J Med Sci
Volume 46, No. 3, September 2014: 108-116

Binaural entrainment of 2000-2040 Hz and 2000-2090 Hz increase Glial Fibrillary Acidic Protein (GFAP) expression of astrocytes in the *CA*1 *rat* hippocampus during operant learning conditioning

Zainuri Sabta Nugraha[1*], Soedjono Aswin[2], Harijadi[3]
[1]Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, [2]Department of Anatomy, [3]Department of Pathology Anatomy, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta

ABSTRACT

Astrocytes of hippocampus contribute in the learning performance. Entrainment of gamma waves can improve learning performance by improving the neurons to astrocytes communication. The aim of this study was to evaluate the effect of binaural entrainment of 2000-2040 Hz and 2000-2090 Hz on Glial Fibrillary Acidic Protein (GFAP) expression in astrocytes of the CA1 region of rat hippocampus during operant learning conditions. Twenty male Wistar rats aged 4-6 weeks with body weight 100-150 g were devided into 4 groups. Group I was given binaural sounds entrainment at 2000-2040 Hz without learning test. Group II was given entrainment as performed in Group I followed by a learning test. Group III was given binaural sounds entrainment at 2000-2090 followed by a learning test. Group IV was not given entrainment nor learning test. The entrainment was performed for 30 minutes everyday for 12 days and the learning test was performed for 10 minutes everyday for 12 days. The GFAP expression was examined imminohistochemically. Astrocytes processes and astrocytes histoscore were also calculated. The results showed that the number of the GFAP-positive astrocytes in Group I (70.96 ± 4.86), II (69.76 ± 3.07) and III (63.10 ± 5.85) were significantly higher than Control (47.33 ± 1.33) ($p < 0.05$). The number of the processes astrocytes in Group I (47.64 ± 3.87), II (60.66 ± 2.07) and III (54.17 ± 6.38) was significantly higher than Control (30.87 ± 2.69) ($p < 0.05$). Moreover, the number of the processes astrocytes in Group II was significantly higher than Group I ($p = 0.016$). The astrocytes histoscore index in the Group II (115.58 ± 14.13) and III (78.32 ± 22.23) were significantly higher than Group I (28.79 ± 9.61) and Control (16.05 ± 1.64) ($p < 0.05$). In conclusion, the binaural entrainment of 2000-2040 Hz and 2000-2090 Hz increase GFAP expression of astrocytes in Cornu Ammonus 1 (CA1) region of rat hippocampus during operant learning conditioning.

ABSTRAK

Astrosit hippocampus berperan dalam kemampuan belajar. Entrainmen gelombang gamma dapat meningkatkan kemampuan belajar dengan memperbaiki komunikasi neuron dengan astrosit. Tujuan penelitian ini adalah mengkaji pengaruh *entrainment* menggunakan metode binaural dengan gelombang suara 2000-2040 Hz dan 2000-2090 Hz terhadap ekspresi Glial Fibrillary Acidic Protein (GFAP) pada astrosit di daerah CA1 hippocampus tikus yang menjalani proses belajar kondisi operan. Dua puluh tikus jantan Wistar berumur 4-6 minggu dengan berat badan 100-150 g dibagi menjadi 4 kelompok. Kelompok I diberi entrainment binaural dengan gelombang suara 2000-2040 Hz tanpa uji belajar. Kelompok II diberi *entrainment binaural* seperti pada Kelompok I

