

Comparison of hippocampal interleukin-6 immunoreactivity after exhaustive exercise in both exercise-trained and untrained rats

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Background/aim: Exhaustive exercise is a strong stress factor and can impact cytokine production in the brain. Interleukin-6 (IL-6) is produced in greater amounts than any other cytokine in response to exercise, and its effects are closely related to both exercise duration and intensity. In this study, we measured the differences between the amount of IL-6 reactivity of the hippocampus after an exhaustive session of running in long-term exercise-trained and untrained rats.

Materials and methods: The exercise-trained group ran on a treadmill for 12 weeks. Both groups were forced to run until exhaustion. Each group of rats was sacrificed immediately, 1 day, or 3 days after exhaustion and the brains were evaluated for IL-6 immunoreactivity in the hippocampus.

Results: Hippocampal IL-6 immunoreactivity was absent in controls, mild to severe in untrained rats, and weak to mild in long-term-trained rats. The most prominent increase in IL-6 was observed in the untrained rats sacrificed 1 day after exhaustion.

Conclusion: Exercise to exhaustion resulted in increased IL-6 levels in brain slices in both groups of rats, but long-term exercise training protected the hippocampus from exposure to an extreme increase in IL-6. The immediate effects of these cytokine levels were observed 1 day after exhaustion.

Key words: Exhaustive exercise, hippocampus, interleukin-6

1. Introduction

Regular physical activity with moderate intensity and duration contributes to the general health of the cardiovascular, respiratory, and cognitive systems and supports many physical processes (1). In recent decades it has been accepted that exercise is indicated in the treatment of many chronic disorders, and in several cases it is as effective as medical treatment (2). However, some types of exercise can be considered as a physical stress factor, since they induce a response in the immune system similar to the subclinical inflammatory response to a pathological condition (3). Long and exhausting exercise may disturb immune cell function due to an increase in the levels of stress hormones and reactive oxygen species. In particular, acute and severe exercise may cause structural muscle damage via muscle fiber inflammation, and it has been shown that moderate to severe exercise also stimulates the hypothalamic–pituitary–adrenal axis (HPA) and the release of cortisol, leading to an inflammatory reaction (4).

An acute, strenuous, and long-term session of exercise, such as running a marathon or ultramarathon, transiently inhibits proper immune function (5). After several hours of strenuous exercise, the immune system produces a strong and adaptive response due to the stimulatory effects of stress hormones. The factors that stimulate the immune system after strenuous exercise include intestinal endotoxin leakage, catecholamines, increased body temperature, glycogen deficit, oxidative stress, and muscle damage (5).

Although the most prominent cytokine produced during exercise is interleukin (IL)-6, which is also known as myokine (6), it has been shown that other pro- or antiinflammatory cytokines are also produced during exercise training (7,8). The sources of this cytokine production during exercise include muscle cells, peritendinous tissue, subcutaneous tissue, and the brain, and cytokine production is closely related to both the duration and the intensity of the exercise (6,9). However,

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regular exercise programs stimulate the pleiotropic antiinflammatory effects of IL-6; increased levels of IL-6 inhibit tumor necrosis factor (TNF)- α (10) and IL-1 but stimulate the expression of TNF-receptor antagonist (ra), IL-10ra, and IL-10 (6,7).

Contrary to the previously held notion that the brain is an immune-privileged organ, studies in recent years have revealed that cytokines produced in the periphery can affect the permeability of the blood brain barrier and may also be produced inside the brain under normal and pathological conditions. It has been shown that acute or chronic stress stimulates the production of TNF- α , IL-1 β , and IL-6 in different regions of the brain, including the hippocampus (11). Pathological conditions in the central nervous system (CNS), such as injury, inflammation, hypoxia, and several neurodegenerative diseases, cause IL-6 production to increase in the brain, the main source of which is thought to be astrocytes (12).

