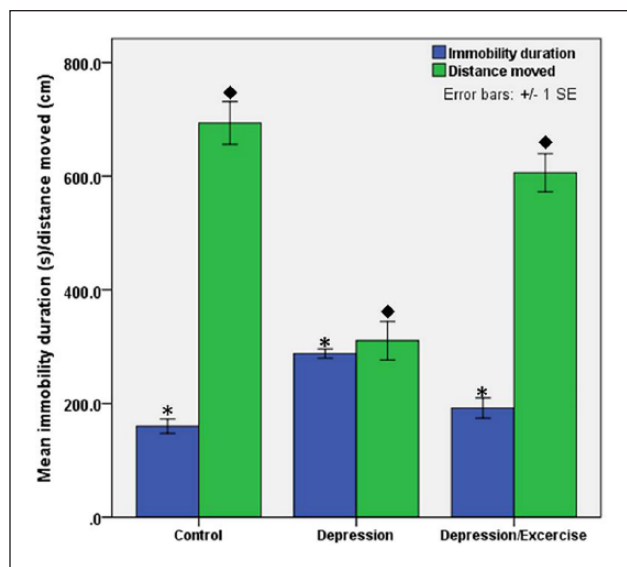
Figure 2. Graph illustrating the immobility duration and the distance moved for control, depression and depression/exercise groups (15 rats each) during the 6-min forced swimming test (means ± SEM).
*Significance for the immobility duration in the successive groups ($P < 0.05$).
♦Significance for the distance moved in the successive groups ($P < 0.05$).

were visualised by immersing the sections in diaminobenzidine. Finally, the sections were counterstained with haematoxylin, dehydrated and cover-slipped using Protex mounting media (DAB-Stock Stain box; Boster Biotechnology, Pleasanton, CA, USA).[33]

A light microscopy examination of the immuno-expression of different myokines was performed in different brain areas, and the immunohistochemical staining was evaluated by measuring the optical densities (ODs) of the stained sections.[34] Briefly, five representative non-overlapping fields were captured as photomicrographs at a magnification of $\times 200$ (microscopic field area of $786.4\,u^2$). The captured images were transferred to a computer for an image analysis. The OD evaluation was performed using the ImageJ (NIH) software, version 2.0.0-beta4 (National Institutes of Health, Bethesda, MD, USA). Each image was first deconvoluted using DAB vector into three different coloured images (i.e. green, brown, and blue). The calibration of the brown DAB image was performed by measuring the mean integrated intensities (mean grey value) of five non-overlapping areas of stained tissue. The intensity numbers were converted into the OD using the following equation: (OD = log (max intensity/mean intensity)), where

the max intensity = 250 and the mean intensity = mean grey value. The results were exported to a Microsoft Excel spreadsheet.

Statistical analysis

The statistical analysis was performed using the IBM SPSS (version 21) statistical package. All data are expressed as the means ± standard errors of the means (SEM). Comparisons between groups were performed using one-way analysis of variance (ANOVA), followed by Fisher's multiple comparison tests as necessary. The statistical significance of the mean comparisons was set at $P < 0.05$. The correlation between different myokines in all groups was done using Pearson correlation test and the P and r values were recorded. The statistical analyses performed are described in the table's captions.

Results

FST before sacrifice

The results of the automatic scoring of the locomotor parameters during the FST in the rats from the different groups are shown in Figure 2.

The mean distance moved by the rats in the depression group ($310.7 \pm 33.7\,cm$) was significantly lower than that in the control rats ($693.6 \pm 37.5\,cm$) ($P < 0.001$). In addition, the mean distance moved by the rats in the depression/exercise group ($606.1 \pm 33.5\,cm$) was significantly higher than that in the rats in the depression group ($P < 0.001$).

The mean immobility duration in the depression group ($288 \pm 8.07\,s$) was significantly higher than that in the control rats ($160.05 \pm 12.7\,s$) ($P < 0.001$). The mean immobility duration in the rats in the depression/exercise group ($192.14 \pm 17.91\,s$) was significantly lower than that in the rats in the depression group ($P < 0.001$).

Histopathological study

DG. According to the examination of the H&E stained sections, the DG in the control rats consisted of the following components: upper limb, lower limb and stem (Figure 3(a)). Each component comprised the following layers: (a) the molecular stratum, which comprised neuronal processes, glial cells and a few neurons; (b) the stratum
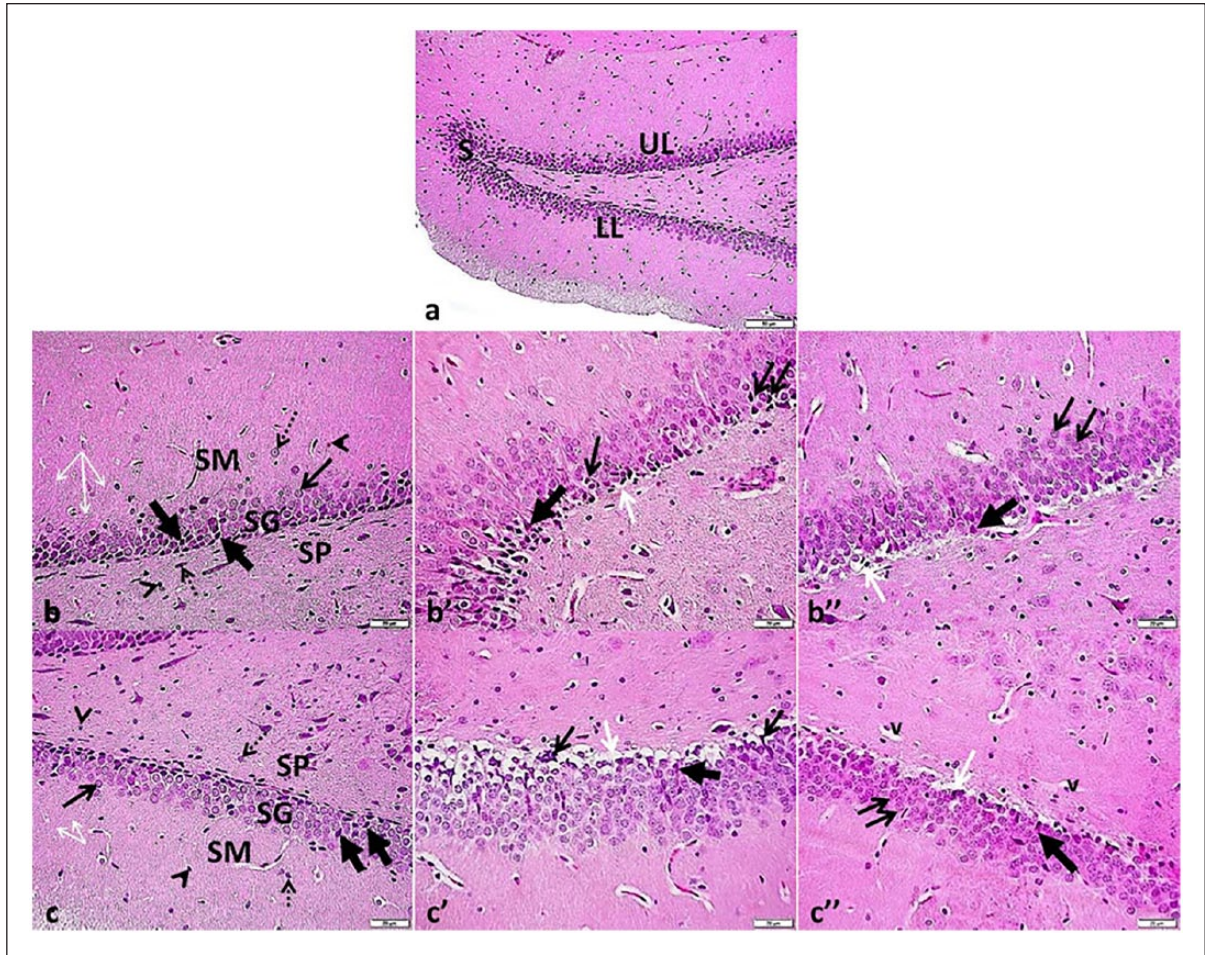
Figure 3. A photomicrograph of sections in dentate gyrus of adult rat. (a) Specimen of control rat shows the dentate gyrus is formed of upper limb (UL), lower limb (LL) and stem (S). (b, c) The upper and lower limbs of control rat consist of three strata. Stratum molecular (SM) contains neuronal processes (white arrows), glial cells (arrow heads) and few neurons (dotted arrow). Stratum granulosum (SG) contains granule cells with rounded vesicular nuclei (thin black arrows). Immature small granular cells with oval nuclei are seen in the sub-granular zone and in between the mature neurons (thick arrows). Stratum pleomorph (SP) contains glial cells (arrow heads) and some neurons (dotted arrows). (b′, c′). The upper and lower limbs of depressed rat show many cells with dark-stained pyknotic nuclei (thin black arrows). Small immature neurons (thick arrows) and apparent vacuolations (white arrows) are present in the sub-granular zone of the lower limb. (b″, c″) The upper and lower limbs of depression/exercise rat show numerous granule cells with vesicular nuclei (thin black arrows). Immature small granular cells (thick arrows) and little vacuolations (white arrows) are seen in the sub-granular zone. Many dilated blood vessels appeared in the pleomorph zone (v) (H&E: a, ×200; b–c″, ×400).

granulosum, which comprised densely packed granular cells with rounded vesicular nuclei and several immature granule cells with oval nuclei that appeared in the sub-granular zone (SGZ) and between the mature neurons; and (c) stratum pleomorph, which comprised glial cells and several neurons (Figure 3(b) and (c)).

In the depression group, the vacuolations in the SGZs appeared in both the upper and lower limbs of the DG, many degenerated neurons exhibited darkly stained pyknotic nuclei, particularly in the upper limb and stem regions, and a few small-sized immature granular cells were observed (Figure 3(b′) and (c′)).