* corresponding author: zainurisabtanugraha@gmail.com

dengan uji belajar. Kelompok III diberi *entrainmen binaural* dengan gelombang suara 2000-2090 Hz dengan uji belajar. Kelompok IV tidak diberi entrainment maupun tanpa uji belajar. Entrainment dilakukan selama 30 menit setiap hari selama 12 hari dan uji belajar dilakukan selama 10 menit selama 12 hari. Ekspresi GFAP diperiksa secara imunohitokimia. Prosesus astrosit dan histoskor astrosit selanjutnya dihitung. Hasil penelitian menunjukkan astrosit dengan GFAP positif pada Kelompok I (70,96 ± 4,86), II (69,76 ± 3,07) dan III (63,10 ± 5,85) lebih tinggi secara nyata dibandingkan Kontrol (47,33 ± 1,33) ($p<0,05$). Jumlah prosesus astrosit Kelompok I (47,64 ± 3,87), II (60,66 ± 2,07) dan III (54,17 ± 6,38) lebih tinggi secara nyata dibandingkan Kontrol (30,87 ± 2,69) ($p<0,05$). Selain itu, jumlah prosesus astrosit pada Kelompok II lebih tinggi secara nyata dibandingkan kelompok I ($p=0,016$). Indeks histoskor pada Kelompok II (115,58 ± 14,13) dan III (78,32 ± 22,23) lebih tinggi secara nyata dari Kelompok I (28,79 ± 9,61) dan Kontrol (16,05 ± 1,64) ($p<0,05$). Dapat disimpulkan, entrainment binaural dengan gelombang suara pada 2000-2040 Hz dan 2000-2090 Hz meningkatkan ekspresi GFAP pada astrosit di CA1 hippocampus tikus yang menjalani proses belajar kondisi operan.

Keywords: entrainment - learning - *Glial Fibrillary Acidic Protein* - Skinner test – astrocytes

INTRODUCTION

Hippocampus plays an important role in the learning process. Adult hippocampus known to have a high plasticity and neurogenesis capabilities.[1] The hippocampus continues growing and developing throughout life. It is believed that the function of neurogenesis in the hippocampus is correlated with learning and memory.[2,3] Neurons of the hippocampus i.e. dentate gyrus (DG), CA3 and CA1 play an important role in the learning process and memory formation or long-term potentiation (LTP).[4] The number of the neurons and synapses is associated with the learning and memory.[5]

Astrocytes are integral components of synapses of central nervous system (CNS). They perform many function, including metabolic support and control of neuron,[6-8] provision of nutrients to the nervous tissue and excretion of waste metabolics,[9] modulation of synaptic transmission and neuron excitability,[10] neurotransmitter uptake and release, maintenance of extracellular ion balance.[9,11,12] Furthermore, astrocytes contribute in the formation of new memories and LTP as well as in the modulating synaptic plasticity through its communication with neuron in the hippocampus.[5,13-15]

The communication between astrocytes and neuron is mediated by the release and regulation several neuroactive molecules such as glutamate, ATP, cytokines, and several other key signaling molecules like adenosine, lactate and D-serine.[13,15,16] D-serine released by astrocytes can affect neuronal activity and modulate the LTP and synaptic plasticity through its interaction with N-methyl D-aspartate (NMDA) receptors in CA1 region.[13] Moreover, astrocyte intermediate filaments play an important role in the neuron to astrocytes communication. Glial fibrillary acidic protein (GFAP) is the principal astrocyte intermediate filament protein that has been associated with the neuronal function.[17,18]

Previous studies showed that the entrainment improved the neurons to astrocytes communication and its function. The study conducted in human showed that auditory and visual stimulation significantly improve in all of the specific cognitive abilities in learning-disabled children.[19] In addition, audiovisual entrainment program was proven to be an effective and affordable treatment for behavior disorders in a school setting.[20] Studies conducted in animal model showed that stimulation of the Schaffer collateral fibers increases the Ca^{2+} concentration in astrocytes

of the stratum radiatum intracellular CA1 hippocampus.[21] This study was performed to evaluate the effect of binaural entrainment of 2000-2040 Hz and 2000-2090 Hz on GFAP expression in astrocytes of the CA1 region of rat hippocampus during operant learning conditions.

