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Correlation between Chronic Pain with BDNF Levels, Histophatology of Hippocampus, and Spatial Memory in Wistar Rats

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Abstract

BACKGROUND: Pain is defined as an unpleasant sensory and emotional experience associated with actural or potential tissue damage. Cognitive impairment can occur due to various processes in the brain, one of which resulted from chronic pain. Brain-derived neurotrophic factor (BDNF) is a neurotropin that plays a role in mediating disinhibition in the excitability of the motor cortex of the brain and inhibitory function in descending pain pathways. Chronic pain of periodontitis causes systemic inflammation that activates microglia resulting in degeneration of CA1 pyramidal neurons in the hippocampus and affects cognitive function, especially spatial memory.

AIM: The objectives of this study were to determine the correlation between BDNF levels, spatial memory, and histopathology of hippocampus on periodontitis Wistar rats.

METHODS: This observational prospective study was conducted between January and April 2022 at Laboratory Negeri Semarang University. Frontal inferior teeth ligation of Wistar rats was carried out to induce periodontitis for 4 weeks. Chronic pain was assessed using Rat Grimace Scale. Morris water maze (MWM) adaptation was applied for 4 weeks and then BDNF levels, spatial memory, and histopathology of hippocampus were investigated. T-test independent and spearman correlation test were used to data analysis.

RESULTS: Among 12 rats, the mean of BDNF levels, spatial memory, and histopathology of hippocampus score were 7.57 ng/mL, 65.08 second, and 3, respectively. There were significant relationships between BDNF levels ($p = 0.028$), spatial memory ($p = 0.001$), and histopathology of hippocampus score ($p = 0.017$) on Wistar rats with chronic pain. Strong correlation ($r = -0.721$, $r^2 = 0.52$, $p = 0.004$) between BDNF levels and spatial memory, and moderate correlation ($r = -0.597$, $p = 0.02$) between BDNF levels and histopathology of hippocampus score was obtained. There was no significant correlation between spatial memory and histopathology of hippocampus score.

CONCLUSIONS: There were significant differences between BDNF levels, spatial memory, and histopathology of hippocampus on periodontitis Wistar rats.

Introduction

Pain is defined by the international association for the study of pain as something experience sensory and emotional which no pleasant which associated with actual or potential tissue damage, or described in terms of such damage [1].

Periodontitis is a chronic inflammatory process, irreversible from periodontium tissue which affects the supporting tissues of the teeth including the gums or gingival tissue, as well as the periodontal ligament and the alveolar bone in more severe forms of the diseases. In chronic conditions, it can cause pain and discomfort so that it will interfere with activities due to the pain, it causes [2], [3].

Intensity pain that occurs in pain range from mild, moderate, and severe this thing result in painful has impact in the form of disturbance to many aspect life such as health, work, relationships social, sleep, mood, and cognitive [4].

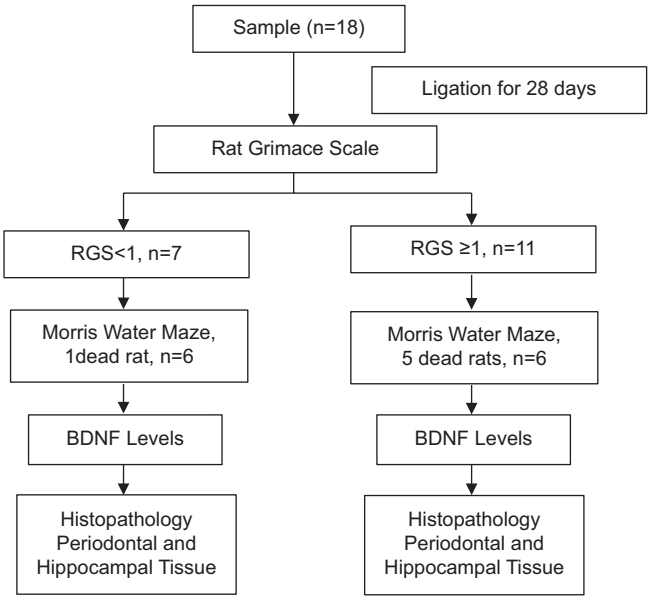
Brain-derived neurotrophic factor (BDNF) is a neurotropin that is widely distributed in the central nervous system. BDNF has several functions, including axonal regulation, dendritic growth, a role in neurotransmitter release, and long-term potentiation. BDNF plays an important role in the inhibitory function of descending pain pathways and cognitive function, especially in the hippocampus [5], [6].

