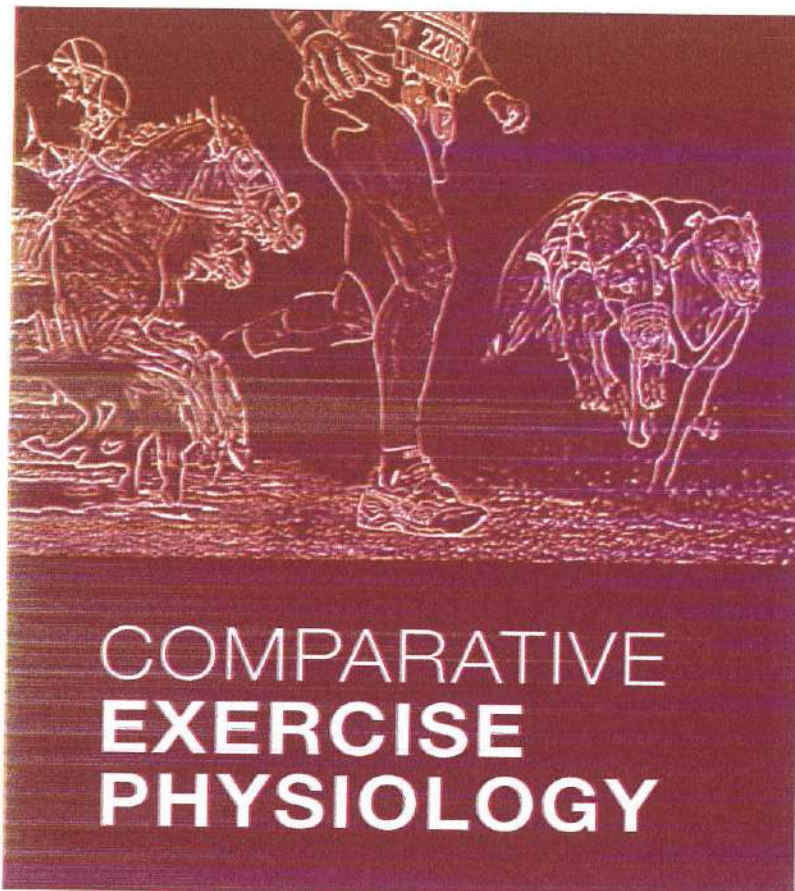


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<https://doi.org/10.3920/CEP200001>

Keywords: equine, cobalt, performance enhancing drugs

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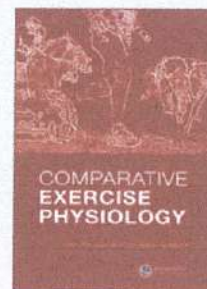
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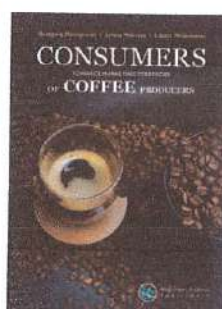
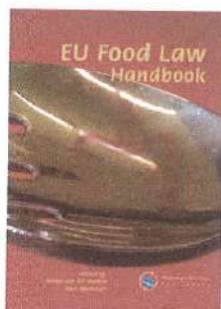
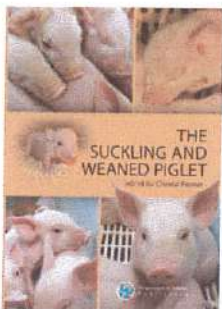
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High intensity exercise increases brain derived neurotrophic factor expression and number of hippocampal neurons in rats