MATERIALS AND METHODS

Animals and experimental design

Twenty male Wistar rats (*Ratus novergicus*) aged from 4 to 6 weeks which were initially weighing from 100 to 150 g were used in this study. Rats were obtained from Animal House of Universitas Gadjah Mada, Yogyakarta. Rats were housed in cages under 12-h of light-dark cycle. Food and water were given *ad libitum*. Rats were then randomly divided into 4 groups with 5 rats in each group. Group I as entrainment control was given entrainment using binaural sounds at 2040 Hz (ë of 0.1681) through rigth ear and at 2000 Hz (ë of 0.1715) through left ear without learning test. Group II as entrainment 1 was given entrainment as performed in Group I followed by a learning test. Group III as entrainment 2 was given entrainment using binaural sounds at 2090 Hz (ë of 0.1641) through rigth ear and at 2000 Hz (ë of 0.1715) through left ear followed by a learning test. Group IV as normal control was not given entrainment nor learning test.

Entrainment and learning test

After 3 days adaptation to the environment continued 3 days adaptation to the Skinner's box for 10 minutes everyday, the entrainment was performed using mini headset binaurally attached to left and right ear of the rats. The entrainment was performed for 30 minutes everyday for 12 days at 4 to 5 PM. A sound wave generator with less than 1% error in accuracy was used in the entrainment. Following the entrainment, the operant condition learning test was directly performed using Skinner's box for 10 minutes everyday for 12 days. The protocol of the study was approved by the Medical and Health Research Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

Brain sectioning

On day 16, all rats were decapitated after chloral-hydrate anesthesia. A midline abdominal incision was made and the brains were removed for immunohistochemical examination. At first the brains were fixed in a solution of 4% buffered formaldehyde, then dehydrated in a graded ethanol series, cleared in clearing agent (ethanol:toluene = 1:1) and embedded in paraffin wax. After histological processing, serial brain sections in stereotaxis coordinates containing CA1 region of hippocampus were cut with a microtome at 6 µm thickness. Coordinates were in accord with Paxinos and Watson.[22]

Immunohistochemical staining and examination

The serial brain sections were mounted on glass slides for immunohistochemical staining using primary of mouse anti-GFAP monoclonal antibody. Slides were deparaffinised and hydrated in graded descending concentration of alcohol for 10 minutes and then incubated with hydrogen peroxide blocking solution (500 µL of H_2O_2 in 50 mL of methanol) for 15 minutes. Slides were incubated with citrate buffer pH 6 and then heated in the microwave oven until heating. After heating, slides were removed from the oven and allowed to cool. Slides were then rinsed again in PBS. Primary antibodies of mouse anti-GFAP polyclonal antibody were then applied on the serial sections and each was incubated for 60 minutes. Slides were then

washed in PBS and incubated with a biotinylated secondary antibody for 30 minutes. Slides were washed twice again in PBS. Slides were incubated with a streptavidine peroxidase for 30 minutes at room temperature, washed in PBS. Slides were incubated with diamino-benzidine (DAB) for 5 to 10 minutes and washed in aquadest. Slides were then ligthtly counterstained by haematoxylin and washed in aquadest. Slides were then dried and coverslipped.

Morphometeric measurements of each slides were carried out using light Olympus BX-41 microscope completed with OLYSIA software a magnification of 400 x. The GFAP-positive astrocytes were identified as astrocytes with blue color nuclei and brown color processes and cytoplasm. They were examined on 3 slides for each rat brain with 10 fields of view for each slides and its number were expressed as mean per field of view. Furthermore, the degree of GFAP expression was analyzed semiquantitatively using image analysis and then grouped according to quality of the binding color in 3 scale i.e. ranging from yellow-brown (1), brown (2) and dark brown (3) .

The astrocytes processes were identified as parts of astrocytes with yellow or brownish color. It was counted in each astrocyte per a field of view. Furthermore, the number of astrocytes that have processes was calculated and grouped according to the number of its processes i.e. 0 (not reactive), 1, 2, 3, 4, 5 and so on. The data of the number of astrocytes, the number of astrocytes processes and staining score were then used to calculate astrocytes histoscore index using formula as follows :

$$\frac{\Sigma \text{ astrocytes processes of} = 3}{\Sigma \text{ astrocytes per field view}} \text{ X degree of GFAP e xpression}$$

Statistical analysis

Data were presented as mean ± standar error of the mean (SEM). The mean of GFAP-positive astrocytes, astrocytes processes and astrocytes histoscore index of CA1 hippocampus among the groups were statistically compared using the one way analysis of variance (ANOVA) or Kruskall-Wallis. A p value <0.05 was considered to be statistically significant. The relationship between the GFAP-positive astrocytes, astrocytes processus and astrocytes histoscore index were then analyzed using Pearson correlation test.