In the past two decades, animal models have become important tools to increase knowledge and understanding about mechanism pain and pharmacologic treatment of pain. One of the models for painful chronic is condition rat with periodontitis. Chronic periodontitis made with ligation of rat inferior frontal teeth during more same with 28 days [7], [8]. There are several ways to assess pain in rats, one of which is through a grimace score which can be measured through gimmick. Grimace score itself is assessed through four parts of the rat's body, namely, the eyes, ears, nose, and whiskers [9]. In the experimental mice, the hippocampus, especially CA1 cells, is the most important part of spatial

memory. Until now, Morris water maze (MWM) is the main choice for the assessment of spatial memory in the experimental animals [10], [11].

Methods

This observational prospective study of Wistar rat divided 18 rats into two group [12]. Wistar rats (*Rattus norvegicus*) were obtained from Semarang University Laboratory. This study was carried out in the laboratory Semarang State University Biology conducted in January 2022.



Implementation flow

Sample used are 18 male Wistar rats (Rattus Norvegicus), 10 weeks old with weight ± 200 g. Every sample inferior frontal tooth ligation was performed for 28 days then rated intensity painful with the rat grimace score. Every sample rat entered cage glass and done recording during 2 min. On assessment intensity, painful conducted with two observers later obtained a test of agreement of 0.98 (Table 1).

After conducted evaluation intensity pain, rat shared into two groups, namely, group rat no pain and group rat with painful chronic.

On examination, memory spatial rat conducted with MWM. After introduction for 3 days, done evaluation memory spatial on each sample with maximum reach platform 120 s as many as six mice dropped out because dead moment introduction nor test MWM.

Then 12 samples rat checked serum BDNF levels taken through the retrobulbar vein. Then, all sample conducted decapitation and checked for histopathology hippocampal CA1 damage.

Results

In this study, a significant difference in BDNF levels was found between the painless rat group and the chronic pain rat group with higher BDNF levels in the painless rat group ($p = 0.028$). There was a significant difference in hippocampal histopathological scores between the painless rat group and the chronic pain rat group with a lower histopathological score in the painless rat group ($p = 0.017$). There was a significant difference in spatial memory function between the painless rat group and the chronic pain rat group with better spatial memory function in the painless rat group (0.000) (Table 2).]