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RESEARCH ARTICLE

Abstract

The decrease in brain derived neurotrophic factor (BDNF) expression and number of hippocampal neurons are two indicators in the decrease of memory function, cognitive, and learning function. The present study aimed to determine BDNF expression and the number of hippocampal neurons on moderate and high intensity exercise by listening to music. Design of the present study was a randomised control group post-test only design. A total of 33 male rats, *Rattus norvegicus* strain Wistar, aged eight weeks, with body weight 160±20 g were randomly divided into three groups: Group 1 (G1) (n=11, control group without intervention), Group 2 (G2) (n=11, performed moderate intensity exercise, treadmill 14-16 m/min for 30 min by listening to pop music with fast tempo of 160 beats/min) and Group 3 (G3) (n=11, high intensity exercise, treadmill 22-25 m/min for 20 min by listening to pop music with fast tempo of 160 beats/min). The intervention was performed between 17:00-21:00 pm, three times per week for 12 weeks. Blood and brain samples were obtained and evaluated 12 h after the end of the last exercise. BDNF serum was measured using ELISA and hippocampal neurons were stained by haematoxylin-eosin and counted using OlyVIA software. Study results showed a BDNF for G1 of 1,098.14±135.31 pg/ml, G2 of 1,113.72±65.87 pg/ml, and G3 of 1,331.56±105.35 pg/ml ($P=0.001$). The total number of hippocampal neurons for G1 was 54.75±6.83 cells, for G2 59.87±7.68 cells, and G3 80.58±9.79 cells ($P=0.001$). According to the study it can be concluded that high intensity exercise combined by listening to music with a fast tempo of 160 beats/min increases BDNF expression and the number of hippocampal neurons.

Keywords: rats, music, brain, memory function

1. Introduction

Exercise is regarded as an interesting and fun activity since it does not only affect the improvement in physical and psychological condition, but also has an important role on prevention and protection of neurological and mental disorders (Liu and Nusslock, 2018; Sugiharto, 2012), such as Alzheimer and dementia (Laurin *et al.*, 2001; Vedovelli *et al.*, 2017). In addition, regular, measurable, and continuous exercise has positive impacts on the increase in brain function characterised by the increase of memory, mood,

function, cognitive, plasticity and learning ability of the brain (Erickson *et al.*, 2011; Phillips *et al.*, 2014; Spalding *et al.*, 2013). However, exercise has an ambiguous character that can have negative effects (Sugiharto, 2012) and this is not been well understood (Liu and Nusslock, 2018).

Exercise has two functions in the body, which can be a stressor that has the potential to cause distress, but on the other hand exercise can also be a stimulator causing eustress (Sugiharto, 2012). This depends on the management of the exercise intensity. If the exercise is performed by the correct,

organised, measured, continuous and pleasant intensity, it can improve brain function (Jeon and Ha, 2017; Wrann *et al.*, 2013). On the contrary, exercise with high intensity and competitive may cause stress (Nayanatara *et al.*, 2005; Sugiharto, 2012); it potentially decreases brain function, marked by the decrease of brain derived neurotrophic factor (BDNF) expression and the number of hippocampal neurons (Jeon and Ha, 2017; Laske *et al.*, 2010). This can increase the risk of neurodegenerative diseases (Soya *et al.*, 2007), such as Alzheimer disease, Parkinson disease, Huntington disease, depression (Bathina and Das, 2015; Liu and Song, 2016), and also dementia (Erickson *et al.*, 2011). In addition, the decrease of BDNF and number of hippocampal neurons also poses risks of reducing memory, cognitive and learning functions (Greenberg *et al.*, 2009; Liu and Nusslock, 2018).

Stress stimulates the hypothalamus-pituitary-adrenal (HPA) axis (Clark and Mach, 2016), that functions to stimulate the increase of corticotrophin-releasing hormone (Sugiharto, 2012) and secretion of adrenocorticotropin hormone (Powers and Howley, 2015). Secretion of adrenocorticotropin can stimulate the secretion of cortisol (Usui *et al.*, 2012), resulting in a decreased BDNF expression in the brain (So *et al.*, 2017). The decrease in BDNF expression activates tyrosine kinase receptor into non-activate Forkhead box O (FoxO) protein by signalling phosphoinositide 3-kinase/kinase B protein (Wang *et al.*, 2015). The non-active FoxO protein causes neuron atrophy, inhibits neurogenesis and synaptogenesis of hippocampus (Wang *et al.*, 2015), resulting in decreasing numbers of hippocampal neurons (Krugers *et al.*, 2010). Consequently, stress must be properly managed, and one method which is very efficient in reducing stress is listening to music (Sugiharto, 2009). Exercise while listening music with appropriate intensity may inhibit the decline of BDNF expression and number of hippocampal neurons (Marzban *et al.*, 2011; Sugiharto, 2009). Moderate intensity exercise with listening to music can inhibit the decline of BDNF expression and number of hippocampal neurons (Fukui and Toyoshima, 2008; Yeh *et al.*, 2015). Exercise while listening music decreases HPA-axis activation (Matrone and Brattico, 2015). A previous study stated that submaximal intensity exercise while listening to music would result in a decrease in cortisol hormone and an increase in endorphins hormone levels (Fukui and Toyoshima, 2008). An increase of endorphin hormones decreases stress responses (Sarkar *et al.*, 2012) and stimulates D- β -hydroxybutyrate (DBHB) synthesis in the liver, which then circulates to the hippocampus (Sleiman *et al.*, 2016). In the hippocampus, DBHB induces an increase of BDNF expression by inhibiting histone deacetylases 2 (HDAC2) and histone deacetylases 3 (HDAC3) (Wang and Holsinger, 2018). BDNF expression binds tropomyosin-receptor-kinase B (TrkB) receptor, which supports the life cycle of the cell (Bathina and Das, 2015), increases neurogenesis and synaptogenesis of hippocampus (Mustroph *et al.*, 2015), increases proliferation and differentiation of neurons (Lee *et al.*, 2016; Sugiharto, 2009), and decreases