In the depression/exercise group, both limbs of the DG showed an apparently normal stratum granulosum with few or no vacuolations in the SGZs. The granular cells had normal rounded vesicular nuclei with many immature granular cells in between and in the SGZ. Few degenerated small cells were observed in the SGZ of several specimens, and numerous dilated blood vessels appeared in the pleomorph superficial zone (Figure 3(b″) and (c″)).
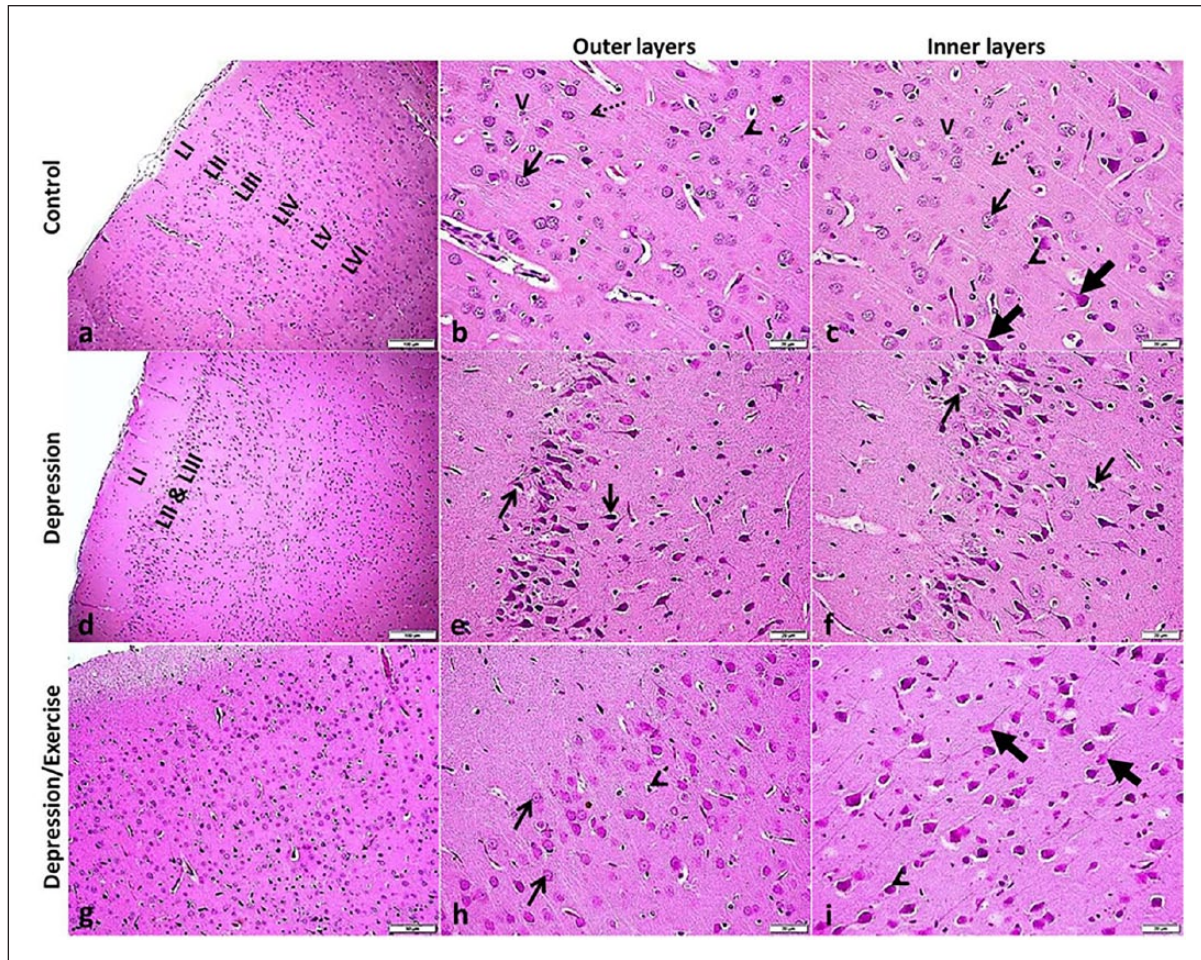
Figure 4. Photomicrographs of sections in the mPFC of adult rat. (a–c) Specimens of control rat illustrate the organised layers of the mPFC; the molecular layer (LI), the outer granular layer (LII), the pyramidal cell layer (LIII), the inner granular layer (LIV), the ganglionic layer (LV) and the multiform layer (LVI). The granule cells have large rounded vesicular nuclei and prominent nucleoli (thin arrows). The pyramidal cells possess vesicular nuclei and long peripheral processes (thick arrows). The glial cells are small in size with dark nuclei (arrow heads). The neuropil contains neuronal and glial cellular processes (dotted arrows) and blood vessels (V). (d–f) Specimens of depressed rat show widening of the layer I (LI) and condensation of layer II (LII) and layer III (LIII). Many neurons appear irregular, shrunken, darkly stained and contain shrunken pyknotic nuclei and wide pericellular space (arrows). (g–i) Specimens of depression/exercise rat show nearly organised layers of the mPFC. Most of the granular (arrows) and pyramidal (thick arrows) cells appear normal. Few neurons appear shrunken and surrounded by haloes (arrow heads) (H&E: a, d, g, ×100; b, c, e–i, ×400).

mPFC. The examination of the H&E stained sections of the mPFC from rats in the control group revealed apparently healthy groups of neurons arranged in the following six organised cortical layers: the molecular layer (LI), the outer granular layer (LII), the pyramidal cell layer (LIII), the inner granular layer (LIV), the ganglionic layer (LV) and the multiform layer (LVI). The granule cells had large rounded vesicular nuclei. The pyramidal cells possessed vesicular nuclei, a slightly basophilic granular cytoplasm and long peripheral processes. The glial cells appeared small with dark nuclei. The neuropil contained neuronal and glial cellular processes and blood vessels with a narrow perivascular space (Figure 4(a)–(c)).

In the depression group, the frontal cortex showed disorganised cortical layers, a widened LI, evident condensation and the merging of LII and LIII. Many neurons appeared irregular, shrunken and darkly stained and possessed shrunken pyknotic nuclei and wide pericellular spaces (haloes). Several neurons contained fragmented nuclei (Figure 4(d)–(f)).

The frontal cortical morphology in the depression/exercise group was restored towards a normal appearance as demonstrated by the apparently
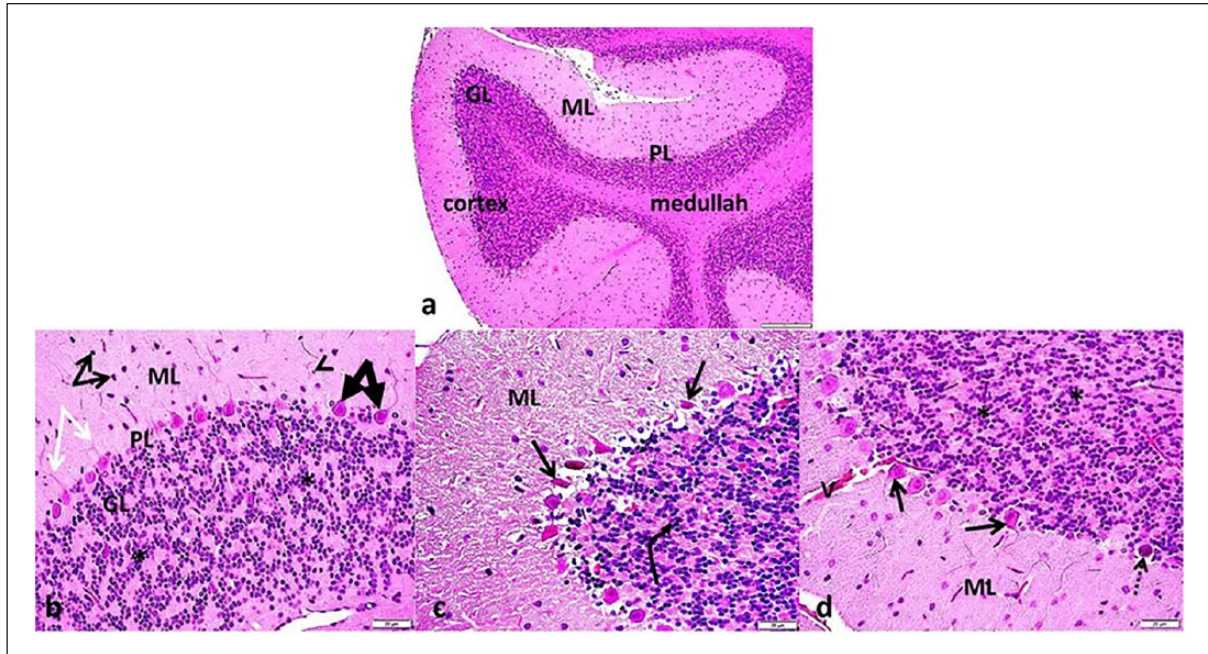
Figure 5. Photomicrographs of sections in cerebellum of adult rat. (a, b) Specimens of control rat illustrate cerebellar folia formed of an outer cortex and inner medulla. The cortex is layered into outer molecular cell layer (ML), middle Purkinje cell layer (PL) and inner granular cell layer (GL). The molecular layer contains basket cells (thin arrows), neuroglia (arrow head) and large apical dendrites of Purkinje cells (white arrows). The Purkinje layer contains large flask-shaped Purkinje cells (thick arrows) arranged in a single row with large rounded vesicular nuclei and prominent nucleoli. The granular cell layer contains groups of small densely packed granule cells (*) with darkly stained nuclei and little cytoplasm. (c) Specimen of depressed rat shows the molecular layer (ML) with apparently decreased neurons and nearly missed dendrites of Purkinje cells. The Purkinje cells appear shrunken with acidophilic cytoplasm and small deeply stained nuclei and are surrounded by pericellular spaces (arrows). The cells of the granular layer appear clumped with some pyknotic nuclei (curved arrow). (d) Specimen of depression/exercise rat shows the molecular layer (ML) with re-establishment of dendrites of Purkinje cells and presence of numerous blood vessels (V). Purkinje cells are seen with large rounded vesicular nuclei, prominent nucleoli and well apparent dendrites (arrows). Some cells are seen with small deeply stained nuclei (dotted arrow). The granular cell layer appears to retain the normal grouping appearance of cells (H&E: a, ×100; b–d, ×400).

increased normal neurons and decreased perineuronal haloes. Numerous pyramidal cells and granular cells appeared normal, but a few neurons remained shrunken and surrounded by haloes (Figure 4(g)–(i)).