Although the metabolic benefits and positive effects of moderate exercise on brain functions are well known, the effects of other types and intensities of exercise on the brain's immune system are not well understood. In this study, we sought to evaluate the effects of exhaustive running on hippocampal IL-6 immunoreactivity. We also attempted to differentiate the effects of this type of intense exercise on the brains of long-term exercise-trained rats and untrained rats. For this purpose, we evaluated hippocampal slices from rats that had undergone regular training as well as untrained rats after exhaustive running using immunohistochemical methods.

2. Materials and methods

The study was approved by the Gazi University Ethics Committee for Animals under Report No. 142-18805 and was confirmed by the Guide of Institutional Animal Care of the Gazi University Experimental Research Center.

2.1. Animals and study design

Three-month-old male Wistar albino rats (n = 48) were housed in standard conditions (21 \pm 2 °C, 12 h light/12 h dark) with free access to tap water and food pellets. The rats were randomly assigned to 7 groups as follows: sedentary controls (C) that did not run during the experiment; untrained rats (UT) that were forced to run to exhaustion acutely and then sacrificed immediately after exhaustion (UT-I), 1 day after exhaustion (UT-1), or 3 days after exhaustion (UT-3); and long-term exercise-trained rats (T) that were forced to run to exhaustion and then sacrificed immediately after exhaustion (T-I), 1 day after exhaustion (T-1), or 3 days after exhaustion (T-3).

At least 6 animals were used in each subgroup. However, due to the risks associated with long-term exercise, each subgroup of the T group consisted of 8 animals. The organization of the groups is shown in Table 1.

2.2. Exercise training and adaptation of the rats on the treadmill

The exercise-trained rats performed 30 min of treadmill running 5 days a week for 12 weeks. Each session included 5-min warm-up and cool-down periods (running 5 m/min on a 0° incline). Between these periods, the rats were forced to run at a speed of 15 m/min on a 15° incline for 20 min (13,14). The untrained rats were adapted to the treadmill by running at a speed of 5 m/min on a 0° incline for 10 min for 5 days during the last week of the experiment. One day after these exercise periods ended, both groups were forced to perform an exhausting run.

For exhausting exercise, the animals were forced to run at a rate of 20 m/min on a 0° incline until they refused to run any further, laid on their backs, and failed to respond to an electrical shock of approximately 1.5 mA. The exhaustion time (15) of each animal was recorded.

Table 1. Groups and running programs of experimental animals.

Groups	Running program	Subgroups according to time of sacrifice	n
Controls (C) (n = 6)	Sedentary group not been subjected to any exercise program or exhaustive running	-	6
Untrained (UT) (n = 18)	Did not run daily but was finally subjected to acute exhaustive running	Immediately (UT-I)	6
		1 day after exhaustion (UT-1)	6
		3 days after exhaustion (UT-3)	6
Trained (T) (n = 24)	Subjected to acute exhaustive running after a training program for 30 min/day, 5 days for 12 weeks -Warm-up and cool-down (each \rightarrow 5 min, 5 m/min, 0° incline) -Running (\rightarrow 20 min, 15 m/min, 15° incline)	Immediately (T-I)	8
		1 day after exhaustion (T-1)	8
		3 days after exhaustion (T-3)	8

2.3. Removal of brains

Subgroups of the T, UT, and C groups were sacrificed immediately after exhaustion, 1 day after exhaustion, or 3 days after exhaustion under ketamine hydrochloride (45 mg/kg) and xylazine hydrochloride (5 mg/kg) anesthesia by drawing 10 mL of blood intraventricularly. The brains were removed from the skulls without injuring the tissue. The hippocampus was removed carefully on ice and prepared for immunohistochemical evaluation.