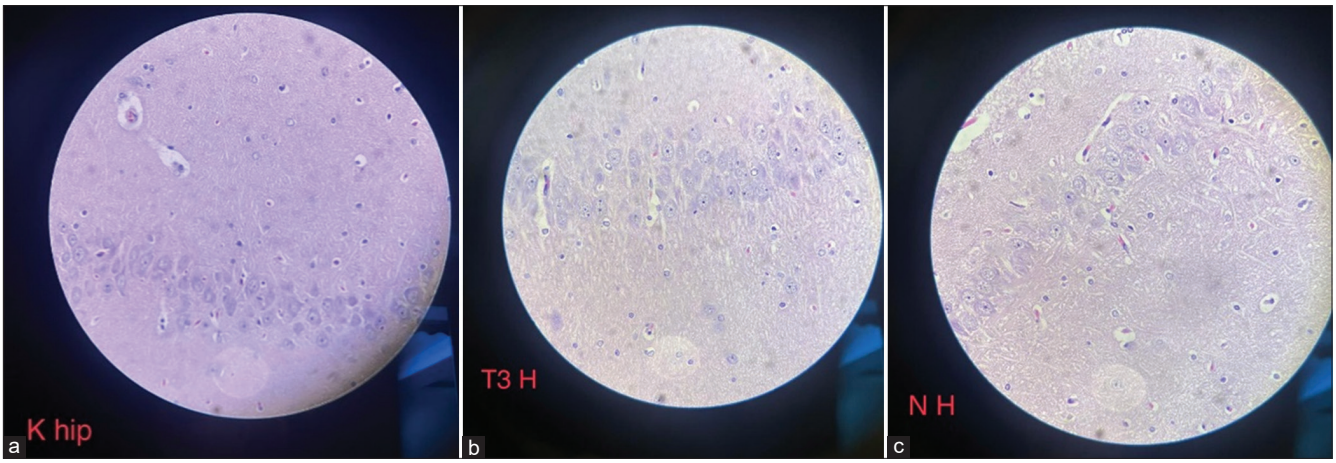


Figure 1: Histopathological features of the hippocampus, (a) histopathological features of control rats, (b) histopathological features of the hippocampus in painless rats, and (c) histopathological features of the hippocampus in chronic pain rats

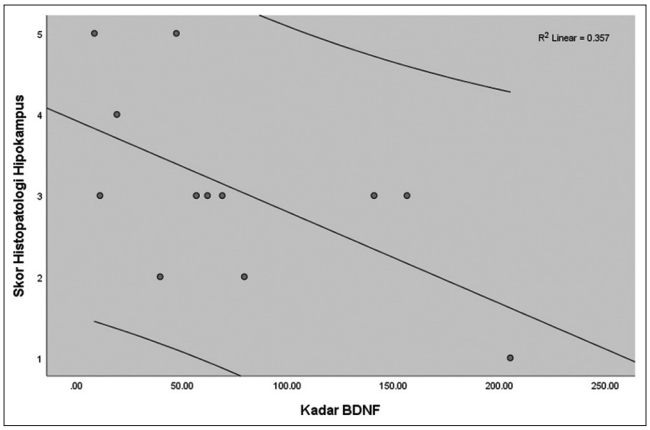


*Figure 2: Scatter plot relationship of BDNF levels with spatial memory function in rats with chronic pain. *Pearson, rho = −0.721; p = 0.004*

Correlation between BDNF levels with histopathology hippocampus in chronic pain group

As shown in Figure 2, an analysis of the relationship between BDNF levels and spatial memory function using Pearson's test, obtained p = 0.004 then the relationship between BDNF levels and the spatial memory function of Wistar rats, has a strong correlation (r = −0.721, r^2 = 0.52). Where the higher the BDNF level, the better the rat's spatial memory function.

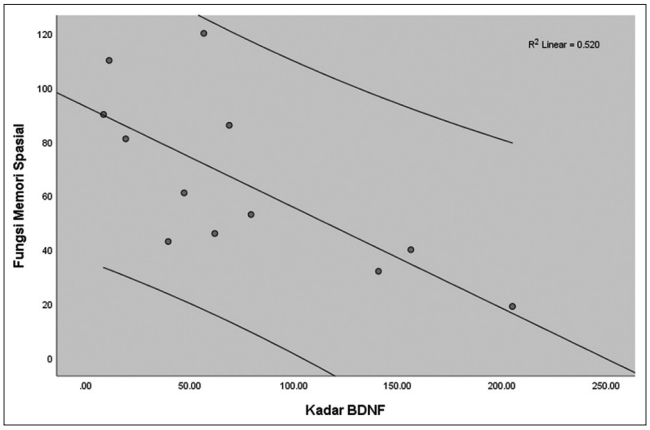


*Figure 3: Scatter plot relationship of BDNF levels with hippocampal histopathological score in rats with chronic pain. *Pearson, rho = −0.721; p = 0.004*

Relationship between BDNF levels and spatial memory function in rats with chronic pain

In this study, an analysis of the relationship between BDNF levels and spatial memory function using Pearson's test, obtained p = 0.004 then the relationship between BDNF levels and the spatial memory function of Wistar rats with a strong correlation (r = −0.721, r^2 = 0.52), was carried out. Where the higher the BDNF level, the better the rat's spatial memory function (Figure 3).

