Acute running stimulates hippocampal dopaminergic neurotransmission in rats, but has no influence on brain-derived neurotrophic factor

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Goekint M, Bos I, Heyman E, Meeusen R, Michotte Y, Sarre S. Acute running stimulates hippocampal dopaminergic neurotransmission in rats, but has no influence on brain-derived neurotrophic factor. J Appl Physiol 112: 535–541, 2012. First published December 1, 2011; doi:10.1152/japplphysiol.00306.2011.—Hippocampal brain-derived neurotrophic factor (BDNF) protein is increased with exercise in rats. Monoamines seem to play a role in the regulation of BDNF, and monoamine neurotransmission is known to increase with exercise. The purpose of this study was to examine the influence of acute exercise on monoaminergic neurotransmission and BDNF protein concentrations. Hippocampal microdialysis was performed in rats that were subjected to 60 min of treadmill running at 20 m/min or rest. Two hours postexercise, the rats were killed, and the hippocampus was dissected. In experiments without microdialysis, hippocampus and serum samples were collected immediately after exercise. Exercise induced a twofold increase in hippocampal dopamine release. Noradrenaline and serotonin release were not affected. Hippocampal BDNF levels were not influenced, whether they were measured immediately or 2 h after the exercise protocol. Serum BDNF levels did not change either, but serum BDNF was negatively correlated to peripheral corticosterone concentrations, indicating a possible inhibitory reaction to the stress of running. Sixty minutes of exercise enhances dopamine release in the hippocampus of the rat in vivo. However, this increase is not associated with changes in BDNF protein levels immediately nor 2 h after the acute exercise bout. An increased corticosterone level might be the contributing factor for the absence of changes in BDNF.

brain-derived neurotrophic factor; exercise; dopamine; serotonin; noradrenaline

Physical activity has significant health benefits, and there is now extensive evidence that it has also substantial benefits for psychological health and cognition (15). Efforts toward understanding the molecular mechanisms behind the influence of exercise on memory function have led to the knowledge of the involvement of neurotrophic factors. The neurotrophin that is most susceptible to regulation through physical activity is brain-derived neurotrophic factor (BDNF), which is abundantly present in the brain (37). The concentration of BDNF protein in animals increases with both acute and chronic exercise protocols (2, 16). Acute exercise protocols mostly use forced running on a treadmill or in a motorized running wheel (16, 30, 35). This approach enables the control of both the speed and duration of running, in contrast to voluntary wheel running, and makes it possible to apply exactly the same protocol (intensity, distance) for every rat. There are, however, few data available on the effects of forced acute exercise on central BDNF protein levels (16, 30, 35). Two studies reported an increase in hippocampal BDNF protein following a forced treadmill exercise of either moderate-intensity (15 m/min for 30 min) (16, 35) or high-intensity (incremental exercise until exhaustion) (16).

The underlying mechanisms of the exercise-induced increase of BDNF are not clear. One of the possible hypotheses is that regulation of BDNF through exercise is mediated by neurotransmission (5). In cell cultures, BDNF protein is increased in response to monoamine application (4, 17). Also, monoaminergic manipulation in rats by means of antidepressant treatment increases both hippocampal BDNF mRNA and protein (6, 8, 26).

Exercise is able to mediate and interact with this monoaminergic neurotransmission. It has already been shown that the monoamine neurotransmission in several brain areas increases as a result of acute exercise and training (10, 21, 22, 28). In the hippocampus, ‘in vivo’ data are only available for serotonin (5-HT). There seems to be a delayed increase, only after 90 min, in extracellular 5-HT concentration with intense exercise (25 m/min) in healthy rats (10). In food-deprived rats, during a lighter exercise (12 m/min), the increase in 5-HT seems to occur faster (23). To our knowledge, no data exist on extracellular dopamine (DA) and noradrenaline (NA) concentrations in the hippocampus during exercise. Other studies indicate an increase of these catecholamines during exercise in certain brain areas, other than the hippocampus (14, 22, 28, 38).

It is not known whether in vivo hippocampal neurotransmission during exercise affects hippocampal BDNF protein levels. Therefore, this study aims to determine whether an acute exercise protocol is able to increase hippocampal BDNF, and whether this is accompanied by changes in monoaminergic neurotransmission. In vivo microdialysis was used to determine NA, DA, and 5-HT concentrations in the hippocampus during exercise. Since BDNF expression can be suppressed by corticosterone, corticosterone concentrations were also measured immediately following exercise.