RESULTS

The GFAP-positive astrocytes and its processes in CA1 region of rat hippocampus of the groups of intervention and control under ligth microscope at 400x magnification are presented in FIGURE 1. The GFAP-positive astrocytes were identified as a blue color of nuclei and a brown color of cytoplasm processes of astrocytes.

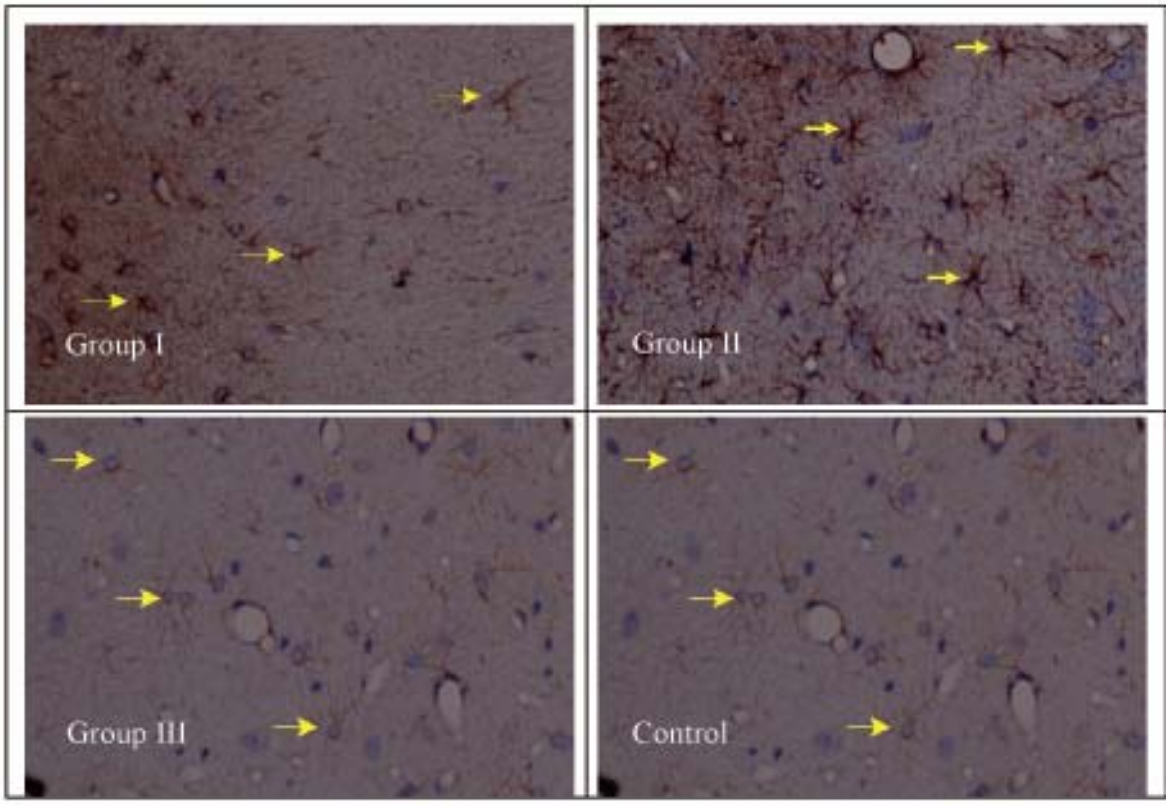

FIGURE 1. GFAP-positive astrocytes (arrow heads) and its processes in CA1 region of rat hippocampus of the groups of intervention and control