Cerebellum. The examination of the H&E stained sections of the cerebellum from rats in the control group revealed normal cerebellar folia within the outer cortex and inner medulla. The cortex, which constitutes the grey matter, comprises the following three layers: the outer molecular layer, middle Purkinje layer and inner granular cell layer (Figure 5(a)). The outer molecular layer contained basket cells, neuroglia, the large apical dendrites of Purkinje cells and several blood vessels. The Purkinje cell layer showed large flask-shaped cells typically arranged in a single row at the junction between the molecular layer and the granular layer,

large rounded vesicular nuclei and prominent nucleoli. The granular cell layer comprised groups of small densely packed granular cells with darkly stained nuclei surrounded by very little cytoplasm (Figure 5(b)).

The molecular level of the cerebellum of the rats in the depression group showed an apparently decreased number of neuronal cells and Purkinje cell dendrites. The Purkinje cell layer showed a marked degeneration, many shrunken Purkinje cells with an acidophilic cytoplasm and small deeply stained nuclei surrounded by marked pericellular spaces. The cells in the granular layer appeared clumped, and several cells exhibited pyknotic nuclei (Figure 5(c)).

After the voluntary exercise, the molecular layer in the depressed/exercise rats exhibited a marked restoration of the dendrites of the Purkinje cells and numerous blood vessels. The Purkinje cell
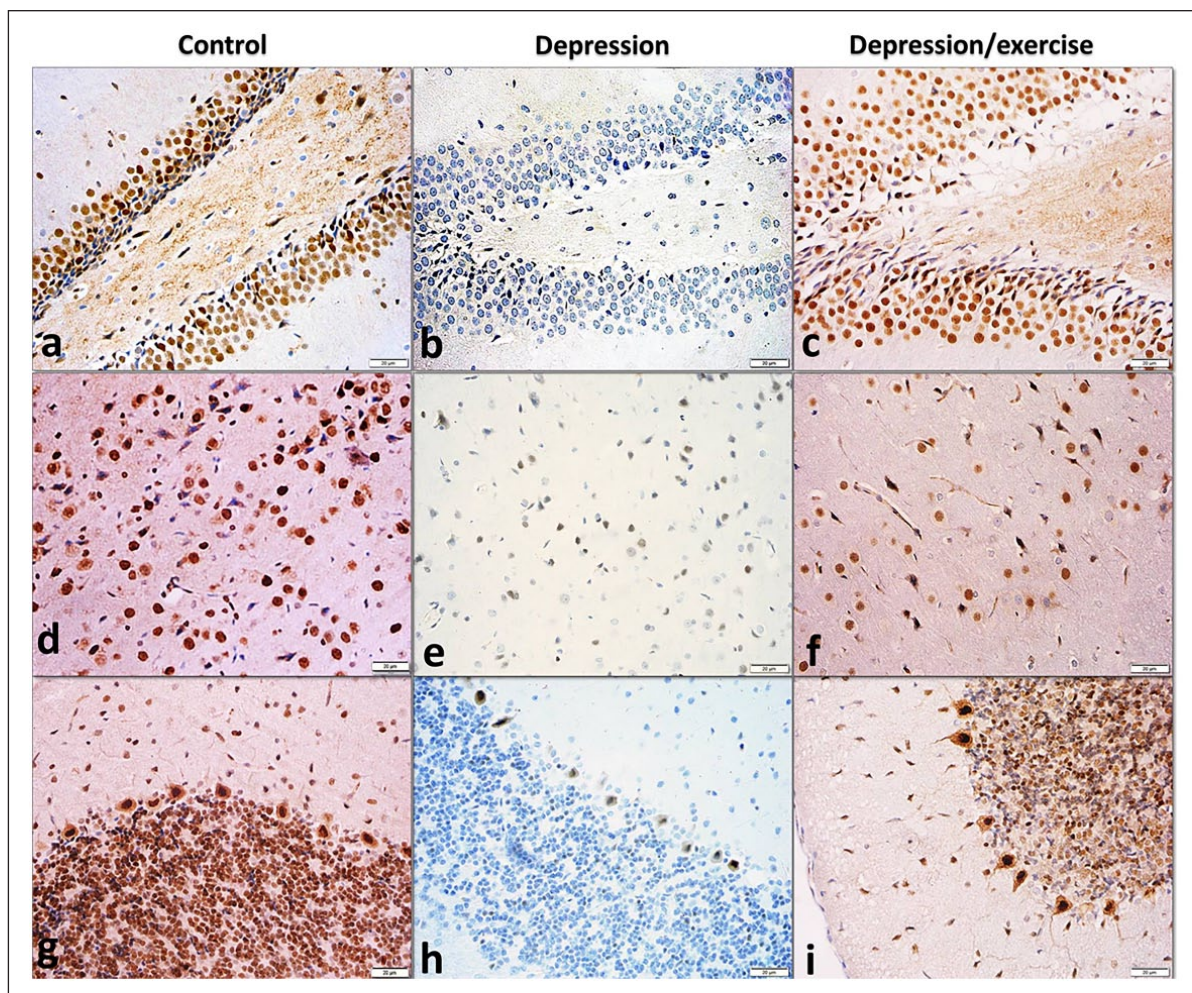
Figure 6. Photomicrographs representing the BDNF immunoexpression in sections of (a–c) the dentate gyrus, (d–f) the medial prefrontal cortex and (g–i) the cerebellum in the studied groups showing its neuronal expression with a marked decrease of the immunoexpression in the depression group, then its obvious increase in the depression/exercise group in all the studied brain areas (anti-BDNF immunostain ×400).

layer had preserved cells with large rounded vesicular nuclei, prominent nucleoli and easily visualised dendrites, but several degenerated cells with small, deeply stained nuclei were also observed. The granular cell layer retained its normal grouping appearance of cells (Figure 5(d)).

### Immunohistochemical study of the expression of myokines

BDNF immunoexpression

DG. In the DG of the control rats, strong nuclear and cytoplasmic BDNF immunoreactions were observed in numerous mature and immature granular cells in all regions. Many immature neurons in the SGZ and between the mature neurons appeared immuno-negative. In the depression group, both limbs of the DG exhibited negative reactions in the mature neurons, but several immunoreactions appeared in the immature neurons in the SGZ. After the voluntary running, a moderate immunoreaction was observed in nearly all mature and immature granular cells in both limbs (Figure 6(a)–(c)).

mPFC. In the mPFC of the control rats, moderate nuclear and cytoplasmic BDNF immunoreactions were observed in numerous granular and pyramidal cells in nearly all layers of the cortex. In the depression group, the reaction was markedly decreased, and only a few neurons exhibited faint immuno-positivity. After the voluntary running, a marked restoration of the immunoreactivity was observed, and many neurons exhibited mild reactions (Figure 6(d)–(f)).
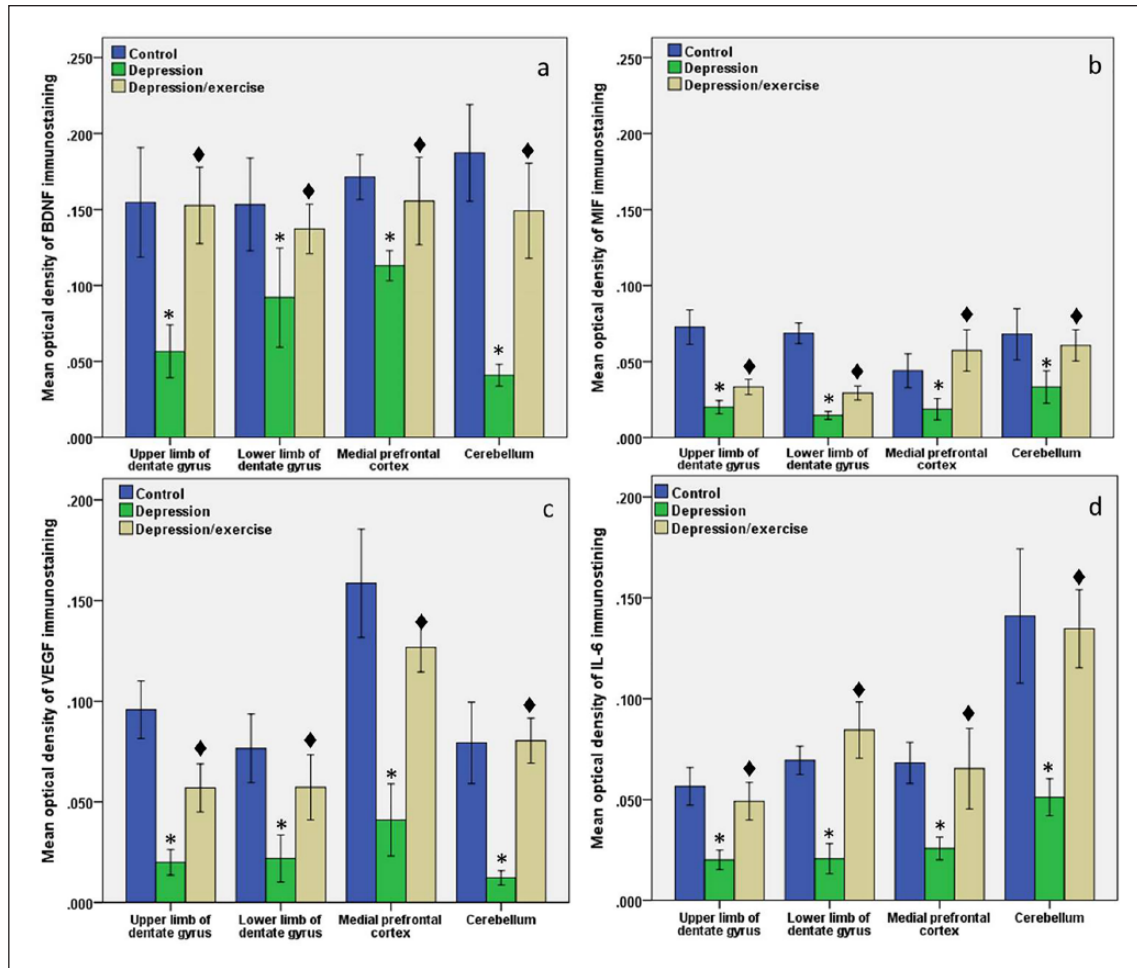
Figure 7. Graphs illustrating the optical density of myokines expression in brain regions of different groups (15 rats each): (a) anti-BDNF-stained sections, (b) anti-MIF stained sections, (c) anti-VEGF stained sections And (d) anti-IL-6-stained sections. *X*-axis shows the optical density and *Y*-axis shows the studied brain areas.
*Significance of depression group compared to control one ($P < 0.05$).
♦Significance of depression/exercise group compared to depression one ($P < 0.05$).