Materials and Methods

Animal Population

Male albino Wistar rats (Charles River Laboratories, Germany) were used for the experiments. Rats were ordered at a weight of 175–200 g (age 45–48 days). On arrival, rats were housed in group in a room on a 12:12-h light-dark cycle for 6 days, before starting treadmill familiarization. Weight at the time of the exercise experii-
ment was between 275 and 350 g. Animals had a standardized diet with food and water “ad libitum”.

Ethical Issues

All procedures in this study were carried out according to the European Guidelines on Animal Experimentation, and the protocol was approved by the ethical committee of the Faculty of Medicine of the Vrije Universiteit Brussel (Project 07-213-1). All efforts were made to minimize animal suffering, and the minimum number of animals necessary to produce reliable scientific data was used.

Treadmill Familiarization

All rats were familiarized for 1 wk on a motor-driven horizontal treadmill (Omnitech Electronics, Columbus, OH) using mild electric shocks (0.8 mA). During 5 consecutive days, exercise intensity and duration were gradually increased until rats were able to run 60 min at 20 m/min. The treadmill familiarization was finished 4 days before the experimental acute exercise protocol.

Microdialysis Experiment

Stereotactic surgery. After 2 days of resting following the treadmill familiarization program, rats undergoing microdialysis (experimental group [EM], n = 7; control group [CM], n = 6) were anesthetized with a mixture of ketamine (50 mg/kg ip; 1000 Ceva, Ceva Sante Animale, Brussels, Belgium) and diazepam (5 mg/kg; Valium, Roche, Brussels, Belgium) and placed on a stereotactic frame. The skull was exposed, and an intracerebral guide (MAB 6.14.1C; Microbiotech) was implanted into the left hippocampus (coordinates relative to bregma: X: −4.6 mm; Y: −5.6 mm; Z: 4.6 mm) (29). The guide cannula was fixed to the skull using dental cement. Postoperative analgesia was provided by giving a single injection of ketoprofen (4 mg/kg ip; Ketofen, Merial, Brussels, Belgium) to each rat. The operation was followed by 2 days of resting before microdialysis experiments were carried out.

Microdialysis during exercise. The day before starting the microdialysis experiment, a probe with a 3-mm membrane (MAB 6.143; Microbiotech) was placed into the cannula. The microdialysis probe was perfused with Ringer solution (147 mM NaCl, 4 mM KCl, 2.3 mM CaCl2·6H2O) at a rate of 2 μl/min (CMA 400 Syringe Pump).

On the day of the experiment, the rat was put onto the treadmill 90 min before starting microdialysate collection. Every 20 min a sample was collected into vials containing 10 μl of antioxidant solution (3.3 mM L-cysteine, 0.27 mM Na2EDTA, 100 mM acetic acid, and 0.125 mM ascorbic acid).

Before starting the experimental protocol, six baseline samples were collected (animal at rest). For each rat, the mean of these six sample concentrations was considered as its resting value at time point zero. These 120 min of baseline sampling were followed by 60 min of experimental intervention (EM, n = 7: running at 20 m/min; CM, n = 6: rest; intervention performed at 12 PM during the light cycle). See Fig. 1. Following the exercise protocol, samples were collected during 120 min while the rat was at rest again.

Rats were killed after the 120 min of recovery following the experimental intervention, as previously indicated by Huang et al. (16), with an overdose of natrium pentobarbital (Nembutal, Ceva Sante Animale, Brussels, Belgium). The brain was excised, and both hippocampi were dissected. Brain tissue was snap frozen and stored at −80°C until analysis.

Serum and Hippocampal BDNF Immediately Following Exercise

To determine the immediate effect of a 60-min exercise bout on hippocampal BDNF protein and serum BDNF concentrations, 12 rats did not undergo microdialysis, but performed either the 60-min exercise protocol [experimental group (E0), n = 6] or stayed at rest for 60 min on the treadmill [control group (C0), n = 6; see Fig. 1]. Exercise protocols were started at 12 PM. All rats were killed immediately afterwards (no recovery of 120 min). Blood serum was collected “postmortem” through decapitation, and hippocampal brain tissue was excised and snap frozen. All samples were stored at −80°C until homogenization and analysis for BDNF.