The mean of the GFAP-positive astrocytes in CA1 region of rat hippocampus of groups of intervention and control groups is presented in FIGURE 2 and TABLE 1. The number of the GFAP-positive astrocytes in the Group I (p=0.001), II (p=0.002) and III (p=0.016) was significantly higher than those of the Control (TABLE 2). However, it was not significantly different between the Group I compared to those the Group II (p=0.840) and the Group III (p=0.199). No significantly different was also observed between the Group II and Group III (p=0.274) (TABLE 2). It was indicated that the entrainment could increase the number of astrocytes in CA1 region of rat hippocampus.

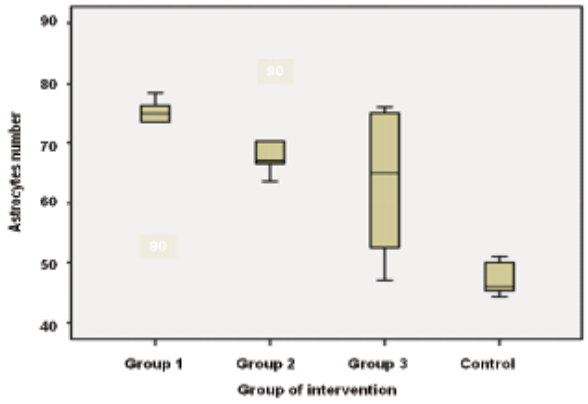

FIGURE 2. Mean of the GFAP-positive astrocytes in CA1 region of rat hippocampus of the groups of intervention and control. Group I: 2000 and 2040 Hz entrainment without learning test; Group II: 2000 and 2040 Hz entrainment with learning test; Group III: 2000 and 2090 Hz entrainment with learning test

TABLE 1. The number of GFAP-positive astrocytes, astrocytes processes and astrocytes histoscore (mean ± SEM) in CA1 region of rat hippocampus of each group and control

Variable	Group I	Group II	Group III	Control	*p*
GFAP-positive astrocytes	70.96 ± 4.86	69.76±3.07	63.10±5.85	47.33±1.33	0.004[a]
Astrocytes processes	47.64 ± 3.87	60.66±2.07	54.17±6.38	30.87±2.69	0.006[b]
Astrocytes-histoscore	28.79 ± 9.61	115.58±14.13	78.32±22.23	16.05±1.64	0.001[a]

Group I: 2000 and 2040 Hz entrainment without learning test; Group II: 2000 and 2040 Hz entrainment with learning test; Group III: 2000 and 2090 Hz entrainment with learning test; Control: without entrainment and no learning test; [a]One way ANOVA analysis; [b]Kruskal-Wallis analysis; significantly different if $p<0.05$.

TABLE 2. The comparison of GFAP-positive astrocytes, processes astrocytes and astrocytes-histoscore among the groups of intervention and control.

Group	p value		
	GFAP-positive astrocytes[a]	Astrocytes processes[b]	Astrocytes histoscore index[a]
Group I vs II	0.840	0.016	0.001
Group I vs III	0.199	0.347	0.013
Group I vs Control	0.001	0.028	0.529
Group II vs III	0.274	0.834	0.224
Group II vs Control	0.002	0.009	0.000
Group III vs Control	0.016	0.009	0.003

Group I: 2000 and 2040 Hz entrainment without learning test; Group II: 2000 and 2040 Hz entrainment with learning test; Group III: 2000 and 2090 Hz entrainment with learning test; Control: without entrainment and no learning test; [a]Least Significant Difference (LSD) test; [b]Mann-Whitney test; significantly different if $p<0.05$

The mean of the astrocytes processes in CA1 region of rat hippocampus of groups of intervention and control groups is presented in FIGURE 3 and TABLE 1. The number of the processes astrocytes in the Group I (p=0.028) II (p=0.009) and III (p=0.009) was significantly higher than those of the Control (TABLE 2). Moreover, the number of the processes astrocytes in the Group II was significantly higher than those of the Group I (p=0.016). However, it was not significantly different between the Group II and the Group III (p=0.834) and between the Group I and the Group III (p=0.347) (TABEL 2). It was indicated that binaural sounds entrainment followed by learning test could increase significantly the processes of GFAP-positive astrocytes in CA1 region of rat hippocampus.