Cerebellum. The BDNF immunoexpression in the cerebellum of the control rats showed strong nuclear and cytoplasmic reactions in numerous Purkinje and granular cells in the cerebellar cortex. After inducing depression, a marked decrease in immunostaining was observed, and only a few neurons expressed faint reactions. After the voluntary exercise, an obvious increase in the immunoexpression was observed in the granular and Purkinje cells and the axon-like fibres of the molecular layer (Figure 6(g)–(i)).

According to the OD measurements of the anti-BDNF stained sections of different brain areas, the BDNF expression in the depression group was significantly lower than that in the control group, and the BDNF expression in the depression/exercise group was significantly higher than that in the depression group (Figure 7(a), Table 1).

MIF immunoexpression

DG. In the DG of the control rats, mild cytoplasmic MIF immunoreactivity was observed in the neurons of both limbs, particularly in the SGZ and immature granular cells. In the depression group, the neurons in both limbs of the DG exhibited negative reactions. Then, after the voluntary running, a mild immunoreaction was detected in the cells in the SGZ, particularly in the upper limb (Figure 8(a)–(c)).

mPFC. In the mPFC of the control rats, a mild cytoplasmic immunoreaction was observed in many neurons. In the depression group, a negative immunoreaction was observed in nearly all cells. Then, in the depression/exercise group, an immunoreaction was detected in several neurons and the neuropil (Figure 8(d)–(f)).

Table 1. The means and the significance of the optical densities of different groups in different studied brain areas.

The myokines	Brain areas	Optical density (mean $\pm$ SE) of control group ($n=15$)	Optical density (mean $\pm$ SE) of depression group ($n=15$)	Optical density (mean $\pm$ SE) of depression/exercise group ($n=15$)	P value
BDNF	Upper limb of DG	0.15 ± 0.018	0.06 ± 0.009	0.15 ± 0.01	$P1 < 0.001\ P2 < 0.001$
	Lower limb of DG	0.15 ± 0.02	0.09 ± 0.02	0.14 ± 0.008	$P1 = 0.003\ P2 = 0.025$
	mPFC	0.17 ± 0.007	0.11 ± 0.005	0.16 ± 0.01	$P1 < 0.001\ P2 = 0.004$
	Cerebellum	0.19 ± 0.02	0.04 ± 0.004	0.15 ± 0.02	$P1 < 0.001\ P2 < 0.001$
MIF	Upper limb of DG	0.07 ± 0.006	0.02 ± 0.002	0.03 ± 0.003	$P1 < 0.001\ P2 = 0.017$
	Lower limb of DG	0.07 ± 0.003	0.01 ± 0.001	0.03 ± 0.002	$P1 < 0.001\ P2 < 0.001$
	mPFC	0.04 ± 0.006	0.02 ± 0.004	0.06 ± 0.007	$P1 = 0.002\ P2 < 0.001$
	Cerebellum	0.07 ± 0.008	0.03 ± 0.005	0.06 ± 0.005	$P1 < 0.001\ P2 = 0.005$
VEGF	Upper limb of DG	0.1 ± 0.007	0.02 ± 0.003	0.06 ± 0.006	$P1 < 0.001\ P2 < 0.001$
	Lower limb of DG	0.08 ± 0.009	0.02 ± 0.006	0.06 ± 0.008	$P1 < 0.001\ P2 = 0.002$
	mPFC	0.16 ± 0.01	0.04 ± 0.009	0.13 ± 0.006	$P1 = 0.029\ P2 < 0.001$
	Cerebellum	0.08 ± 0.01	0.01 ± 0.002	0.08 ± 0.006	$P1 < 0.001\ P2 < 0.001$
IL-6	Upper limb of DG	0.06 ± 0.005	0.02 ± 0.002	0.05 ± 0.005	$P1 < 0.001\ P2 < 0.001$
	Lower limb of DG	0.07 ± 0.004	0.02 ± 0.004	0.08 ± 0.007	$P1 < 0.001\ P2 < 0.001$
	mPFC	0.07 ± 0.005	0.03 ± 0.003	0.07 ± 0.01	$P1 < 0.001\ P2 < 0.001$
	Cerebellum	0.14 ± 0.02	0.05 ± 0.005	0.13 ± 0.01	$P1 < 0.001\ P2 < 0.001$

SE: standard error; DG: dentate gyrus; BDNF: brain-derived neurotrophic factor; mPFC: medial prefrontal cortex; MIF: migration inhibitory factor; VEGF: vascular endothelial growth factor; IL-6: interleukin-6; n: sample number.
Significance is considered at $P > 0.05$. $P1$ is the significance between control and depression groups. $P2$ is the significance between depression and depression/exercise groups.

Cerebellum. In the anti-MIF immunostained sections of the cerebellum of the control rats, a moderate immuno-positivity was observed in the cytoplasm and processes of the Purkinje cells. After inducing depression, only a weak reaction appeared in several Purkinje cells. Then, after the voluntary running, a moderate expression was observed in the Purkinje cells and their processes (Figure 8(g)–(i)).

According to the OD measurements in photographs of anti-MIF stained sections of the studied brain areas, the MIF expression in the depression group was significantly lower than that in the control group, and the MIF expression in the depression/exercise group was significantly higher than that in the depression group (Figure 7(b), Table 1).

VEGF immunoexpression

DG. The examination of the anti-VEGF immunostained sections of the DG of the control rats revealed strong immunoreactions in the cytoplasm of numerous granular cells in both limbs and the neuropil of both the molecular and polymorph layers. In the depression group, the expression was markedly decreased, and negative neurons were observed in both limbs of the DG. The stem region in several sections showed strong reactions. In the depression/exercise group, the immuno-positivity was obvious in the granular cells in both limbs and the molecular layer (Figure 9(a)–(c)).

mPFC. In the mPFC of the control rats, strong VEGF immunoreactions were observed in several neurons and the neuropil. In the depression group, a weak reaction was observed in many neurons, while in the depression/exercise sections, the restoration of the immuno-positivity was obvious in several neurons and the neuropil (Figure 9(d)–(f)).

Cerebellum. In the cerebellum of the control rats, a strong VEGF immunoexpression was observed in the Purkinje cells and their dendrites and the neuropil in the molecular layer. After inducing depression, only a few Purkinje cells were faintly stained. In the depression/exercise group, the Purkinje cells and their dendrites expressed strong immunoreactions. In addition, the neuropil in the granular cell layer showed strong reactions (Figure 9(g)–(i)).