2.4. Histological procedure

Hippocampal slices were sectioned at a thickness of 4 μ m. The sections were stained initially with hematoxylin and eosin to investigate the morphology of the neurons under light microscopy. Additional serial sections were stained for immunohistochemistry using the peroxidase-antiperoxidase (PAP) method with a goat polyclonal antibody against mouse IL-6 (M-19, sc-1265, Lot: G 3008, Santa Cruz Biotechnology), and the slides were examined with a photo-light microscope (DCM4500 Image Analysis System and QWin V3 software, Leica, Germany) at the Gazi University Faculty of Medicine Histology and Embryology Laboratories. The immunoreactivity of the stained tissues was scored semiquantitatively as the percentage of retention density in the brains from each group. The values were reported as H scores and were classified as 0 (no retention), 1 (+, weak immunoreactivity), 2 (++ , moderate immunoreactivity), or 3 (+++ , strong immunoreactivity). The immunoreactivities of each area were summated and formulated as $\sum Pi(I + 1)$, where Pi is the percentage of retention and I is the density of retention.

2.5. Statistical analysis

The statistical analyses were carried out using SPSS 16.0, and $P < 0.05$ was accepted as significant. The mean exhaustion times of the UT and T groups were compared using the Mann–Whitney U test. The calculated immunoreactivity values obtained for the controls and the groups that were sacrificed immediately after exhaustion, 1 day after exhaustion, and 3 days after exhaustion were evaluated using the Kruskal–Wallis test. Any differences established between the groups were evaluated using the Bonferroni correction with the Mann–Whitney U test. The comparisons between the exhausted rats and the controls

for the trained and untrained groups were performed using the Mann–Whitney U test.

3. Results

The mean exhaustion time of the rats was 149 ± 35 min for the T group and 84 ± 26 min for the UT group, and the difference between these groups was statistically significant ($P < 0.05$). The levels of IL-6 immunoreactivity in the hippocampus from rats in the UT and T groups were 224.34 ± 4.06 and 114.63 ± 2.62 , respectively (Table 2).

IL-6 immunoreactivity was calculated using H scores for each subgroup, and the results are shown in Table 3. In the hippocampal slices of the controls, the IL-6 immunoreactivity was very weak or negative (Figure 1). In the hippocampal slices of the untrained rats, the IL-6 immunoreactivity ranged from mild to severe (Figure 2). In the hippocampal slices from the long-term trained rats, the IL-6 immunoreactivity ranged from weak to mild (Figure 3). As compared to sedentary controls, the IL-6 immunoreactivity in the UT and T groups was found to be elevated significantly after exhaustive running ($P < 0.05$). When the subgroups from the UT and T groups were compared in regards to the time of sacrifice after exhaustion, there were statistically significant differences in the levels of IL-6 between the rats sacrificed immediately after exhaustion, those that were sacrificed 1 day after exhaustion, and those that were sacrificed 3 days after exhaustion in both groups ($P < 0.05$). The level of IL-6 immunoreactivity was significantly higher in the rats that were sacrificed 1 day after exhaustion in both groups as compared to the other subgroups sacrificed at different time points ($P < 0.05$). A decrease in IL-6 immunoreactivity on the third day after exhaustion was most evident in the T group. The calculated IL-6 immunoreactivity in the subgroups of rats sacrificed at each of the 3 time points was significantly higher in the UT groups as compared to the T groups ($P < 0.05$) (Figure 4). The mean values of IL-6 immunoreactivity and the data from the statistical evaluations are shown in Tables 2 and 3.

4. Discussion

In this study, we investigated the immune response in the brain following exhaustive exercise in long-term exercise-

Table 2. Mean IL-6 immunoreactivities and exhaustion time differences between the UT and T groups of rats (data are presented as mean \pm SEM).

	Untrained group (UT) ⁺	Trained group (T) ⁺
Exhaustion time (min) ⁺⁺	84 ± 26	149 ± 35
IL-6 immunoreactivity ^{*,++}	224.34 ± 4.06	114.63 ± 2.62

*: IL-6 immunoreactivity = percentage of retention \times (density of retention + 1).

†: $r = -0.304$ for UT group and $r = -0.487$ for T group. ††: UT–T groups: $P < 0.05$.