apoptosis of hippocampal neurons as well (Van Praag, 2005) by signalling p38-mitogen activated kinase protein (p38-MAPK) (Zheng *et al.*, 2018); consequently, the number of hippocampal neurons will increase (Shors *et al.*, 2014).

The present study aimed to reveal the effects of moderate and high intensity exercise by listening to music toward BDNF expression and the number of hippocampal neurons. The result of the present study can be used as a basic for management in improving memory, cognitive and learning functions.

2. Materials and methods

Experimental design

The present study used a randomised control post-test only group design. The animal models were 33 males *Rattus norvegicus* strain Wistar aged 8 weeks with a body weight of 160 ± 20 grams. They were randomly divided into three groups: control group (G1); intervention group with moderate intensity exercise by listening to fast tempo music (G2); and intervention group with high intensity exercise by listening to fast tempo music (G3). All procedures of the present study were approved by the Ethical Committee of the Faculty of Medicine, Brawijaya University, Jawa Timur, Indonesia, number 261/EC/KEPK-S1/07/2017. The present study followed animal welfare principles in experimental science published by the European Convention for the Protection of Vertebrate Animals.

Exercise protocol

Moderate intensity exercise was performed by placing animal models in a treadmill, where they had to run at a speed 14–16 m/min for 30 min while listening to pop music with a fast tempo of 160 beats/min. High intensity exercise was performed at a speed of 22–25 m/min for 20 min while listening to pop music with a fast tempo of 160 beats/min (Kim *et al.*, 2013; Sugiharto, 2009). This intervention was applied between 17:00–21:00 pm (Vinicius *et al.*, 2007) three times per week for 12 weeks.

Biochemical analysis

Five ml blood was taken from left ventricle of the animal models. Brain retrieval was carried out by dissecting calvaria and the brain was placed to formalin liquid 10% in a tube. Blood and brain samples were taken 12 h after the last intervention. Measurement of BDNF serum was performed by a BT-Lab Enzyme-Linked Immunosorbent Assay kit BT-E0476Ra (Biossay Technology Laboratory, Inc., Shanghai, China P.R.) with a BDNF standard curve range of 0.05–10 ng/ml and sensitivity level of 0.01 ng/ml. Measurement of hippocampal neurons used hematoxylin-eosin staining method which was then counted by OlyVIA

software (Olympus, Tokyo, Japan) from five fields of view of each sample under the magnification 400 times.

Statistical analysis

The data were analysed by using SPSS software (Chicago, IL, USA). Test for normality used the Shapiro-Wilk test, while test for homogeneity used the Levene test. Normal distribution of data with Varian homogen was tested by ANOVA and afterwards by Tukey honestly significant difference (HSD) post hoc test for significance ($P < 0.01$).

3. Results

The results of the analysis of mean BDNF expression and number of hippocampal neurons after the last intervention are presented in Table 1. A histological overview of the number of hippocampal neurons after the last exercise is shown in Figure 1. The expression of BDNF and the number of hippocampal neurons of G3 were greater than those of the G1 and G2 groups. ANOVA showed significant differences in the mean BDNF expression ($P = 0.001$) and hippocampal neurons ($P = 0.001$). Tukey HSD post hoc test showed significant differences in BDNF expression and the number of hippocampal neurons between G3 and G2 groups ($P = 0.001$), G3 and G1 groups ($P = 0.001$), while G2 and G1 (BDNF, $P = 0.954$; hippocampal neurons, $P = 0.438$) did not show significant differences. However, the G2 group has a higher mean BDNF expression and number of hippocampal neurons than G1.