Serum and Hippocampal BDNF Following a Short, Moderate Exercise

For comparison, a protocol including a more moderate exercise of shorter duration, as previously described (16, 35), was also evaluated. Indeed, with a less prolonged, moderate exercise protocol, the concern of a stress-induced response is reduced.

For these experiments, rats performed a 30-min acute running exercise on the treadmill at a speed of 15 m/min [experimental group (ES), n = 6]. Rats were killed after 120 min of recovery following exercise (16). The control group for these trials was again the C0 group from the previous experiments (n = 6; intervention: rest). Blood serum was collected postmortem through decapitation, and hippocampal brain tissue was excised and snap frozen. All samples were stored at −80°C until homogenization and analysis for BDNF.

Analytic Procedures

Monoamines. NA, 5-HT, and DA were analyzed by means of microbore LC with electrochemical detection, as described before (33). The mobile phase consisted of 33 ml acetonitrile and 200 ml of buffer (0.5 mM Na2EDTA, 2 mM sodium 1-decane-sulfonate, 100 mM sodium acetate trihydrate, and 20 mM citric acid monohydrate, adjusted to pH 5.5). The detection limit was ~0.03 nM for all three monoamines.

BDNF. Hippocampal BDNF protein content was determined in duplicate using an enzyme-linked immunosorbent assay kit (CYT306, ChemiKine, Millipore, Billerica, MA). Hippocampal tissue was homogenized in homogenization buffer consisting of 100 mM Tris-HCl,
pH 7, and containing 2% bovine serum albumin, 1 M NaCl, 4 mM Na$_2$EDTA, 2% Triton X-100, 0.1% sodium azide, and the protease inhibitors (Sigma) 5 μg/ml aprotinin, 0.5 μg/ml antipain, 157 μg/ml benzamidine, 0.1 μg/ml pepstatin A, and 17 μg/ml phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 10,000 g for 30 min. The resulting supernatant was used for BDNF assay (dilution 1:2). Serum was diluted 200 times with sample diluent (PBS 1% bovine serum albumin 0.05% Tween 20) before the BDNF analysis was performed. All samples were added in duplicate to the plate, together with a standard series (7.8–500 pg/ml), and incubated overnight. The next day, biotinylated mouse anti-BDNF monoclonal antibody and streptavidin-horseradish peroxidase conjugate solution was added, with washing steps and incubation in between. Color reaction was started with TMB/E solution. Absorbance was measured using a Bio-Rad microplate reader at a wavelength of 450 nm.

**Serum corticosterone.** Corticosterone was determined in the serum samples of rats using an enzyme immunoassay kit (900-097, Assay Designs, Ann Arbor, MI). Steroid displacement reagent was added to the serum samples (2.5 parts of reagent for 97.5 parts of serum). These samples were diluted five times with standard diluents (Assay Buffer 15) and added in duplicate to the plate, together with a standard series (32–20,000 pg/ml).

For analysis, a polyclonal antibody was added to bind, in a competitive manner, corticosterone in the standard or sample, or an alkaline phosphatase molecule, which has corticosterone covalently attached to it. After a simultaneous incubation at room temperature, the excess reagents were washed away, and pH$_{pp}$ substrate was added. After a short incubation time, the enzyme reaction was stopped, and the optical density was read on a microplate reader at 405 nm.

**Statistical Analysis**

Statistical analysis was performed using Statistica 6.0. To check normality of the data, we used the Kolmogorov-Smirnov test. Monoamine concentrations (parametric for all) were compared between and within groups by two-way ANOVAs (group EM + CM × time) with repeated measures for the second factor. If any significant effect came forward, Duncan’s post hoc analysis was performed. All groups were compared using an independent $t$-test (BDNF, corticosterone). Correlations were verified using Pearson’s $r$ correlation. Significance level was set at $P < 0.05$.

**RESULTS**

**Mean Basal Neurotransmitter Concentrations in the Hippocampus**

By means of microdialysis, we measured extracellular NA, DA, and 5-HT concentrations in the hippocampus. Basal hippocampal dialysate concentrations were 0.08 ± 0.02 nM for NA ($n = 10$), 0.14 ± 0.02 nM for DA ($n = 13$), and 0.17 ± 0.03 nM for 5-HT ($n = 13$).