Table 3. Comparison of IL-6 immunoreactivity between the C, UT, and T groups of rats sacrificed at different time points after exhaustion (data are presented as mean \pm SEM).

Time of sacrifice	Untrained group (UT)			Trained group (T)			Controls (C)
	Immediately after exhaustion (UT-I)	1 day after exhaustion (UT-1)	3 days after exhaustion (UT-3)	Immediately after exhaustion (T-I)	1 day after exhaustion (T-1)	3 days after exhaustion (T-3)	Nontreated
IL-6 immunoreactivity*	222.20 \pm 3.13	334.33 \pm 7.82	116.50 \pm 1.25	43.40 \pm 1.2	228.67 \pm 4.96	71.83 \pm 1.69	33.00 \pm 3.60

*: IL-6 immunoreactivity = percentage of retention \times (density of retention + 1). UT-I = a, T-I = a₁, UT-1 = b, T-1 = b₁, UT-3 = c, T-3 = c₁, C = x. P < 0.05 for a-a₁, b-b₁, c-c₁; P < 0.05 for a-b, a-c, b-c; P < 0.05 for a₁-b₁, a₁-c₁, b₁-c₁; P < 0.05 for x-a, b, c, a₁, b₁, and c₁.

trained and untrained rats. We found increased IL-6 immunoreactivity in the brain slices from both groups after exhaustive running, although this brain immunoreactivity after exhaustive exercise was more severe in the UT group as compared to the T group.

Exercise represents a form of physical stress that challenges homeostasis (16). In humans, strenuous, prolonged physical activity has been shown to increase the concentrations of several cytokines in the circulation. For example, previous studies have observed increases in the plasma concentrations of various substances that are known to influence leukocyte function, including proinflammatory cytokines, such as TNF- α , macrophage inflammatory protein-1, and IL-1 β ; antiinflammatory cytokines, such as IL-6, IL-10, and IL-1ra; and acute phase proteins, such as C-reactive protein (17). Of these proinflammatory cytokines, 3 are secreted in a cascade-like fashion, with TNF- α appearing first, IL-1 appearing second, and IL-6 appearing third (16). Among these cytokines, IL-6 is most abundantly expressed during exercise (13). Athletes suffering from underperformance

syndrome, a state including various symptoms such as fatigue and a decrease in performance capacity, often exhibit elevated levels of cytokines (18). Moreover, many studies have reported that various immune cell functions are temporarily impaired following acute bouts of prolonged, continuous heavy exercise (19), and athletes engaged in intensive periods of endurance training appear to be more susceptible to minor infections (17). In our study, by acting as a strong stress factor, exhaustive exercise promoted IL-6 immunoreactivity in the hippocampal slices from both experimental groups as compared to the controls. Based on the intensity of the IL-6 immunoreactivity, it may be suggested that the effect of exhaustive exercise-induced stress on the hippocampus was milder in the T group as compared to the UT group.

Long-lasting strenuous exercise can be deleterious to certain organs and systems of the body. In addition, cytokine production is known to be modulated by various stimuli, including trauma, infection, and physical activity. IL-6 is a pleiotropic cytokine that can produce both pro- and antiinflammatory effects; as such, this cytokine has diverse activities, such as the modulation of proliferation and differentiation and regulation of the immune response. IL-6 also mediates fever and is the main inducer of the acute-phase response to infection (20). IL-6 is released from the brain as well as the muscles during prolonged exercise in humans, and it appears that the duration of the exercise, rather than the increase in body temperature, dictates the cerebral IL-6 response (21). Nybo et al. (21) examined whether exercise-induced increases in IL-6 were linked to central fatigue, which was enhanced by hyperthermia. However, after conducting a study in humans undergoing prolonged exercise, the authors found that the elevated core temperature during the hyperthermic trial was not associated with altered cerebral IL-6 release as compared to the control trial (21). Furthermore, the augmented release of IL-6 from the brain during exercise may be ascribed to the elevated arterial adrenaline concentration due to stress, as IL-6 production by astrocytes is stimulated by adrenaline (22).