4. Discussion

The present study aimed to compare the effects of moderate intensity exercise and high intensity exercise while listening to music towards BDNF expression and the number of hippocampal neurons. The novelty of the present study is provided by the use of pop music with fast tempo (allegro). In order to complete the purpose, moderate and high intensity exercises were combined with listening to pop music at a fast tempo of 160 beats/min. The results showed that the BDNF expression and the number of hippocampal neurons in group G3 was greater than in group G2 and G1.

There was a significant difference in BDNF expression between group G3 and G1. This result is in line with a previous study that used Wistar rats performing high-intensity interval training on a treadmill with an intensity of 85-100% of the maximum rate of oxygen consumption for 30 min per day, six times per week for six weeks. This study showed that high-intensity interval training increased BDNF expression significantly compared to non-trained rats (Freitas *et al.*, 2018). However, the results were not supported by the study conducted by Almeida *et al.* (2013) using Wistar rats with intervention high-intensity exercise treadmill with similar speed 30 min per day for 10 days, which showed no increased BDNF expression of hippocampus between high intensity exercise and control.

The difference is probably due to the use of music with fast tempo of 160 beats/min in the present study, whereas the previous study did not use music. According to the study conducted by Marzban *et al.* (2011) in Wistar rats, listening music for 60 days in sequence could increase BDNF expression significantly. The combination of exercise and music can improve motor skills (Van Dyck *et al.*, 2015), motivation, and affect mood (Terry and Karageorghis, 2006), and also create a sense of comfort and psychophysiological effect to switch fatigue sensation during exercise (Almeida *et al.*, 2015). In addition, the combination between exercise and listening to music can reduce stress response which is characterised by the decrease in cortisol secretion (Sugiharto, 2009) that stimulates DBHB synthesis in the liver, after which DBHB is transported to the hippocampus via circulation (Sleiman *et al.*, 2016). In the hippocampus, DBHB induces BDNF expression by inhibiting HDAC2 and HDAC3 (Wang and Holsinger, 2018). However, there was no significant increase in BDNF expression detected by a previous study with an acute high-intensity exercise intervention (Domínguez-Sánchez *et al.*, 2018). This result could be caused by the difference in the intervention type: the present study used repeated exercise (chronic), while the study by Domínguez-Sánchez *et al.* (2018) only tested one intervention (acute). In order to increase BDNF expression repeated intervention (chronic) is necessary (Shahandeh *et al.*, 2013).

Table 1. Mean brain derived neurotrophic factor (BDNF) expression and number of hippocampal neurons by respective exercise groups.¹

Group	n	Control (G1)	Moderate intensity exercise with music (G2)	High intensity exercise with music (G3)	ANOVA P-values
BDNF (pg/ml)	11	1,098.14±135.31 ^a	1,113.72±65.87 ^a	1,331.56±105.35 ^b	0.001
Hippocampal neurons (number of cells)	11	54.75±6.83 ^a	59.87±7.68 ^a	80.58±9.79 ^b	0.001

¹ Different superscript shows significant differences in the Tukey HSD post hoc test with ($P < 0.01$).