**Effect of Exercise on Monoamine Release**

Dialysate concentrations in the hippocampus showed a significant interaction for time and group for DA ($P < 0.001$). At rest, baseline DA concentrations were comparable between both EM and CM. Throughout exercise, a significant increase in extracellular DA concentrations occurred in the EM group ($P < 0.001$), while DA did not change in the CM group (Fig. 2A). In the EM group, the DA release remained higher compared with baseline levels until 40 min after the end of the treadmill exercise. Extracellular DA levels at the end of the exercise protocol did not correlate with postmortem BDNF levels in EM ($P = 0.20$).

Both NA and 5-HT release in the hippocampus were not affected by acute treadmill running (Fig. 2, B and C). For these monoamines, no significant differences were found either between groups or within time, and no significant interaction between time and group was detected.

**Effect of Exercise on BDNF Protein Expression**

Two hours following the exercise protocol (16), rats that underwent microdialysis were killed, and the BDNF content was determined in the hippocampal homogenates. No difference in hippocampal BDNF protein content was found between CM and EM ($P = 0.39$) (Fig. 3, top). When BDNF was measured immediately following the running protocol (no microdialysis), hippocampal BDNF protein content in the E0 rats did not differ from that of the sedentary C0 group (Fig. 3, top). As a comparison to human studies, serum BDNF was also determined in these rats. However, no exercise-induced changes were present in the E0 rats completing the 60 min of treadmill exercise (Fig. 3, top). Also, no correlation was present between serum and hippocampal BDNF levels, or between BDNF and DA concentrations.

There was no significant effect of the microdialysis procedure on hippocampal BDNF concentrations in the intact hippocampus (no differences between CM and C0; $P = 0.31$; data not shown).

One hundred and twenty minutes following the less prolonged, moderate exercise protocol, hippocampal BDNF tended to increase by ~17% (data not shown). This change, however, did not reach statistical significance ($P = 0.07$). No significant change in serum BDNF was detected 2 h after stopping the exercise bout.

**Effect of Exercise on Corticosterone Levels**

Serum corticosterone was determined immediately following acute exercise in rats, to evaluate the stress component of running (Fig. 4). We found significantly higher corticosterone levels in the experimental E0 rats compared with the C0 group ($P = 0.007$), indicating a stress reaction in these exercising animals.

There was a negative correlation between serum BDNF and corticosterone levels in the E0 and C0 group ($r = -0.60, P = 0.039$). Hippocampal BDNF content, however, did not correlate with serum corticosterone levels.

**DISCUSSION**

This is the first study to determine in vivo extracellular DA and NA levels in the hippocampus of rats during an exercise protocol. Previously, these neurotransmitters have been studied in the striatum, the preoptic area and anterior hypothalamus (PO/AH), and in the frontal cortex during exercise (12, 22, 28). In the hippocampus, during a 60-min intense treadmill exercise, we observed an increase in DA release, while NA and 5-HT were unaffected. However, this enhanced dopaminergic neurotransmission was not accompanied by significant changes in hippocampal BDNF protein levels.

The increase in DA levels in the hippocampus is in agreement with the data found in the striatum (14, 22) and the PO/AH (36). They also confirm previous data from hippocampal homogenates following exercise (3). The function of this raise in hippocampal DA with exercise remains to be deter-
mined. The hippocampus is part of the dopaminergic mesolimbic pathway, involved in depression and schizophrenia, disorders that are both associated with lower BDNF levels (1). Whether an acute, short-term DA increase is involved in the regulation of BDNF is rather unclear. Since no correlation was found between DA and BDNF concentrations, it cannot be substantiated that DA is acutely involved in BDNF induction. However, with repetitive exercise bouts, as performed in training programs, the recurring increases in DA could still act on BDNF and thus contribute to the benefits of exercise on the brain. This assumption should be taken into consideration and deserves attention in future studies. After all, DA receptors are known to play an important neuromodulatory role in the hippocampus where, at a cellular level, they influence the expression of synaptic plasticity (24). Also, effects of antidepressant treatment on BDNF mRNA mostly require a longer period of time (27). NA is known to increase in the striatum (22) and the frontal cortex (28) with exercise. Release of NA has been shown to influence multiple aspects of plasticity, including cellular excitability and induction and maintenance of long-term potentiation, and could therefore affect the alterations in synaptic strength that are hypothesized to underlie learning and memory (9). However, NA was not acutely enhanced in the hippocampus with exercise, as previously shown in the PO/AH (12). Our running speed was slightly lower than these previous studies (20 vs. 25–26 m/min), but intensity is probably not the most important factor for inducing NA release, since Hasegawa et al. (12) also used a running speed of 26 m/min. Whole brain NA levels after acute bouts of exercise mostly found a decrease or no effect (21). Also, for the hippocampus, a decrease is observed postmortem (21). Further research is necessary to clearly map the NA levels in several brain areas during different types of exercise and the role of possible changes.