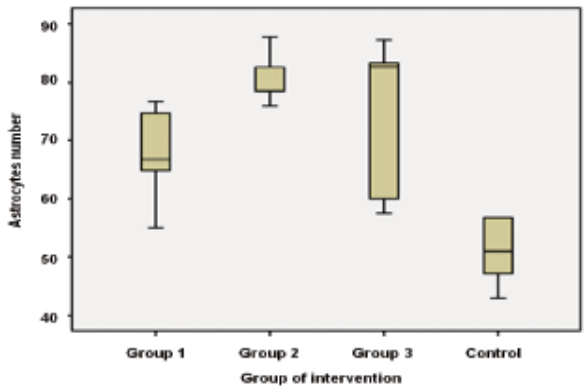

FIGURE 3. Mean of astrocytes processes in CA1 region of rat hippocampus of the groups of intervention and control. Group I: 2000 and 2040 Hz entrainment without learning test; Group II: 2000 and 2040 Hz entrainment with learning test; Group III: 2000 and 2090 Hz entrainment with learning test

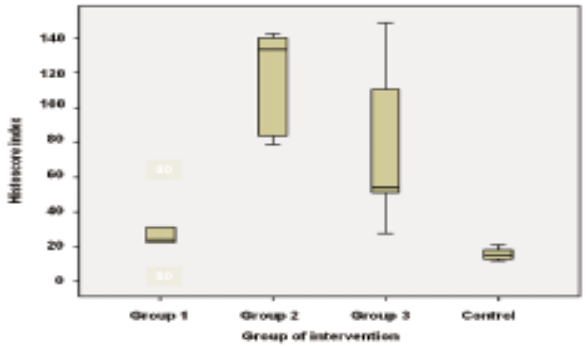

FIGURE 4. Astrocytes histoscore index in CA1 region of rat hippocampus groups of intervention and control. Group I: 2000 and 2040 Hz entrainment without learning test; Group II: 2000 and 2040 Hz entrainment with learning test; Group III: 2000 and 2090 Hz entrainment with learning test

The mean of the astrocytes histoscore index in CA1 region of rat hippocampus of groups of intervention and control groups is presented in FIGURE 3 and TABLE 1. The number of the astrocytes histoscore index in the Group II (p=0.000) and III (p=0.003) was significantly higher than those of the Control (TABLE 2). Moreover, the astrocytes histoscore index in the Group II (p=0.001) and Group III (p=0.013) were also significantly higher than those of the Group I (TABLE 2). However, it was not significantly different between the Group II and the Group III (p=0.224) and between the Group I and the Control (p=0.529) (TABEL 2). It was indicated that binaural sounds entrainment followed by learning test could increase significantly the astrocytes histoscore index of the CA1 region of rat hippocampus.

DISCUSSION

Entrainment is a principle of physics that defined as the synchronization of two or more rhythmic cycles. When the brain is given a stimulus, for example through ears with different sound frequency of 40 and 90 Hz, it emits an electrical charge in response called a Cortical Evoked Response.[23-25] If rhythm of the stimulus become fast and consistent enough, it can start to resemble the natural internal rhythms of the brain. When this happens, the brain responds by synchronizing its own electric cycles to the same rhythm. This is commonly called the Frequency Following Response (FFR).

The FFR is used to regulate human brain wave of the both hemispheres in order to increase the expression of astrocytes GFAP in CA1 region of hippocampus.[25-27] Learning process involves a conscious behavior that controled by the brain. Conversely, the brain will function properly when it is stimulated by the learning process.[28] This study proved that the GFAP-positive astrocytes of the groups with

learning test were higher than those groups without learning test.