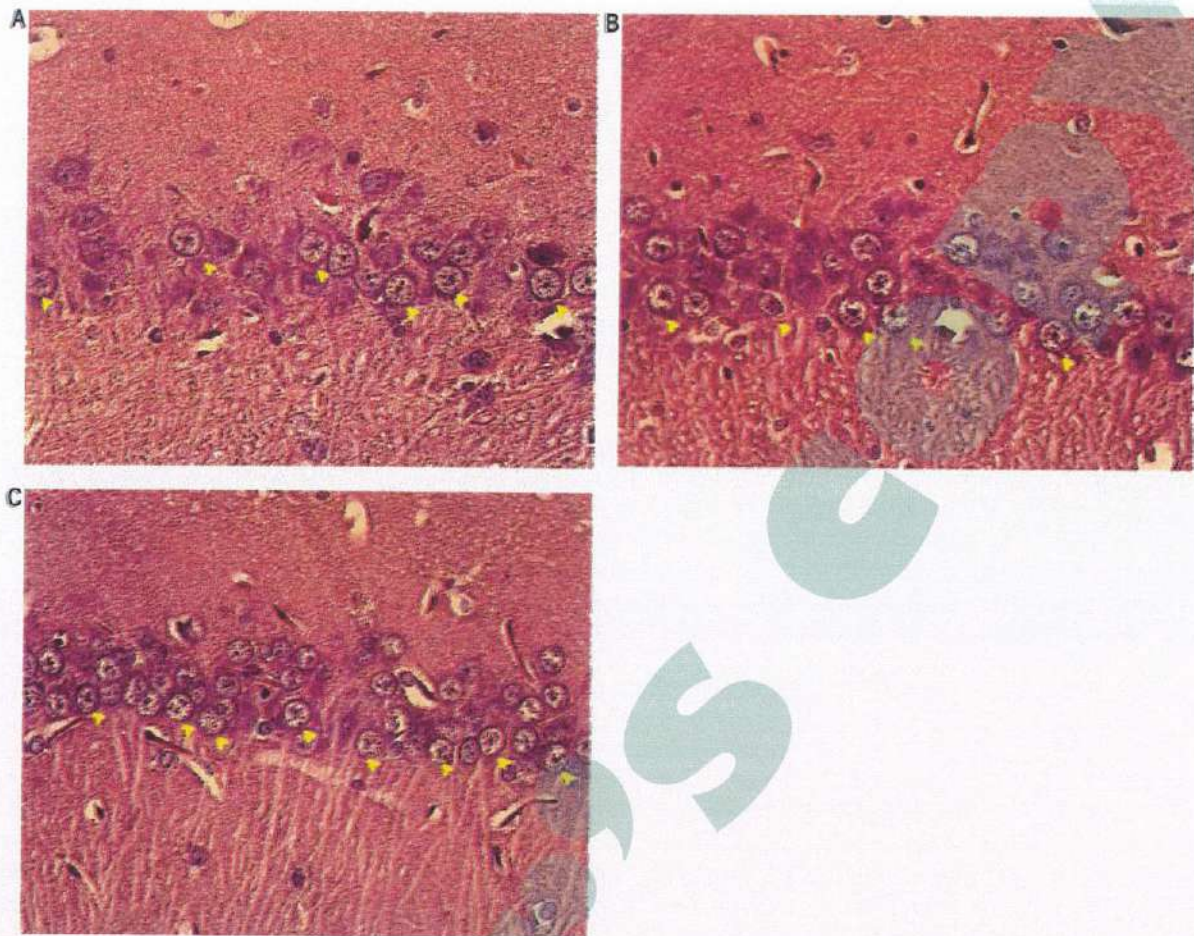


Figure 1. A cross section of hippocampus under haematoxylin-eosin staining (A) Control group (G1); (B) moderate intensity exercise while listening to pop music group (G2); (C) high intensity exercise while listening to pop music group (G3). Photomicrograph of hippocampal neurons shows that G3 has denser neurons which describe the number of hippocampal neurons with branches as pointed by yellow arrows compared to G2 and G1. Magnification 400 \times . Arrows show a number of neurons with branches.

There is a significant difference in the number of hippocampal neurons between G3 and G1 groups ($P=0.001$). This is in line with the study by Jin *et al.* (2017) using Sprague-Dawley rats treated with treadmill intervention (30 m/min for 30 min per day for six weeks) which showed increased hippocampal neurogenesis compared to the control group. In contrast, the study by Nokia *et al.* (2016) using Wistar rats with high intensity exercise for 6-8 weeks did not show any significant increase of adult hippocampal neurogenesis. Likewise, Almeida *et al.* (2013) using Wistar rats and performed high-intensity treadmill exercise (22 m/min for 30 min per day for 10 days in sequence) concluded that there was no significant difference in cell proliferation of dentate gyrus of the hippocampus between the high intensity exercise and control groups. These different results were obtained from studies that only used high-intensity exercise intervention, while our study also included listening to music. The increase in the number of hippocampal neurons may be caused by music with fast tempo of 160 beats/min, as listening to music has a positive impact on improving hippocampus

neurogenesis (Fukui and Toyoshima, 2008). Furthermore, listening to music for one hour per day for 28 days can significantly increase cell proliferation in the hippocampus (Lee *et al.*, 2016). Listening to music can influence heart condition and increase mood (Almeida *et al.*, 2015), cause peace of mind and reduce stress followed by the decrease in HPA-axis stimulus and cortisol secretion (Chafin *et al.*, 2004), which in turn stimulates DBHB synthesis. The increase in BDNF expression will bind TrkB receptor (Lee *et al.*, 2016). BDNF and TrkB receptor binding promotes the life cycle of cells, improves neurogenesis (Van Praag, 2005), synaptogenesis and synaptic plasticity (Bathina and Das, 2015), as well as inhibits neuron apoptosis through p38-MAPK dependent signalling (Zheng *et al.*, 2018), so it may increase the number of hippocampal neurons (Shors *et al.*, 2014).