The stress system is activated even when the body is at rest, as it responds to many distinct circadian,

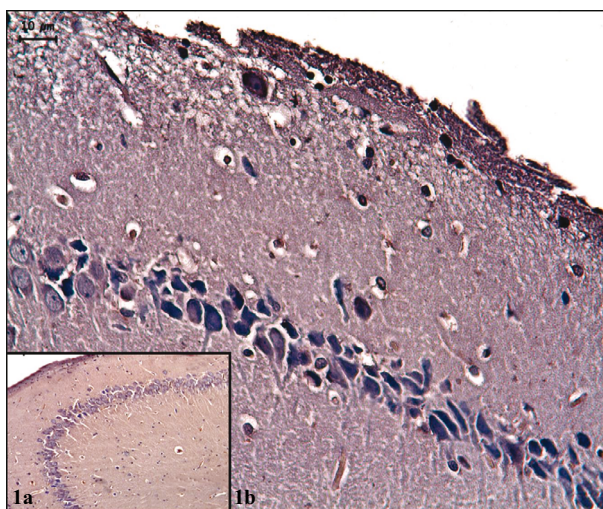


Figure 1. IL-6 immunoreactivity in hippocampal slices from rats in the control group, immunoperoxidase and hematoxylin: a) 100 \times , b) 400 \times .

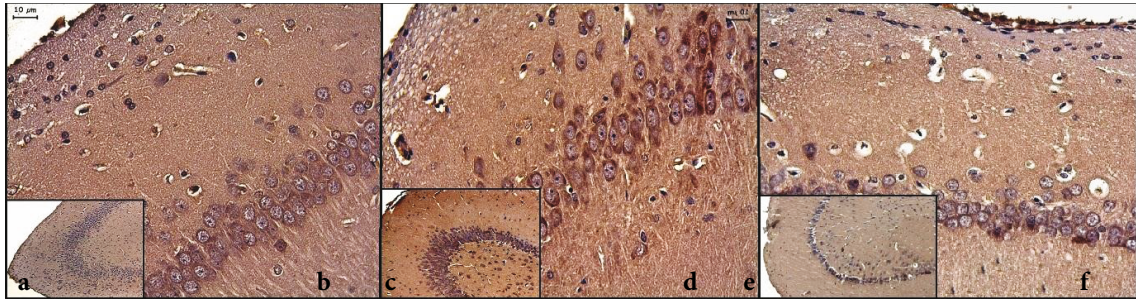


Figure 2. IL-6 immunoreactivity in hippocampal slices from untrained rats sacrificed immediately (a, b), 1 day (c, d), and 3 days (e, f) after exhaustion, immunoperoxidase and hematoxylin: a, c, e) 100 \times , b, d, f) 400 \times .

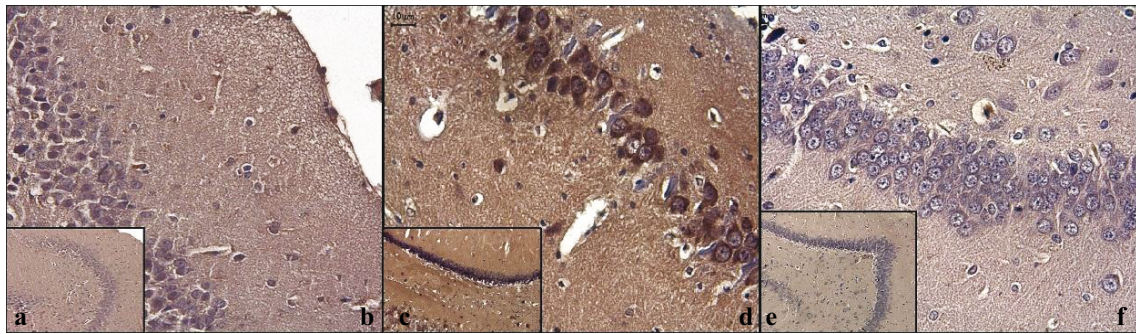


Figure 3. IL-6 immunoreactivity in hippocampal slices of trained rats sacrificed immediately (a, b), 1 day (c, d), and 3 days (e, f) after exhaustion, immunoperoxidase and hematoxylin: a, c, e) 100 \times ; b, d, f) 400 \times .