It is widely accepted that cognitive function, learning and memory in the cerebral cortex and hippocampus are influenced by gamma waves with a frequency of 30-80 Hz.[29] In addition, entrainment using the gamma waves with a frequency of 40 Hz can improve communication and strengthen synapses in the hippocampus. The entrainment can also increase the interaction between neuron and astrocytes.[19,20] This study showed that the binaural entrainment of 2000-2040 Hz and 2000-2090 Hz increased GFAP expression of astrocytes and number of astrocytes processes in CA1 region of rat hippocampus. It was indicated an increase the interaction between neuron and astrocytes. The increased of GFAP expression of astrocytes might be due to the increase of astrocytes autocrine endhothelin-1 (ET1) activity. Previous study reported that the activation of ET-1 receptors increased the astrocytes proliferation and GFAP levels.[30-34]

This study showed that the binaural entrainment at the frequency difference of 40 Hz (2000-2040 Hz) had better effect compare to those of 90 Hz (2000-2090 Hz). Previous study concerning auditory stimulation at various frequencies in the gamma waves showed that the optimal stimulation was achieved at frequency of 40 Hz. Furthermore, the optimal stimulation was related to increased cortical synaptic activity. Moreover, the stimulation at 40 Hz selectively activated the auditory region of the pontocerebellum, a brain structure with important roles in cortical inhibition and timing.[35]

CONCLUSION

In conclusion, the binaural entrainment of 2000-2040 Hz and 2000-2090 Hz increase GFAP expression of astrocytes and number of astrocytes processes in CA1 region of rat hippocampus during operant learning conditioning. In addition, the binaural entrainment of 2000-2040 Hz has better effect compare to those of 2000-2090 Hz.

ACKNOWLEDGEMENTS

We would like to thank all technicians from Laboratory of Anathomy and Laboratory Pathology Anatomy, Faculty of Medicine, Universitas Gadjah Mada for valuable assistances during laboratory works.

REFERENCES

1. Palmer TD, Willhoite AR, Gage FH. Vascular niche for adult hippocampal neurogenesis. J Comp Neurol 2000; 425(4):479-94.
2. Aberg MA. Learning, memory and plasticity. J Neurosci 2001; 21(18):7153-60.
3. Bischofberger J. Young and excitable: new neurons in memory networks. Nat Neurosci 2007; 10(3):273.
4. Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS. Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. J Neurobiol 1999; 39(4):569-78.
5. Temburni MK and Jakob SK. New function for glia in the brain. PNAS 2001; 98(7):3631-2.
6. Conejo NM, Pardo HG, Cimadevilla JM, Seco GV, Arias JL. Maturation of astrocytes in the rat hippocampus: potential behaviour implication. Psicothema 2003; 15(2):216-20.
7. Gruetter R. Glycogen: the forgotten cerebral energy store. J Neurosci Res 2003; 74:179-83.
8. Magistretti PJ, Oliver S, Martin JL. Regulation of glycogen metabolism in astrocytes: physiological, pharmacological and pathological aspects. In: Murphy S editor. Astrocytes pharmacology and function. San Diego: Academic Press, 1993; 243-65.
9. Perea G, Araque A. Communication between astrocytes and neurons: a complex language. J Physio Par 2002; 96:199-207.
10. Keyser DO, Pellmar TC. Synaptic transmission in the hippocampus: critical role for glial cells. GLIA 1994; 10(4):237-43