According to the OD measurements of photographs of anti-VEGF stained sections, the VEGF expression in the depression group was significantly lower than that in the control group, and the VEGF expression in the depression/exercise group
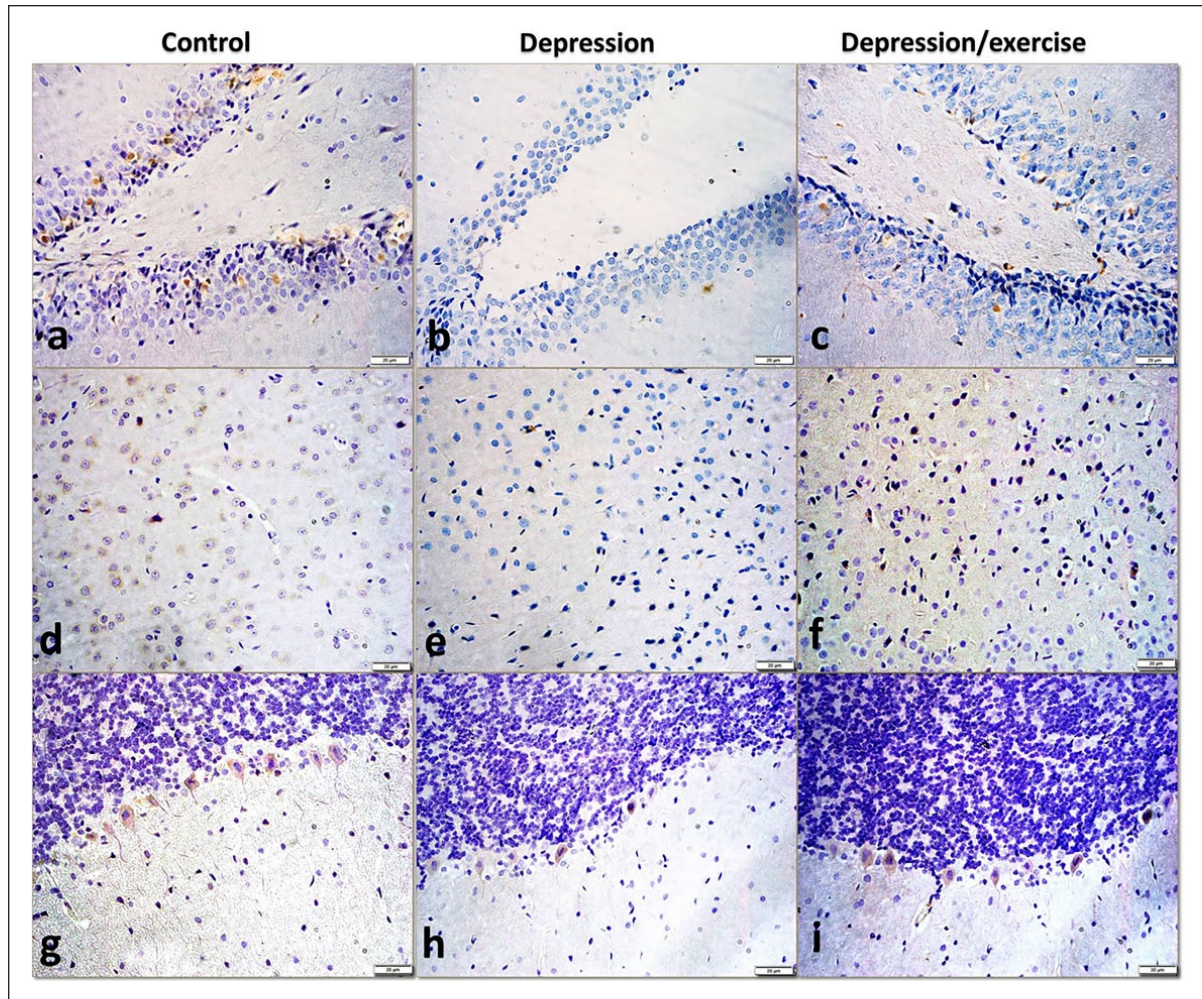
Figure 8. Photomicrographs representing the MIF immunoexpression in sections of (a–c) the dentate gyrus, (d–f) the medial prefrontal cortex and (g–i) the cerebellum in all the studied groups showing its neuronal expression with a markedly decreased or negative expression in depression group, and then its restoration in the depression/exercise group. Note the expression in the neuropil of the medial frontal cortex and in the Purkinje cells processes of the cerebellum in the depression exercise group (anti-MIF immunostain, ×400).

was significantly higher than that in the depression group in all studied areas (Figure 7(c), Table 1).

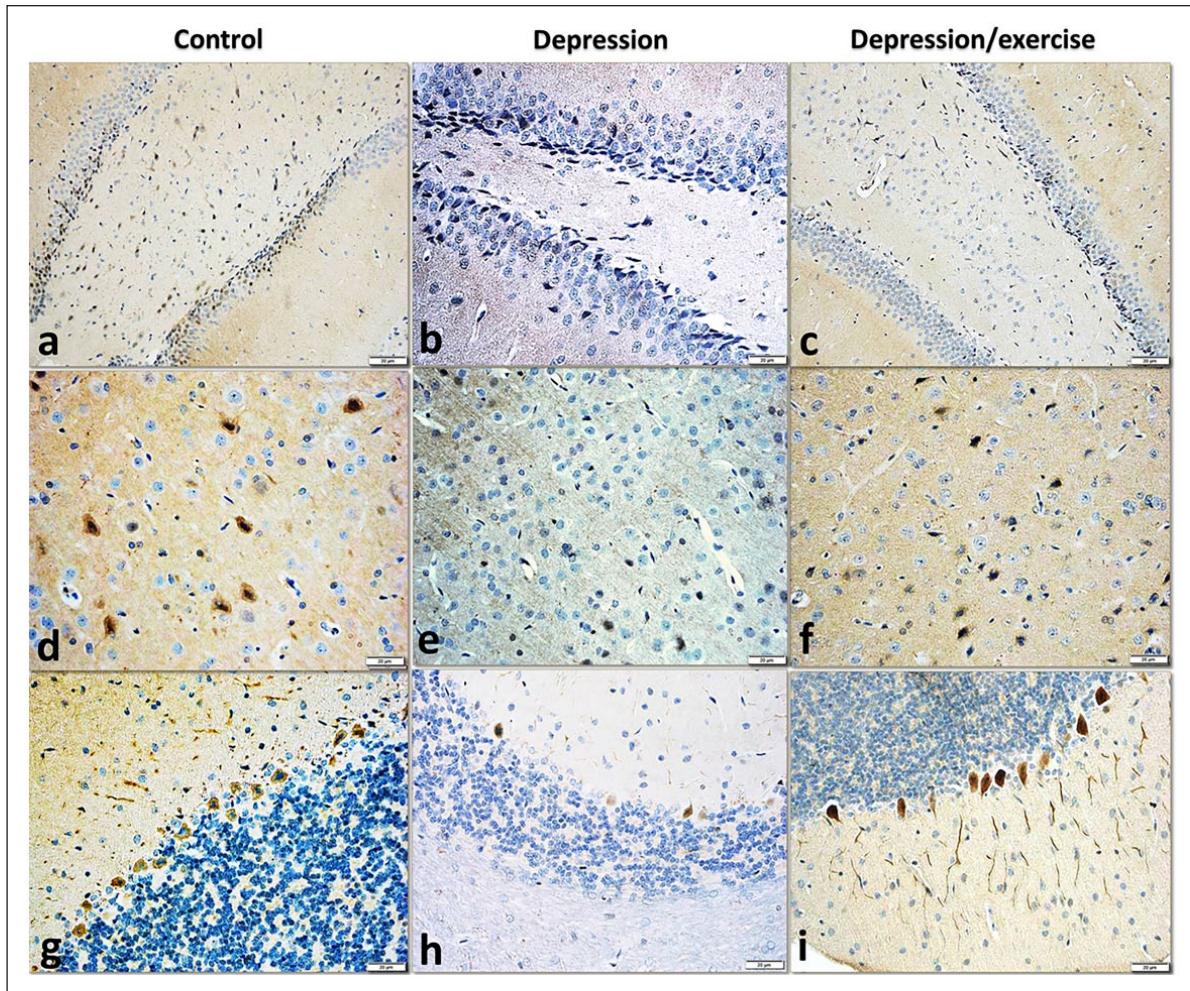*IL-6 immunoexpression*

DG. The examination of anti-IL-6 immunostained sections of the DG of the control rats revealed a mild cytoplasmic immunoexpression in several granular cells, particularly in the SGZ and the neuropil in the molecular and polymorph layers. No IL-6 immunoexpression was observed in the DG of the rats in the depression group, but in the depression/exercise group, a mild cytoplasmic reaction was observed in the granular cells, particularly in the lower limb, and the reaction extended to the polymorph and molecular layers (Figure 10(a)–(c)).

mPFC. A mild IL-6 expression was observed in the mPFC of the control rats, primarily in the neuropil, glial cells and epithelium of blood vessels. The nerve cells in the control sections appeared negative. In the depression group, only a few endothelial cells expressed IL-6 immunoreactions. In the depression/exercise group, the IL-6 immunoreaction was restored in the neuropil and glial cells. In addition, a strong reaction was exhibited by the endothelial cells of blood vessels (Figure 10(d)–(f)).

Cerebellum. In the cerebellum of the control rats, a moderate cytoplasmic IL-6 immunoexpression was observed in the Purkinje cells and their processes. Immuno-positivity was also observed between groups of cells in the granular and molec-

Figure 9. Photomicrographs representing the VEGF immunoexpression in sections of (a–c) the dentate gyrus, (d–f) the medial prefrontal l cortex and (g–i) the cerebellum in all the studied groups showing its neuronal expression with a marked decrease of the immunoexpression in the depression group, then its obvious increase in the depression/exercise group in all the studied brain areas. Note the increased immunoexpression in the neuropil of the medial frontal cortex and the granular layer of the cerebellum of the depression/exercise group (anti-VEGF immunostain, ×400).

ular zones. In the depression group, a weak reaction was expressed by a few Purkinje cells. In the depression/exercise group, a strong immunoreaction was detected in the cytoplasm of the Purkinje cells and their processes, and an immunoreaction also appeared in the neuropil in the granular zone (Figure 10(g)–(i)).

According to the OD measurements of photographs of anti-IL-6-stained sections, the IL-6 expression in the depression group was significantly lower than that in the control group, and the IL-6 expression in the depression/exercise group was significantly higher than that in the depression group in all studied brain areas (Figure 7(d), Table 1).

The correlation between values of optic density of different expressed myokines revealed significant correlation between the immune-expression of different myokines in all examined areas of the brain except for the non significant correlation found between MIF and BDNF in the lower limb of DG ($P = 0.069$) (Table 2).