neurosensory, blood-borne, and limbic signals (23). These signals include cytokines produced by immune-mediated inflammatory reactions, such as TNF- α , IL-1, and IL-6. Systemic IL-6 concentrations increase in a similar fashion

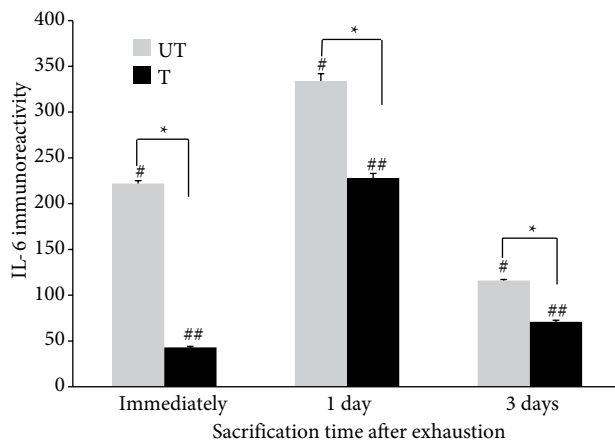


Figure 4. IL-6 immunoreactivity [percentage of retention \times (density of retention + 1)] in T and UT groups of rats sacrificed immediately, 1 day, and 3 days after exhaustion. The data are presented as mean \pm SEM. UT: Untrained group, T: trained group. *: $P < 0.05$ between UT and T groups; #: $P < 0.05$ between UT groups according to time of sacrifice; ##: $P < 0.05$ between T groups according to time of sacrifice.

during stress that is unrelated to pathogenic conditions, presumably stimulated by catecholamines through β 2-adrenergic receptors (16). Strenuous physical activity to the point of exhaustion may cause an inflammatory response in the brain, as it does in muscle (24). For example, it was shown that at least 3 areas of the brain (hippocampus, cerebellum, and cortex) release IL-6 during prolonged exercise and during recovery, and data from studies in mice suggest that concurrent changes in IL-6, mRNA, and glycogen levels make the hippocampus a likely source of the IL-6 released from the brain (25). During exercise, the release of IL-6 from the brain would be expected to have the same biological effect as the release of IL-6 from exercising muscles. Moreover, an increased IL-6 level in the brain at the end of a prolonged bout of exercise may contribute to the excess postexercise oxygen consumption that persists for hours after a prolonged exercise session (26), and it has been speculated that the cerebral release of IL-6 during exercise may be somehow connected to the effect of physical activity on the balance between energy expenditure and energy intake. In addition, the increased cerebral release of IL-6 may result from elevated expression of IL-6 synthesis in the brain, and an increased IL-6 level in the brain could affect the sensation of fatigue during prolonged exercise (27).

We found very low levels of or no IL-6 immunoreactivity in the brains of sedentary control animals that did not

run in our experiments (Figure 1), and previous studies support this claim that, within the CNS, the IL-6 levels remain low under normal conditions. These studies show that during brain injury, inflammation, hypoxia, certain diseases, and long-term stress, the IL-6 level becomes elevated due to the activation of astrocytes (22).