11. Catalani A, Sabbatini M, Consoli C, Cingue C, Tommassoni D, Azmitia E, *et al*. Glial fibrillary acidic protein immunoreactive astrocytes in developing rat hippocampus. Mech Ageing Dev 2002; 123(5):481-90.
12. Wu Y, Zhang AQ, Yew DT. Age related changes of various markerof astrocytes in senescence-accelerated rat hippocampus. Neurochem Int 2005; 46(7):565-74.
13. Yang Y, Ge W, Chen Y, Zhang Z, Shen W, Wu C, *et al*. Contribution of astrocytes to hippocampal long term potentiation through release of D-serin. PNAS 2003; 100(25):15194-9.
14. Araque A, Carmignoto G, Haydon PG. Dynamic signaling between astrocytes and neurons. Annu Rev Physiol 2001; 63:795-813.
15. Ota Y, Zanetti AT, Hallock RM. The role of astrocytes in the regulation of synaptic plasticity and memory formation. Neural Plasticity 2013; 2013:1-11.
16. Theodosis DT, Poulain DA, Oliet SHR. Activity dependent structural and functional plasticity of astrocyte-neuron interactions. Physiol Rev 2008; 88(3): 983–1008.
17. McCall MA, Gregg RG, Behringer RR, Brenner M, Delaney CL, Galbreath EJ, *et al*. Targeted deletion in astrocyte intermediate filament (GFAP) alters neuronal physiology. *PNAS* 1996; 93(13):6361-6.
18. Pekny M and Pekna M, Astrocyte intermediate filaments in CNS pathologies and regeneration. J Pathol 2004; 204(4)428-37.
19. Olmstead R. Use of auditory and visual stimulation to improve cognitive abilities in learning-disabled children. J Neurother 2005; 9(2):49-61.
20. Joyce M, Siever D. Audivisual entrainment program as treatment for behavior disorders in a school setting. J Neurother 2000; 4(2):9-25.
21. Antanitus D. A theory of cortical neuron-astrocyte interaction. Philadelphia: Williams & Wilkins Inc, 1998.
22. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates, 2nd ed. San Diego, California: Academic Press Inc, 1986.
23. Frederick JA. EEG coherence and amplitude effects of rhythmic auditory and visual stimulatio with an emphasis in computational method. [Dissertation]. Knoxville: The University of Tennessee; 2001.
24. Larry SM. The use of auditory and visual stimulation for the treatment of attention deficit hyperactivity disorder in children [Dissertation]. Texas: University of Houston; 1999.
25. Teplan M. Fundamentals of EEG measurement. Measur Sci Rev 2002; 2(2):1-11.
26. Turow G. Auditory driving as a ritual technology: A review and analysis, religious studies honors [Thesis]. Stanford: Stanford University; 2005.
27. Ossebaard HC. Stress reduction by technology? An experimental study into the effects of brainmachines on burnout and state anxiety. Appl Psyc Biofeed 2000; 25(2):93-101.
28. Baharuddin. Teori belajar dan pembelajaran. Yogyakarta: Ar-Ruzmedia, 2007.
29. Montgomery SM, Buzsáki G. Gamma oscillations dynamically couple hippocampal CA3 and CA1 regions during memory task performance. PNAS 2007; 104(36): 14495-500.
30. Gadea A, Schinelli S, Gallo V. Endothelin-1 regulates astrocyte proliferation and reactive gliosis via a JNK/c-Jun signaling pathway. J Neurosci 2008; 28(10):2394-408.
31. Hasselblatt M, Bunte M, Dringen R, Tabernero A, Medina JM, Giaume C, *et al*. Effect of endothelin-1 on astrocytic protein content. GLIA 2003; 42:390-7.
32. Zhang WW, Badonic T, Hoog A, Jiang MH, Ma KC, Nie XJ, *et al*. Astrocytes in Alzheimer's disease express immunoreactivity to the vasoconstrictor endothelin-1. J Neurosci 1994; 122:90-6.
33. Ridet JL, Malhotra SK, Privat A, Gage FH. Reactive astrocytes: cellular and molecular cues to biological function. Trends Neurosci 1997; 20:570-7.
34. Couraud PO, Durieu-Trautmann O, Le Nguyen P, Marin P, Glibert F, Strosberg AD. Functional endothelin-1 receptors in rat astrocytoma C6. Eur J Pharmacol 1991; 206:191-8.
35. Pastor MA, Artieda J, Arbizu J, Marti-Climent JM, Peniuelas I, Masdeu JC. Activation of human cerebral and cerebellar cortex by auditory stimulation at 40 Hz. J Neurosci 2002; 22(23):10501-6.