Discussion

In this study, the induction of depression caused a marked degeneration in the DG, and voluntary running markedly repaired this degeneration. These changes could be due to the changed expression of neurotrophic and vascular neurogenic myokines in brain cells, which is consistent with our results.[35–38]

The histological study of the mPFC of depressed rats showed a disorganisation of the cortical layers and degeneration of many neurons. This finding is
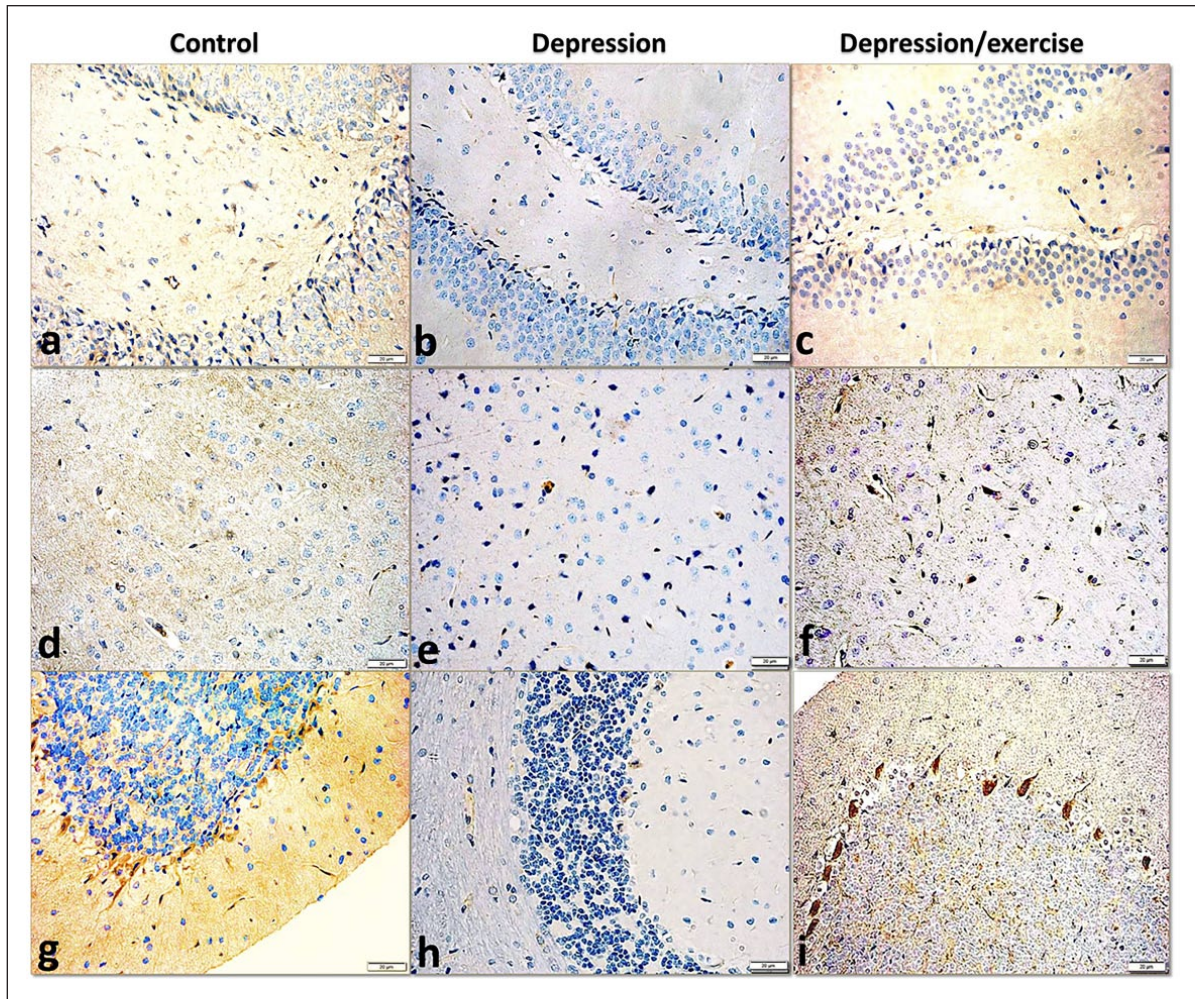
Figure 10. Photomicrographs representing the IL-6 immunoexpression in sections of (a–c) the dentate gyrus, (d–f) the medial frontal cortex and (g–i) the cerebellum in all groups showing a marked decrease of the immunoexpression in the depression group, then its obvious increase in the depression/exercise group in all the studied brain areas. The expression appears in the neurons and neuropil of the dentate and cerebellum, but only in the neuropil, glial cells and endothelium of blood vessels of the medial frontal cortex (anti-IL-6 immunostain, ×400).

consistent with a recent study in which a reduced neuronal size and glial cell density were observed in the prefrontal cortex of subjects with major depression.[39] Also, some stereological studies have reported a decrease in cortical thickness in depression,[40,41] suggesting neuronal loss along with a reduction in the neuropil.[42] Consistent with our hypothesis and findings, Rajkowska[43] suggested that neurotrophic factors play a role in the cellular changes that occur in the mPFC in depression.

This study revealed that exercise likely has a protective effect on the neurons and neuropil in the mPFC, which was evident by the restoration of the normal morphology. Although many studies have reported the exercise-induced proliferation and enhanced survival of hippocampal neurons in

rodents,[44–46] no reports thus far have shown the protective role of exercise on the mPFC.

In this study, the cerebellar cortex of the depressed rats showed a marked degeneration in the Purkinje cells with decreased dendritic extensions. This finding could denote the possible contribution of the cerebellum to the pathophysiology of depression and may be consistent with the reported cerebellar atrophy and reduced cerebellar size in patients with depression.[47,48]

After exercise, the restored dendrites of the Purkinje cells and the increased blood vessels in the molecular layer of the cerebellar cortex can denote the beneficial effect of running which is consistent with the previously reported enhancement of the synaptogenesis of Purkinje cells and

Table 2. Correlation between the optical densities of BDNF, VEGF, MIF and IL-6 in different areas of the brain in the studied groups.

Upper limb of dentate gyrus (n = 15)				Lower limb of dentate gyrus (n = 15)			
	MIF	VEGF	IL-6		MIF	VEGF	IL-6
BDNF	$r = 0.383^{**}$ $P = 0.009$	$r = 0.487^{**}$ $P = 0.001$	$r = 0.584^{**}$ $P < 0.001$	BDNF	$r = 0.273$ $P = 0.069$	$r = -0.480^{**}$ $P = 0.001$	$r = 0.342^{*}$ $P = 0.022$
MIF		$r = 0.635^{**}$ $P < 0.001$	$r = 0.512^{**}$ $P < 0.001$	MIF		$r = -0.537^{**}$ $P < 0.001$	$r = 0.435^{**}$ $P = 0.003$
VEGF			$r = 0.595^{**}$ $P < 0.001$	VEGF			$r = 0.437^{**}$ $P = 0.003$

Medial prefrontal cortex (n = 15)				Cerebellum (n = 15)			
	MIF	VEGF	IL-6		MIF	VEGF	IL-6
BDNF	$r = 0.398^{**}$ $P < 0.001$	$r = 0.582^{**}$ $P < 0.001$	$r = 0.578^{**}$ $P < 0.001$	BDNF	$r = 0.304^{*}$ $P = 0.043$	$r = 0.658^{**}$ $P < 0.001$	$r = 0.590^{**}$ $P < 0.001$
MIF		$r = 0.564^{**}$ $P < 0.001$	$r = 0.520^{**}$ $P < 0.001$	MIF		$r = 0.446^{**}$ $P = 0.002$	$r = 0.141^{**}$ $P = 0.357$
VEGF			$r = 0.488^{**}$ $P = 0.001$	VEGF			$r = 0.564^{**}$ $P < 0.001$

MIF: migration inhibitory factor; VEGF: vascular endothelial growth factor; IL-6: interleukin-6; BDNF: brain-derived neurotrophic factor; *n*: sample number.
*Correlation is significant at the 0.05 level (two-tailed).
**Correlation is significant at the 0.01 level (two-tailed).

improved motor performance after chronic treadmill exercise.[49] Nevertheless, this beneficial effect of exercise might not be mediated via neurogenesis because, in contrast to the hippocampus, many studies have failed to detect neurogenesis in Purkinje cells after exercise.[50,51]

Recently, physical exercise has proved to upregulate certain cytokines and immune cells while downregulating others.[2] Recently, many researchers have investigated the effects of different types of exercise on the expression of these myokines in brain areas.[2] In this study, we investigated the effect of voluntary running on the localization and the intensity of myokines in different brain areas.

The OD values of BDNF immune-expression in the DG, mPFC and cerebellum were significantly lower in the depression group compared to control one, and its values in the depression/exercise group increased significantly than in the depression group.

The downregulation of BDNF expression in brain areas of depressed rats highlights its role in the pathophysiology of depression[52] and is in consistent with some previous studies that reported its decrease in the hippocampus of depressed rats.[53,54] On the other hand, its upregulation in these areas after voluntary running reflects the role of BDNF

in alleviating depression symptoms, which is similar to the effect of antidepressant drugs in animals and human.[55–59]

The mPFC has recently been considered associated with depression and BDNF expression in response to different types of antipsychotic and antidepressant treatments.[60–62] Therefore, the increased expression of BDNF in the mPFC detected in this study could be a contributing factor mediating the relieving effect of voluntary running on depression. This hypothesis is further supported by the findings that electroconvulsive therapy (ECT) alleviates symptoms of depression and increases the BDNF level in both the hippocampus and frontal cortex in a rodent model of depression.[63–66] However, conflicting evidence exists regarding whether the increased expression of BDNF in the frontal cortex after chronic treatment of depression is a direct effect on the frontal cortex or an indirect effect caused by the remodelling of hippocampal neuronal terminations in the mPFC.[67,68]

The functional relationship between the cerebellum and brain areas associated with psychiatric diseases is well recognised.[69,70] However, limited studies have provided evidence regarding the direct role of the cerebellum in depression.[71,72] In our

study, depression caused a marked decrease, whereas voluntary running resulted in a noticeable increase in the immunoexpression in the three cerebellar cortical layers. This changed expression of BDNF in the cerebellar cortex provides evidence of the role of the cerebellum in mood disorders.

The increased BDNF expression in the Purkinje cells and their dendrites after voluntary running is consistent with the improvement in the motor performance of rats after treadmill running[38,49] and can reflect the promoting effect of voluntary exercise on synaptogenesis and neuronal networks in the cerebellum.[51]

Recently, MIF has been considered a promising cytokine that plays a significant role in the pathophysiology of depression and has potential implications for depression management.[73] In this study, the OD values of MIF immune-expression, in all brain regions, decreased significantly in the depression group than in control one and significantly increased in the depression/exercise group than in depression one.