Cytokines, and also chemokines, are modulatory molecules that regulate the bidirectional cross-talk between the immune system and the brain. Their important roles in all critical hippocampal functions, including learning and memory, synaptic plasticity, neurogenesis, and brain aging, have been demonstrated previously (28). Physical exercise has favorable effects in the balance between hippocampal pro- and antiinflammatory cytokines during aging and is thought to play a preventive and curative role on various inflammatory nervous system disorders through these compensatory effects (29). In recent years, there have been many studies published on the proinflammatory functions of the cytokine IL-6 in the pathogenesis of several neurodegenerative disorders. However, due to the pleiotropic functions (acting both as a proinflammatory and an antiinflammatory) of IL-6, it is thought that the production of a determined level of IL-6 during moderate exercise can reverse the detrimental effects of uncontrolled inflammation by triggering the antiinflammatory effects of other cytokines (30). In an animal model showing the death of the hippocampal dentate granule cell layer after a chemical application, exercise attenuated neuronal death and diminished the elevation of the level of TNF- α , but this effect was undetectable in IL-6 knock-out mice (31). This study suggested that IL-6 may have protective effects in the CNS, as it does in peripheral tissues, and that it will be necessary to further investigate these beneficial mechanisms (31). In our study, the long-term exercise-trained rats presented low to moderate IL-6 immunoreactivity in their brains after a very stressful and exhausting run (Figure 3). On the other hand, running to exhaustion resulted in a high level of IL-6 immunoreactivity in the brains of untrained rats (Figure 2). At the same time, we observed that rats with the highest IL-6 levels in the brain showed the lowest performance and became exhausted much more quickly (Table 2).

Although it has been shown that strenuous exercise can cause oxidative stress, proinflammatory cytokine expression, and apoptosis in related tissues, the net effect of acute exercise on resident immune cells in the CNS remains unclear. In a recent study that investigated the effects of a single bout of strenuous exercise on the hippocampus of healthy mice (32), IL-6 levels were found to be significantly increased in contrast to the decreased levels of TNF- α . This result may be related to the antiinflammatory effect of exercise on the hippocampus.

Under inflammatory conditions, including viral infections, the levels of proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6 are increased, and it has been suggested that a particular type of neuronal death depends on TNF- α receptor activation. Therefore, a reasonable elevation of IL-6 in the hippocampus following exercise acts as an antiinflammatory mediator by inhibiting TNF- α , and this regulatory effect of IL-6 on TNF- α is thought to protect neurons from cell death (33).

In our study, we found that the level of IL-6 immunoreactivity in the brain was highest on the day after exhaustion, both in the UT and T groups, and that this level decreased to approach that observed in the controls after 3 days in the T but not the UT group. Wallberg et al. (34) studied the plasma IL-6 response during ultraendurance events and found that the IL-6 level began to increase at 6 h, increased further at 12 h, and then remained unchanged at 24 h. According to their results, the authors claimed that the relative intensity of the exercise was the main determining factor and that exercise duration did not affect the accumulation of IL-6 in the blood (34). These results are similar to those observed in our immunohistochemical brain evaluations, as we observed the highest level of IL-6 immunoreactivity at 24 h after exhaustive exercise as compared to immediately or 3 days after exhaustion ($P < 0.05$). However, because we did not evaluate the brains at 12 h after exhaustion, we could not determine whether this increase in IL-6 occurred at 12 or 24 h after exhaustion. However, the levels of IL-6 immunoreactivity in most of the brains from rats in the T group decreased to approach the levels detected in the control animals after 3 days, and this decrease was lower and insignificant in the UT group (Figure 4).

Fischer proposed that duration and intensity were equal determinants of the IL-6 response to exercise (9). However, similar to the results described by Wallberg et al. (34), we observed that there was a negative relationship between the increased IL-6 levels in the brain and the duration of running; animals with higher levels of IL-6 immunoreactivity in the brain could not continue to run for long periods, and they rapidly became exhausted. Although we were unable to provide data indicating why the exhaustive exercise-induced IL-6 retention was highest at 1 day after exhaustion, we propose that another factor, possibly cortisol, may have retarded the synthesis of IL-6 during the acute period of harmful exercise. Adrenocorticotrophic hormone and cortisol are the primary stress hormones that are released due to physical or emotional stress. Cortisol provides necessary energy to the muscles during strenuous physical activity and also affects the immune system (35), and previous studies have shown the inhibitory effects of cortisol on the peripheral production of IL-6 (36). These data