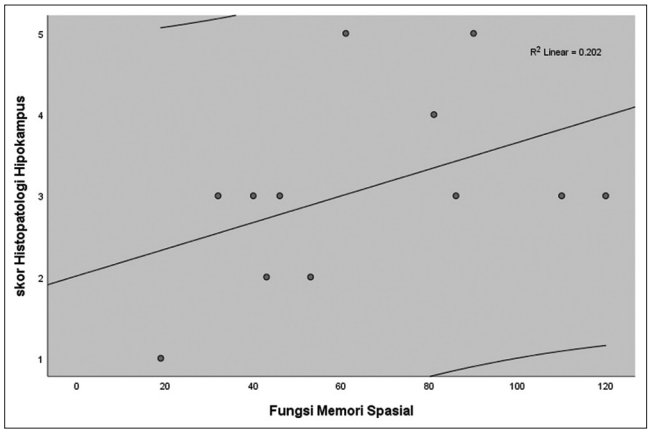


*Figure 4: Scatter plot relationship of hippocampal histopathological scores with spatial memory function in rats with chronic pain. *Pearson, rho = 0.45; p = 0.071*

Correlation between hippocampal histopathology score and spatial memory function in rats with chronic pain

In this study, the histopathological score of the hippocampus with the spatial memory function of rats with chronic pain did not have a significant relationship (p = 0.071) (Figure 4).

Table 1: Interobserver agreement (IOA)

Observer	Observer 1		Amount
	Painful	No pain	
Observer 2			
Painful	7	5	12
No pain	6	6	12
Amount	13	11	24

Discussion

In this study, there was a significant difference in BDNF levels in the painless rat group and the chronic pain rat group (p = 0.028). These results indicate that rats with chronic pain have decreased serum levels of BDNF. This is in accordance with the conclusion by Duric and McCarson (2006) regarding acute and chronic pain. Each group was examined for BDNF levels and found a significant difference between the control group, acute pain, and chronic pain (p < 0.05) [13]. Research by Stefani *et al.* related to the relationship between BDNF levels and pain threshold, higher BDNF levels were found associated with lower pain intensity (p < 0.001) [14], [15].

These results show a significant difference between spatial memory function in the painless rat group and the chronic pain rat group. Mice with chronic pain took longer to reach the baseline, which was associated with poorer spatial memory function. The study of Saffarpour *et al.* also said that if there was a

Table 2: Differences in BDNF levels, histopathology hippocampus, and memory spatial in groups rat no pain with group rat with chronic pain

Information	Group		Statistics	p	Levenne's test
	No pain	Chronic pain	t		
	n = 6	n = 6			
BDNF levels	113.8 ± 63.56 ng/mL	35.34 ± 25.64 ng/mL	2.804	0.028	0.015
Histopathology hippocampus	2.33 ± 0.82	3.83 ± 0.98	−2.875	0.017	0.448
Memory spatial	38.33 ± 11.92 s	91.33 ± 21.1 s	−5.305	0.000	0.254

*Independent t-test, significant if $p < 0.05$

decrease in spatial memory function in rats with chronic pain ($p < 0.001$); in this study, the levels of BDNF and glutamate decreased significantly [16], [17].

Likewise, Zhu *et al*. (2022) analyzed rats with postoperative pain and assessed their spatial memory function. Mice with chronic post-operative pain experienced a significant decrease in spatial memory function ($p < 0.05$). BDNF levels affect the spatial memory of mice through the mechanism of increased expression of synaptic vesicle proteins such as synapsin-1 and neurogenesis in these significantly [4], [18].