The decreased immunoexpression of MIF during depression is consistent with the increased depression-like behaviour in MIF knockout mice and the significant reduction in the number of proliferative cells in the DG after the pharmacological inhibition of MIF.[74,75] The increased MIF expression after the voluntary running programme is consistent with the findings reported by Bloom and Al-Abed, who highlighted the central role of MIF in the exercise-mediated alleviation of depressive behaviour.[73]

VEGF is considered the master regulator of angiogenesis.[76] Recently, the neuroprotective role of VEGF in psychiatric diseases has attracted attention.[2,77] In this study, the OD values of VEGF immune-expression were significantly lower in depression group than that in control in all studied brain areas, and after voluntary running, the OD values increased significantly compared with the depression group in all areas.

The changed expression of VEGF during depression and after exercise might reflect the important role of this cytokine in signalling pathways engaged in behaviour and neurogenesis.[77,78] The increased expression after the voluntary running is consistent with several recent studies[79–81] and may reflect the neurotrophic action of this cytokine during exercise.[2,82] This finding could be consistent with a new hypothesis that physical exercise triggers angiogenic genes[83–85] and supports the suggestion of using pro-angiogenesis factors, including VEGF, in the treatment of neurodegenerative diseases.[86]

IL-6 is commonly known as a proinflammatory cytokine, but several authors consider IL-6 an anti-inflammatory immunosuppressive factor.[87] Recently, IL-6 has been considered to play a role in depression by changing the connectivity of the hippocampus to the anterior cingulate cortex.[88] In this study, depression decreased the expression significantly in all studied brain areas, whereas voluntary running significantly increased the expression, but different patterns were observed in different brain areas.

The low expression of IL-6 in the studied brain areas and its localisation in glial and endothelial cells are consistent with a recent study reporting that IL-6 is primarily expressed in astrocytes and minimally expressed in neurons.[89] The changing expression of this myokine in the studied brain areas during depression and after the improvement of the depressive behaviour reflects its involvement in the pathophysiology of depression and the modulation of neurogenesis.[89]

The significant correlation between the immune-expression of different myokines in all examined areas of the brain (DG, mPFC and cerebellum) can denote the harmony in reaction of these areas during induction and/or relief of depression and so, this can confirm the hypothesis that the mPFC and cerebellum are involved in the pathophysiology of depression.

In conclusion, this study explored the histopathological changes in the DG, mPFC and cerebellum both in depression and after voluntary running. To the best of our knowledge, this is the first study to immunohistochemically investigate the cellular expression of myokines (i.e. BDNF, MIF, VEGF and IL-6) and whether they are upregulated in particular areas of the brain after practising voluntary running. This study clearly reveals the involvement of the mPFC and cerebellum in the pathophysiology of depression.

Author's contribution

ORCID iD

Magda A Eldomiaty https://orcid.org/0000-0003-4899-2005

References

1. Krogh J, Speyer H, Gluud C, et al. (2015) Exercise for patients with major depression: A protocol for a systematic review with meta-analysis and trial sequential analysis. *Systematic Reviews* 4: 40.
2. Vivar C, Potter MC and van Praag H (2013) All about running: Synaptic plasticity, growth factors and adult hippocampal neurogenesis. *Current Topics in Behavioral Neurosciences* 15: 189–210.
3. Moon HY, Kim SH, Yang YR, et al. (2012) Macrophage migration inhibitory factor mediates the antidepressant actions of voluntary exercise. *Proceedings of the National Academy of Sciences of the United States of America* 109: 13094–13099.
4. Pedersen BK (2011) Muscles and their myokines. *Journal of Experimental Biology* 214: 337–346.
5. Pedersen BK, Akerstrom TC, Nielsen AR, et al. (2007) Role of myokines in exercise and metabolism. *Journal of Applied Physiology* 103: 1093–1098.
6. Pedersen BK and Febbraio MA (2008) Muscle as an endocrine organ: Focus on muscle-derived interleukin-6. *Physiological Reviews* 88: 1379–1406.
7. Frodl T, Schule C, Schmitt G, et al. (2007) Association of the brain-derived neurotrophic factor Val66Met polymorphism with reduced hippocampal volumes in major depression. *Archives of General Psychiatry* 64: 410–416.
8. Ward NL and Hagg T (2000) BDNF is needed for postnatal maturation of basal forebrain and neostriatum cholinergic neurons in vivo. *Experimental Neurology* 162: 297–310.
9. Musil R, Schwarz MJ, Riedel M, et al. (2011) Elevated macrophage migration inhibitory factor and decreased transforming growth factor-beta levels in major depression–no influence of celecoxib treatment. *Journal of Affective Disorders* 134: 217–225.
10. Clark-Raymond A, Meresh E, Hoppensteadt D, et al. (2014) Vascular Endothelial Growth Factor: A potential diagnostic biomarker for major depression. *Journal of Psychiatric Research* 59: 22–27.
11. Campbell S and Macqueen G (2004) The role of the hippocampus in the pathophysiology of major depression. *Journal of Psychiatry & Neuroscience* 29: 417–426.
12. Kempermann G, Krebs J and Fabel K (2008) The contribution of failing adult hippocampal neurogenesis to psychiatric disorders. *Current Opinion in Psychiatry* 21: 290–295.
13. Zikopoulos B and Barbas H (2007) Circuits for multisensory integration and attentional modulation through the prefrontal cortex and the thalamic reticular nucleus in primates. *Reviews in the Neurosciences* 18: 417–438.
14. Zikopoulos B and Barbas H (2007) Parallel driving and modulatory pathways link the prefrontal cortex and thalamus. *PLoS ONE* 2: e848.
15. Ressler KJ and Mayberg HS (2007) Targeting abnormal neural circuits in mood and anxiety disorders: From the laboratory to the clinic. *Nature Neuroscience* 10: 1116–1124.
16. Schmahmann JD, Weilburg JB and Sherman JC (2007) The neuropsychiatry of the cerebellum – insights from the clinic. *Cerebellum* 6: 254–267.
17. Huang Y, Ko H, Cheung ZH, et al. (2012) Dual actions of brain-derived neurotrophic factor on GABAergic transmission in cerebellar Purkinje neurons. *Experimental Neurology* 233: 791–798.
18. Kleim JA, Markham JA, Vij K, et al. (2007) Motor learning induces astrocytic hypertrophy in the cerebellar cortex. *Behavioural Brain Research* 178: 244–249.
19. Black JE, Isaacs KR, Anderson BJ, et al. (1990) Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proceedings of the National Academy of Sciences of the United States of America* 87: 5568–5572.
20. Manabe Y, Miyatake S and Takagi M (2012) Myokines: Do they really exist? *The Journal of Physical Fitness and Sports Medicine* 1: 51–58.
21. Andrus BM, Blizinsky K, Vedell PT, et al. (2012) Gene expression patterns in the hippocampus and amygdala of endogenous depression and chronic stress models. *Molecular Psychiatry* 17: 49–61.
22. Garrett L, Lie DC, Hrabe de Angelis M, et al. (2012) Voluntary wheel running in mice increases the rate of neurogenesis without affecting anxiety-related behaviour in single tests. *BMC Neuroscience* 13: 61.

23. Eldomiaty MA, Almasry SM, Desouky MK, et al. (2017) Voluntary running improves depressive behaviours and the structure of the hippocampus in rats: A possible impact of myokines. *Brain Research* 1657: 29–42.

24. Porsolt RD, Anton G, Blavet N, et al. (1978) Behavioural despair in rats: A new model sensitive to antidepressant treatments. *European Journal of Pharmacology* 47: 379–391.

25. Porsolt RD, Bertin A and Jalfre M (1977) Behavioral despair in mice: A primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et de Therapie* 229: 327–336.

26. Sun MK and Alkon DL (2003) Open space swimming test to index antidepressant activity. *Journal of Neuroscience Methods* 126: 35–40.

27. Jonsson J (2012) *Effect of Voluntary Exercise on BDNF/TrkB Gene Expression and Alcohol Intake*. Linköping: Linköping University.

28. Porsolt RD (2000) Animal models of depression: Utility for transgenic research. *Reviews in the Neurosciences* 11: 53–58.

29. Mehrpouya S, Nahavandi A, Khojasteh F, et al. (2015) Iron administration prevents BDNF decrease and depressive-like behavior following chronic stress. *Brain Research* 1596: 79–87.

30. Vega-Rivera NM, Fernandez-Guasti A, Ramirez-Rodriguez G, et al. (2014) Forced swim and chronic variable stress reduced hippocampal cell survival in OVX female rats. *Behavioural Brain Research* 270: 248–255.

31. Eisch AJ, Bolanos CA, de Wit J, et al. (2003) Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: A role in depression. *Biological Psychiatry* 54: 994–1005.

32. Spijker S (2011) Dissection of Rodent brain regions. In: Li KW (ed.) *Neuroproteomics*. New York: Humana Press, pp. 13–26.

33. Grizzle WE, Fredenburgh JL and Myers RB (2008) Fixation of tissues. In: Bancroft JD and Gamble M (eds) *Theory and Practice of Histological Techniques*, 6th edn. Philadelphia, PA: Churchill Livingstone, pp. 53–74.

34. Varghese F, Bukhari AB, Malhotra R, et al. (2014) IHC Profiler: An open source plugin for the quantitative evaluation and automated scoring of immunohistochemistry images of human tissue samples. *PLoS ONE* 9: e96801.

35. van Praag H (2008) Neurogenesis and exercise: Past and future directions. *Neuromolecular Medicine* 10: 128–140.

36. Vadodaria KC and Jessberger S (2014) Functional neurogenesis in the adult hippocampus: Then and now. *Frontiers in Neuroscience* 8: 55.

37. Kuhn HG, Dickinson-Anson H and Gage FH (1996) Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation. *Journal of Neuroscience* 16: 2027–2033.

38. Hyer MM, Hunter TJ, Katakam J, et al. (2016) Neurogenesis and anxiety-like behavior in male California mice during the mate's postpartum period. *European Journal of Neuroscience* 43: 703–709.

39. Cotter D, Mackay D, Chana G, et al. (2002) Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. *Cerebral Cortex* 12: 386–394.