The result of the present study shows a significant difference of BDNF expression between G3 and G2 groups ($P=0.001$). This finding supports the study of Luo *et al.* (2019) which used Wistar rats as animal models treated

with high-intensity interval training (HIIT) and moderate intensity continuous training (MICT) indicating that HIIT significantly increased the mature ratio BDNF/precursor BDNF. However, Gomes *et al.* (2016) found that moderate intensity exercise for eight weeks significantly increased BDNF expression. The study by So *et al.* (2017) also showed that moderate intensity exercise significantly increased BDNF level compared to high-intensity exercise. Again, these different conclusions were drawn from studies that only used moderate and high intensity exercise, while our study used a combination of moderate or high intensity exercise and listening to music with a fast tempo of 160 beats/min. According to the study conducted by Yeh *et al.* (2015), doing exercise by listening to music for 12 weeks can significantly increase BDNF expression in the hippocampus. It may enhance enjoyment and reduce anxiety so that it can inhibit physical and psychological stress because listening to music changes distress to eustress (Jurcău and Jurcău, 2012). In addition, listening to music also reduces stress which is characterised by cortisol secretion decline (Sugiharto, 2009), with previously mentioned effects.

There are significant differences in the number of hippocampal neurons between G3 and G2 ($P=0.001$). This is in line with the results of a study using Wistar rats treated with high-intensity exercise intervention (25 m/min for 30 min). The study showed that high intensity exercise significantly increases expression of doublecortin positive cells in the ventral hippocampus (Nishii *et al.*, 2017). However, some other studies using Wistar rats treated with moderate and high intensity exercise found different results. These showed that moderate intensity exercise significantly improved hippocampal neurogenesis in adults and increases in the number of new hippocampal neurons compared to high-intensity exercise (Inoue *et al.*, 2015; Nokia *et al.*, 2016). Also, the use of music with a fast tempo of 160 beats/min in our study is the different factor here.

A study conducted by Kirste *et al.* (2015) on Wistar rats with audio stimuli concluded that listening to music can significantly increase hippocampal neurogenesis in adults. A combination between exercise and music can influence body physiological (Norheim *et al.*, 2014) and psychological function (Prasetyo, 2006), and can cause an autonomic nervous system activity changes (Arazi *et al.*, 2015). Listening to music during exercise promotes the limbic and autonomic nervous system, creating a sense of relaxation, safety, comfort, and pleasure (Gómez-Villafuertes *et al.*, 2001). It causes the midbrain to secrete γ -amino butyric acid, enkephalin and β -endorphin, thereby improving the mood, creating a sense of comfort and reducing anxiety (Angelucci *et al.*, 2007; Prasetyo, 2006; Stefano *et al.*, 2004). In addition, exercise combined with music can also reduce stress response characterised by the decline of cortisol secretion (Joshi and De Sousa, 2012; Sugiharto, 2009).

The decrease in cortisol secretion may increase BDNF expression (Chafin *et al.*, 2004; Gomes *et al.*, 2016; Yeh *et al.*, 2015). The increase in BDNF expression binds TrkB receptor (Lee *et al.*, 2016), then promotes improvement of neurogenesis, synaptogenesis, proliferation and differentiation of hippocampal neurons (Mustroph *et al.*, 2015; Tarfarosh *et al.*, 2018). It improves the life cycle of neurons (So *et al.*, 2017), synaptic development, synaptic plasticity and dendritic complexes (Reinsberger, 2015), and inhibits neuronal apoptosis by p38-MAPK dependent signalling (Zheng *et al.*, 2018); thus it increases the number of hippocampal neurons (So *et al.*, 2017).

No significant difference between G2 and G1 in BDNF expression and the number hippocampal neurons ($P=0.954$ and $P=0.438$, respectively) were found.