Significant differences were also found in the histopathology of the experimental animal hippocampus in this study. Hippocampal histopathology scores were higher in the group of rats with chronic pain, in agreement with Duric and McCarson (2006) who investigated the relationship between acute and chronic pain in the rat hippocampus. In this study, there was a decrease in hippocampal CA1 cells in the chronic pain group of rats, because, in chronic pain, there was damage to hippocampal CA1 cells so that spatial memory function decreased [13], [19].

The results showed that there was a relationship between BDNF levels and spatial memory function using the Pearson correlation test ($p = 0.008$, $r = 0, -721$, $r^2 = 0.52$). This is in accordance with Bechara *et al*. (2014) [20] in their research on Wistar rats found a relationship between BDNF levels and spatial memory function ($p = 0.0132$). In this study, rats doing exercise showed an increase in BDNF along with an increase in spatial memory function compared to the group of rats that did not exercise. This also proves that the increase in synapses in the hippocampus is influenced by BDNF levels [17], [20].

After the Pearson correlation test, it turns out that there is an also BDNF levels with hippocampal histopathological scores in rats with chronic pain ($p = 0.02$, $r = -0.597$, $r^2 = 0.357$). The higher the level of BDNF, the less damage occurred to the rat hippocampus. This is in accordance with Tayler *et al.* [21] conducted a study by administering anti-BDNF in mice. The results showed a significant relationship with a moderate correlation between BDNF and hippocampal CA1 cells. It is said that anti-BDNF inhibits CA1 cells in the rat hippocampus and affects long-term memory and short-term memory [19], [21], [22].

In this study, it was found that the relationship between hippocampal CA1 histopathological cells and spatial memory function was not significant ($p = 0.08$), this is in accordance with O'Keefe *et al.* [23] who conducted a study that spatial memory in rats is not only influenced by the hippocampus but also can be affected by the surrounding temporal lobe. There is a grid cell located in the medial entorhinal cortex which allows the rats to know the direction and area to be targeted. The entorhinal cortex receives signals from and projects back to the frontal cortex, insula, and cingulate cortex, and it is the main brain region that transmits input to the hippocampus. So the enthorinal cortex with the hippocampus can determine the location and orientation of animals [19], [22].

Limitations of the research

In this research, there was no measurement of BDNF level and rat grimace scale (RGS) assessment in the acute phase of periodontitis. This is to determine changes in BDNF and RGS levels in the pain process.

Conclusions

There was a significant difference in BDNF levels between the painless rat group and the chronic pain rat group with higher BDNF levels in the painless rat group. There was a significant difference in spatial memory function between the painless rat group and the chronic pain rat group with better spatial memory function in the painless rat group. There was also a significant difference in hippocampal histopathological scores between the painless rat group and the chronic pain rat group with a lower histopathological score in the painless rat group.

There is a significant correlation between BDNF levels and spatial memory function in Wistar rats with chronic pain. There was a significant correlation between BDNF levels and hippocampal histopathological scores in wistar rats with chronic pain group. There is no significant relationship between hippocampal histopathological scores and spatial memory function in Wistar rats with chronic pain.