40. Bouras C, Kovari E, Hof PR, et al. (2001) Anterior cingulate cortex pathology in schizophrenia and bipolar disorder. *Acta Neuropathologica* 102: 373–379.

41. Rajkowska G, Halaris A and Selemon LD (2001) Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biological Psychiatry* 49: 741–752.

42. Chana G, Landau S, Beasley C, et al. (2003) Two-dimensional assessment of cytoarchitecture in the anterior cingulate cortex in major depressive disorder, bipolar disorder, and schizophrenia: Evidence for decreased neuronal somal size and increased neuronal density. *Biological Psychiatry* 53: 1086–1098.

43. Rajkowska G (2000) Histopathology of the prefrontal cortex in major depression: What does it tell us about dysfunctional monoaminergic circuits? *Progress in Brain Research* 126: 397–412.

44. Clark PJ, Kohman RA, Miller DS, et al. (2010) Adult hippocampal neurogenesis and c-Fos induction during escalation of voluntary wheel running in C57BL/6J mice. *Behavioural Brain Research* 213: 246–252.

45. Holmes MM, Galea LA, Mistlberger RE, et al. (2004) Adult hippocampal neurogenesis and voluntary running activity: Circadian and dose-dependent effects. *Journal of Neuroscience Research* 76: 216–222.

46. Snyder JS, Glover LR, Sanzone KM, et al. (2009) The effects of exercise and stress on the survival and maturation of adult-generated granule cells. *Hippocampus* 19: 898–906.

47. Magavi SS, Leavitt BR and Macklis JD (2000) Induction of neurogenesis in the neocortex of adult mice. *Nature* 405: 951–955.

48. Pillay SS, Yurgelun-Todd DA, Bonello CM, et al. (1997) A quantitative magnetic resonance imaging study of cerebral and cerebellar gray matter volume in primary unipolar major depression: Relationship to treatment response and clinical severity. *Biological Psychiatry* 42: 79–84.

49. Huang TY, Lin LS, Cho KC, et al. (2012) Chronic treadmill exercise in rats delicately alters the Purkinje cell structure to improve motor performance and toxin resistance in the cerebellum. *Journal of Applied Physiology* 113: 889–895.

50. Lee A, Kessler JD, Read TA, et al. (2005) Isolation of neural stem cells from the postnatal cerebellum. *Nature Neuroscience* 8: 723–729.

51. Lee KS, Lim BV, Chang HK, et al. (2005) Liuweidihuang-tang improves spatial memory function and increases neurogenesis in the dentate gyrus in rats. *Fitoterapia* 76: 514–519.

52. Kimpton J (2012) The brain derived neurotrophic factor and influences of stress in depression. *Psychiatria Danubina* 24(Suppl. 1): S169–S171.

53. Roceri M, Hendriks W, Racagni G, et al. (2002) Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. *Molecular Psychiatry* 7: 609–616.

54. Smith MA, Makino S, Kvetnansky R, et al. (1995) Effects of stress on neurotrophic factor expression in the rat brain. *Annals of the New York Academy of Sciences* 771: 234–239.

55. Duman RS and Monteggia LM (2006) A neurotrophic model for stress-related mood disorders. *Biological Psychiatry* 59: 1116–1127.

56. Warner-Schmidt JL and Duman RS (2006) Hippocampal neurogenesis: Opposing effects of stress and antidepressant treatment. *Hippocampus* 16: 239–249.

57. Cotman CW and Berchtold NC (2002) Exercise: A behavioral intervention to enhance brain health and plasticity. *Trends in Neurosciences* 25: 295–301.

58. Cotman CW, Berchtold NC and Christie LA (2007) Exercise builds brain health: Key roles of growth factor cascades and inflammation. *Trends in Neurosciences* 30: 464–472.

59. Novak CM, Burghardt PR and Levine JA (2012) The use of a running wheel to measure activity in rodents: Relationship to energy balance, general activity, and reward. *Neuroscience and Biobehavioral Reviews* 36: 1001–1014.

60. Balu DT, Hoshaw BA, Malberg JE, et al. (2008) Differential regulation of central BDNF protein levels by antidepressant and non-antidepressant drug treatments. *Brain Research* 1211: 37–43.

61. Cotter D, Mackay D, Landau S, et al. (2001) Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. *Archives of General Psychiatry* 58: 545–553.

62. Rajkowska G and Miguel-Hidalgo JJ (2007) Gliogenesis and glial pathology in depression. *CNS & Neurological Disorders Drug Targets* 6: 219–233.

63. Chen AC, Shin KH, Duman RS, et al. (2001) ECS-Induced mossy fiber sprouting and BDNF expression are attenuated by ketamine pretreatment. *Journal of ECT* 17: 27–32.

64. Dyrvig M, Christiansen SH, Woldbye DP, et al. (2014) Temporal gene expression profile after acute electroconvulsive stimulation in the rat. *Gene* 539: 8–14.

65. Luo J, Min S, Wei K, et al. (2015) Behavioral and molecular responses to electroconvulsive shock differ between genetic and environmental rat models of depression. *Psychiatry Research* 226: 451–460.

66. Ryan KM, O'Donovan SM and McLoughlin DM (2013) Electroconvulsive stimulation alters levels of BDNF-associated microRNAs. *Neuroscience Letters* 549: 125–129.

67. Zhong YM, Yukie M and Rockland KS (2006) Distinctive morphology of hippocampal CA1 terminations in orbital and medial frontal cortex in macaque monkeys. *Experimental Brain Research* 169: 549–553.

68. Fuchs E, Flugge G and Czeh B (2006) Remodeling of neuronal networks by stress. *Frontiers in Bioscience: A Journal and Virtual Library* 11: 2746–2758.

69. Anand BK, Malhotra CL, Singh B, et al. (1959) Cerebellar projections to limbic system. *Journal of Neurophysiology* 22: 451–457.

70. Heath RG, Dempesy CW, Fontana CJ, et al. (1978) Cerebellar stimulation: Effects on septal region, hippocampus, and amygdala of cats and rats. *Biological Psychiatry* 13: 501–529.

71. Konarski JZ, McIntyre RS, Grupp LA, et al. (2005) Is the cerebellum relevant in the circuitry of neuropsychiatric disorders? *Journal of Psychiatry & Neuroscience* 30: 178–186.

72. Smith KA, Ploghaus A, Cowen PJ, et al. (2002) Cerebellar responses during anticipation of noxious stimuli in subjects recovered from depression: Functional magnetic resonance imaging study. *The British Journal of Psychiatry: The Journal of Mental Science* 181: 411–415.

73. Bloom J and Al-Abed Y (2014) MIF: Mood Improving/Inhibiting Factor? *Journal of Neuroinflammation* 11: 11.

74. Conboy L, Varea E, Castro JE, et al. (2011) Macrophage migration inhibitory factor is critically involved in basal and fluoxetine-stimulated adult hippocampal cell proliferation and in anxiety, depression, and memory-related behaviors. *Molecular Psychiatry* 16: 533–547.

75. Swant JD, Rendon BE, Symons M, et al. (2005) Rho GTPase-dependent signaling is required for macrophage migration inhibitory factor-mediated expression of cyclin D1. *The Journal of Biological Chemistry* 280: 23066–23072.

76. Crafts TD, Jensen AR, Blocher-Smith EC, et al. (2015) Vascular endothelial growth factor: Therapeutic possibilities and challenges for the treatment of ischemia. *Cytokine* 71: 385–393.

77. Nowacka MM and Obuchowicz E (2012) Vascular endothelial growth factor (VEGF) and its role in the central nervous system: A new element in the neurotrophic hypothesis of antidepressant drug action. *Neuropeptides* 46: 1–10.

78. van Praag H, Shubert T, Zhao C, et al. (2005) Exercise enhances learning and hippocampal neurogenesis in aged mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 25: 8680–8685.

79. Clark PJ, Brzezinska WJ, Puchalski EK, et al. (2009) Functional analysis of neurovascular adaptations to exercise in the dentate gyrus of young adult mice associated with cognitive gain. *Hippocampus* 19: 937–950.

80. Fabel K, Fabel K, Tam B, et al. (2003) VEGF is necessary for exercise-induced adult hippocampal neurogenesis. *The European Journal of Neuroscience* 18: 2803–2812.

81. Van der Borght SF, Clevenbergh P, Rijckborst H, et al. (2009) Mortality and morbidity among HIV type-1-infected patients during the first 5 years of a multicountry HIV workplace programme in Africa. *Antiviral Therapy* 14: 63–74.

82. Ogunshola OO, Antic A, Donoghue MJ, et al. (2002) Paracrine and autocrine functions of neuronal vascular endothelial growth factor (VEGF) in the central nervous system. *The Journal of Biological Chemistry* 277: 11410–11415.

83. Arany Z, Foo SY, Ma Y, et al. (2008) HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. *Nature* 451: 1008–1012.

84. Raschke S and Eckel J (2013) Adipo-myokines: Two sides of the same coin–mediators of inflammation and mediators of exercise. *Mediators of Inflammation* 2013: 320724.

85. Thom R, Rowe GC, Jang C, et al. (2014) Hypoxic induction of vascular endothelial growth factor (VEGF) and angiogenesis in muscle by truncated peroxisome proliferator-activated receptor gamma coactivator (PGC)-1alpha. *The Journal of Biological Chemistry* 289: 8810–8817.

86. Pereira AC, Huddleston DE, Brickman AM, et al. (2007) An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proceedings of the National Academy of Sciences of the United States of America* 104: 5638–5643.

87. Pedersen BK, Steensberg A, Fischer C, et al. (2004) The metabolic role of IL-6 produced during exercise: Is IL-6 an exercise factor? *The Proceedings of the Nutrition Society* 63: 263–267.

88. Greicius MD, Flores BH, Menon V, et al. (2007) Resting-state functional connectivity in major depression: Abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biological Psychiatry* 62: 429–437.

89. Arisi GM (2014) Nervous and immune systems signals and connections: Cytokines in hippocampus physiology and pathology. *Epilepsy & Behavior* 38: 43–47.