5. Conclusions

High intensity exercise by listening to the music with fast tempo (allegro) significantly increases BDNF expression and the number of hippocampal neurons. High intensity exercise by listening to music with fast tempo of 160 beats/min which is performed 20 min, three times per week for 12 weeks shows positive impacts on the increase in BDNF expression and the number of hippocampal neurons compared to moderate intensity exercise by listening to music. The improvement of the present study can be subjected by adding study group and observe other variables such as TrkB receptor, cortisol, and endorphins hormone.

Conflict of interest

The authors declare that they have no competing interests.

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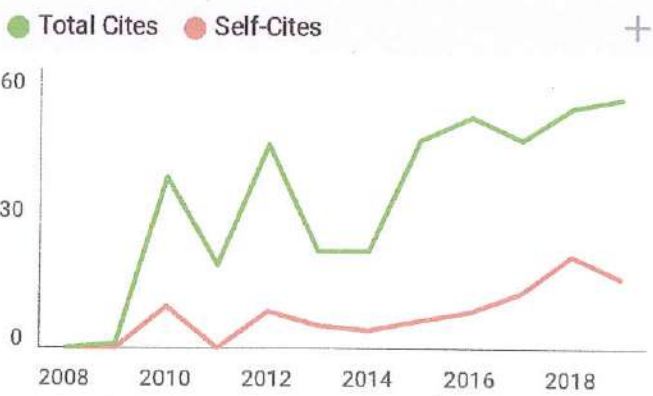
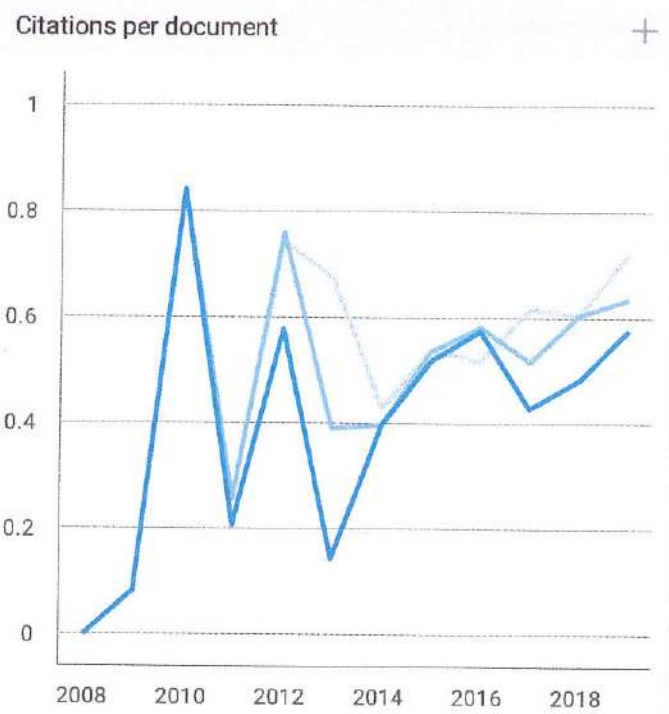
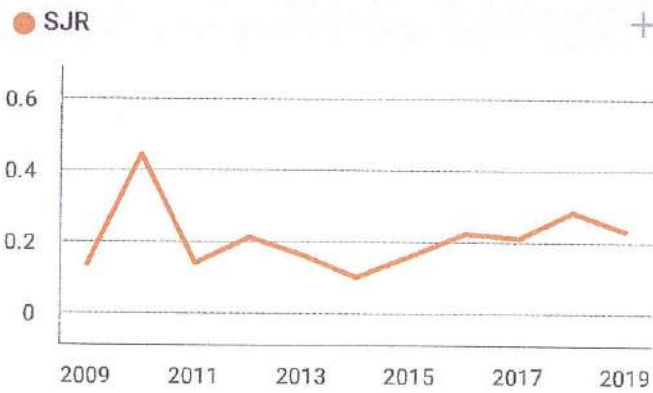
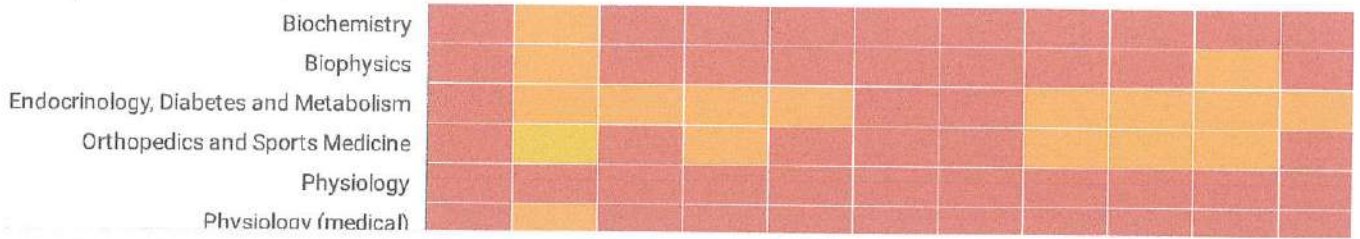
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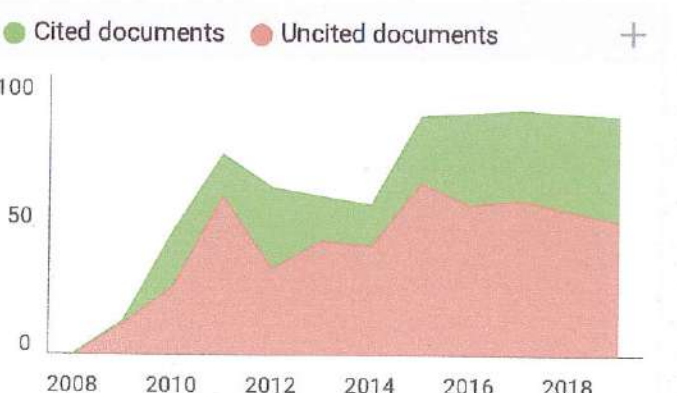
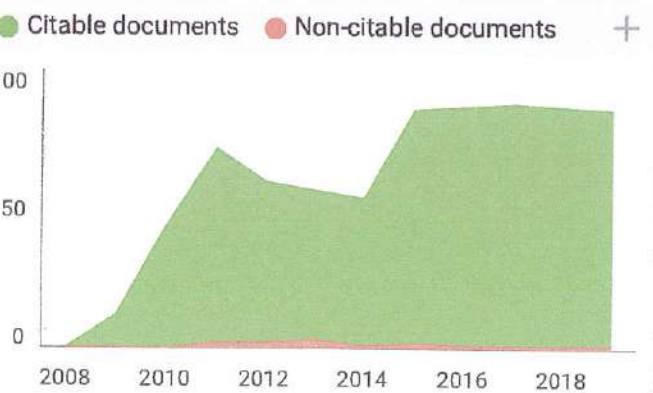
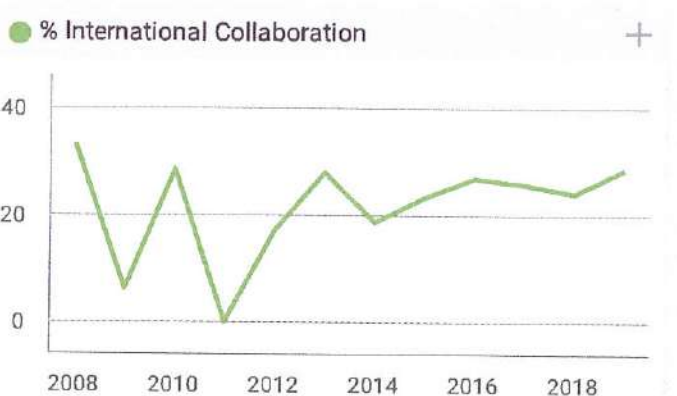
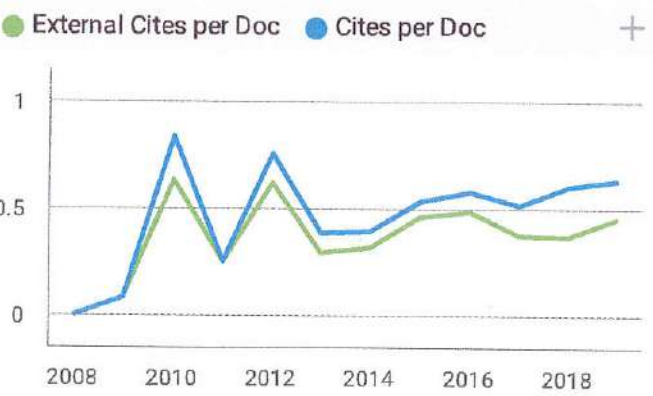


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