Fig. 2. Changes in extracellular dopamine (DA; A), noradrenaline (NA; B), and serotonin (5-HT; C) concentrations in the hippocampus following 60-min exercise at 20 m/min. The average concentration of 6 microdialysis samples before treadmill exercise (Ex) was taken as the baseline and was defined as 100%. Values are means ± SE (%baseline values). For DA levels, a significant difference was present between the EM and CM group ($P < 0.01$). Within the experimental group, DA release increased during exercise compared with baseline ($* P < 0.05$; $** P < 0.01$). For NA and 5-HT levels, no significant differences were present between the EM and CM group. Within the experimental group, no changes were detected in the release of these transmitters with exercise.
In our study, 5-HT release was not affected by the acute exercise bout. This is in correspondence with the data of Gomez-Merino et al. (10). These authors only measured a delayed increase in hippocampal 5-HT, 90 min after an intense exercise (25 m/min). In our study, the exercise protocol could have been too short and/or not intense enough to elicit an increase in 5-HT. And yet, Meeusen et al. (23) found an increase in 5-HT during a less intense exercise. However, their rats were food deprived, a major difference from our protocol, which may explain the difference in results. Other than the hippocampus, no changes in 5-HT levels in the PO/AH were observed during exercise (12).

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In contrast to the study of Huang et al. (16), BDNF content in the hippocampus was not increased immediately or 2 h following exercise. Previous data on BDNF and acute exercise in healthy animals are limited to two studies. Both Soya et al. (35) and Huang et al. (16) found an increase in BDNF protein concentration following 30 min of treadmill running in rats with a moderate intensity (15 m/min) or following an intensive exercise protocol (incremental exercise to exhaustion). For practical reasons, our experimental exercise protocol consisted of 60-min running at 20 m/min instead of 30 min. This allowed us to collect several microdialysates during the experiment. Exercise duration could be of major importance when comparing our results to other studies. Previously, only 30 min of acute exercise were applied in healthy animals, while exercise duration is one of the major parameters that could influence the BDNF protein induction with exercise. In the endothelin-1 rat model for transient focal ischemia, Ploughman et al. (30) observed an increase in BDNF protein when rats were walking for 30 min at 11 m/min. When increasing the duration to 60 min, this enhancement of BDNF concentration disappeared. However, this research involved rats in which a stroke was induced, a pathological condition that is already associated with changes in BDNF, so these results cannot be generalized to a healthy study population. Therefore, clear dose-response studies in healthy animals are necessary to investigate the influence of acute exercise duration on the induction of BDNF protein levels.

Next to duration, intensity of exercise might also play a role in the induction of changes in BDNF in hippocampal homogenates. The intensity of 20 m/min in the present study corresponds to the published lactate threshold intensity for Wistar rats (35). Our laboratory recently performed oxygen uptake measurements during a gradually increasing treadmill exercise in rats and measured that, at a running speed of 20 m/min during 20 min, they performed at ~80% of maximum O2 uptake (13). Thus running for a longer period of time (60 min) at this intensity can elicit a great increase in blood lactate, which may partly prevent the positive effect of exercise on BDNF. Similarly, Soya et al. (35) found a significant increase in hippocampal BDNF when the acute exercise was performed at an intensity below the lactate threshold. Higher intensity exercise above the lactate threshold and inducing higher lactate and corticosterone levels, however, did not lead to further increments in both BDNF mRNA and protein levels and can even be inhibitory.

Since, in humans, peripheral BDNF is increased immediately following exercise (31, 32), we also measured BDNF in serum immediately following the running protocol. In contrast to results in humans, serum BDNF was not increased in rats. Furthermore, we did not observe a correlation between serum and hippocampal BDNF levels in these rats. Previous reports on the correlation between central and peripheral BDNF are quite ambiguous (7, 18, 19). This can indicate that serum BDNF levels in rats do not completely reflect brain concentrations and suggests the involvement of peripheral mechanisms in serum BDNF induction, which act independently from central changes in BDNF protein.