support our assertion that increased cortisol synthesis and the stressful effect of exhaustive exercise inhibited IL-6 synthesis during the acute phase. Furthermore, it is possible that as soon as the cortisol levels begin to decrease, the IL-6 levels start to increase in various organs, and a previous study found that an increase in IL-6 levels led to an increase in antiinflammatory interleukins, such as IL-1ra and IL-10. Thus, IL-6 can limit the potentially harmful effects of inflammation and attenuate the inflammatory process (37). In addition, the study by Mastorakos et al. (16) showed acute increases in the plasma concentration of glucocorticoids in response to submaximal, maximal, and supramaximal exercise. During inflammatory stress, the proinflammatory cytokines TNF- α , IL-1, and IL-6 stimulate hypothalamic corticotropin-releasing hormone and/or arginine-vasopressin secretion leading indirectly, through glucocorticoid secretion, to limitation of an inflammatory reaction. These previous authors claimed that cortisol prevented IL-6 levels from peaking approximately 300 min after the inflammatory stress (16). Similarly, Gleeson (17) suggested that prolonged bouts of strenuous exercise led to a temporary depression of various aspects of immune function (e.g., neutrophil respiratory burst, lymphocyte proliferation, monocyte antigen presentation) lasting approximately 3 to 24 h after exercise, depending on the intensity and duration of the exercise. In support of our hypothesis that immunoreactivity increases 1 day after exhaustive exercise and not immediately after exhaustion, several studies have mentioned that in experimental animals, stress and catecholamines stimulate endogenous IL-6 secretion, whereas glucocorticoids inhibit it (16). Catecholamines are known to stimulate IL-6 secretion via the β -adrenergic receptor (38). However, in human studies, it has been shown that physical conditioning in highly trained athletes is associated with a decreased HPA response to exercise (16). Catecholamines are end products of the stress system and have a major role in the control of inflammation by stimulating IL-6 production. During exercise-induced stress, glucocorticoids inhibit the production of IL-6 in vitro and in vivo in animals as well as humans (39).

Pervaiz and Hoffman-Goetz (40) investigated the expression of several proinflammatory cytokines, including IL-6, in rats that underwent 16 weeks of voluntary wheel running. As a pleiotropic cytokine, IL-6 has also been shown to play an antiinflammatory role (40), and these previous results revealed a significant increase in IL-6 and a decrease in TNF- α , while there was not any difference in IL-1 β and IL-10 levels. Moreover, this increase in IL-6 was lower in exercise-trained rats as compared to the untrained rats in our study. Therefore, we suggest that long-term exercise training enabled the rats to gain physical endurance and adapt to exercise. In contrast to our findings regarding the neuroprotective effects of optimal IL-6 levels in the CNS, it has also been suggested that uncontrolled inflammatory reactions and related high levels of proinflammatory cytokines, including IL-6, could have destructive effects on cognitive processes. The pleiotropic actions of IL-6 as both a pro- and an antiinflammatory cytokine are manifest via trans-signaling and classical pathways, respectively (41). Chronic mild stress, which impairs memory in rats, stimulates the production of proinflammatory cytokines, including IL-6, TNF- α , and IL-1 β (42). Similar to chronic stress in rats, psychosocial stress in humans impairs cognitive function and is related to several mood disorders (42).

In summary, exhaustive exercise (acting as a strong stress factor) promoted IL-6 cytokine immunoreactivity in brain slices from both the T and UT groups of rats. However, the destructive effect of this stress on the hippocampus was low or moderate in the T group as compared to the UT group. The beneficial effects of exercise are known to arise gradually, and these include long-term positive effects on both physical and mental health. Moreover, a regular exercise schedule, rather than irregular programs or acute forced exercise, contributes to controlled activation of the immune system in both the CNS as well as peripheral tissues, and this control mechanism is especially important because it protects against the detrimental effects of exacerbated immune system activation.

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