![BDNF Content](image1.png)

![Corticosterone Level](image2.png)
A last item that can influence BDNF protein levels is a possible stress factor with treadmill running (25). Both stress and high-intensity exercise are known to elicit an increase in corticosterone, a steroid hormone that inhibits BDNF induction (34). This stress, however, is minimized by performing a familiarization period on the treadmill (16). We measured serum corticosterone immediately following exercise. In exercising animals, corticosterone levels were significantly higher than in control animals. Furthermore, serum BDNF and corticosterone levels were negatively correlated in these groups. Although a 1-wk familiarization period was performed in this study, we believe that the increased corticosterone levels may explain why acute running was not associated with changes in BDNF.

The hippocampal BDNF concentration, however, did not correlate with serum corticosterone, again suggesting that peripheral and central mechanisms act independently from each other.

Since there might be a stress reaction with our prolonged exercise protocol, and we could not confirm the results of previous studies, we investigated whether a more moderate running protocol, applied previously (16, 35), had an effect on BDNF levels. This reduces the stress of longer duration exercise and enlarges the adaptation reached within the familiarization period of 5 days.

With this more moderate exercise of shorter duration, BDNF levels showed a tendency to increase compared with the sedentary control levels. This indicates that the statement previously made in this discussion about the stress imposed on the animals during prolonged exercise is worth considering. Clearly, there is a need for dose-response studies in healthy animals to assess the influence of acute exercise duration and intensity on BDNF levels.

Next to the physiological parameters discussed above, a possible confounding factor might be postexercise sleep. It is not clear whether the lack of effects, observed in this study, may be due to differences in the amount, length, or type of sleep epochs occurring after the exercise bout. Indeed, rapid eye movement sleep has been positively associated with neurogenic changes in the hippocampus (20), and, therefore, a difference in sleeping pattern following the exercise bout might be of influence.

Also, exercise in our study was performed during the light phase of the day-night cycle. Since rodents are mostly active during the dark phase, the imposition of an exercise bout during the light phase could be an issue: it has been shown that BDNF expression in the hippocampus is reduced after 48 h of sleep deprivation, and that this decrement in BDNF is correlated with rapid eye movement sleep loss (11). This is, however, a parameter that is difficult to monitor in our experimental setting. Still, it should be considered in future studies.

Within our study, we should also keep in mind that we measured BDNF protein. While BDNF protein levels do not change, exercise can still cause alterations in the expression of BDNF mRNA. Indeed, Huang et al. (16) found that intense exercise (incremental exercise to exhaustion) increased BDNF mRNA, while moderate intensity (30 min at 15 m/min) was ineffective in this regard. To bring on any effect, the raise in BDNF mRNA still needs to be translated into changes of protein concentrations.

**Conclusion**

Sixty minutes of intense forced running exercise in rats was shown to enhance extracellular DA concentrations in the hippocampus, but not those of NA or 5-HT. Hippocampal BDNF protein content was not influenced when measured immediately or 2 h following the exercise protocol. Similarly, serum BDNF protein was not increased immediately following intense exercise. It seems that central and peripheral BDNF are not strongly related, since no correlation was present between serum and hippocampal BDNF levels. BDNF in serum was negatively correlated to peripheral corticosterone levels, indicating that stress remains an important aspect in forced exercise running protocols. A more moderate exercise protocol is recommended and could slightly reduce this presence of stress. BDNF seems to increase following a shorter and less intensive exercise protocol, as shown in previous studies; however, in our study, this change did not reach significance.

Future research should focus on different acute and chronic exercise protocols and their influence on BDNF protein content and on the functional outcome of changes in BDNF due to exercise. A main focus area can be the application of (dose-response) studies with acute exercise to investigate the influence of both intensity and duration on the induction of BDNF protein levels. Also, the relation between both human and animal studies should be investigated.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: M.G., E.H., R.M., Y.M., and S.S. conception and design of research; M.G. and I.B. performed experiments; M.G. and I.B. analyzed data; M.G., I.B., and E.H. interpreted results of experiments; M.G. prepared figures; M.G. drafted manuscript; M.G., E.H., R.M., Y.M., and S.S. edited and revised manuscript; M.G., E.H., R.M., Y.M., and S.S. approved final version of manuscript.

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