References

1. Cohen M, Quintner J, van Rysewyk S. Reconsidering the international association for the study of pain definition of pain. Pain Rep. 2018;3(2):e634. https://doi.org/10.1097/PR9.0000000000000634
   PMid:29756084
2. Taylor JJ. Protein biomarkers of periodontitis in saliva. ISRN Inflamm. 2014;2014:593151. https://doi.org/10.1155/2014/593151
   PMid:24944840
3. Herrera BS, Martins-Porto R, Maia-Dantas A, Campi P, Spolidorio LC, Costa SK, et al. iNOS-derived nitric oxide stimulates osteoclast activity and alveolar bone loss in ligature-induced periodontitis in rats. J Periodontol. 2011;82(11):1608-15. https://doi.org/10.1902/jop.2011.100768
   PMid:21417589
4. Marchand S. The physiology of pain mechanisms: From the periphery to the brain. Rheum Dis Clin North Am. 2008;34(2):285-309. https://doi.org/10.1016/j.rdc.2008.04.003
   PMid:18638678
5. Miranda M, Morici JF, Zanoni MB, Bekinschtein P. Brain-Derived neurotrophic factor: A key molecule for memory in the healthy and the pathological brain. Front Cell Neurosci. 2019;13:363. https://doi.org/10.3389/fncel.2019.00363
   PMid:31440144
6. Lu B, Nagappan G, Lu Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. Handb Exp Pharmacol. 2014;220:223-50. https://doi.org/10.1007/978-3-642-45106-5_9
   PMid:24668475
7. Ionel A, Lucaciu O, Moga M, Buhatel D, Ilea A, Tabaran F, et al. Periodontal disease induced in Wistar rats-experimental study. Hum Vet Med. 2015;7(2):90-5.
8. Lee JH, Lin JD, Fong JI, Ryder MI, Ho SP. The adaptive nature of the bone-periodontal ligament-cementum complex in a ligature-induced periodontitis rat model. Biomed Res Int. 2013;2013:876316. https://doi.org/10.1155/2013/876316
   PMid:23936854
9. Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, et al. The rat grimace scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol Pain. 2011;7(1):1-10. https://doi.org/10.1186/1744-8069-7-55
   PMid:21801409
10. Vorhees CV, Williams MT. Morris water maze: Procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006;1(2):848-58. https://doi.org/10.1038/nprot.2006.116
    PMid:17406317
11. Barnhart CD, Yang D, Lein PJ. Using the Morris water maze to assess spatial learning and memory in weanling mice. PLoS One. 2015;10(4):e0124521. https://doi.org/10.1371/journal.pone.0124521
    PMid:25886563
12. Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. Malays J Med Sci. 2017;24(5):101-5. https://doi.org/10.21315/mjms2017.24.5.11
    PMid:29386977
13. Duric V, McCarson KE. Persistent pain produces stress-like alterations in hippocampal neurogenesis and gene expression. J Pain. 2006;7(8):544-5. https://doi.org/10.1016/j.jpain.2006.01.458
    PMid:16885011
14. Stefani LC, Torres IL, de Souza IC, Rozisky JR, Fregni F, Caumo W. BDNF as an effect modifier for gender effects on pain thresholds in healthy subjects. Neurosci Lett. 2012;514(1):62-6. https://doi.org/10.1016/j.neulet.2012.02.057
    PMid:22395087
15. Smith PA. BDNF: No gain without pain? Neuroscience. 2014;283:107-23. https://doi.org/10.1016/j.neuroscience.2014.05.044
    PMid:24887639
16. Saffarpour S, Shaabani M, Naghdi N, Farahmandfar M, Janzadeh A, Nasirinezhad F. In vivo evaluation of the hippocampal glutamate, GABA and the BDNF levels associated with spatial memory performance in a rodent model of neuropathic pain. Physiol Behav. 2017;175:97-103. https://doi.org/10.1016/j.physbeh.2017.03.025
    PMid:28336100
17. Jain A. Mechanisms underlying exercise-induced BDNF stimulated memory improvements in rodents. Undergrad Res Nat and Clin Sci Technol J. 2021;5:1-7.
18. Zhu W, Yao Y, Hao J, Li W, Zhang F. Short-term postoperative pain and function of unilateral biportal endoscopic discectomy versus percutaneous endoscopic lumbar discectomy for single-segment lumbar disc herniation: A systematic review and meta-analysis. Appl Bionics Biomech. 2022;2022:5360277. https://doi.org/10.1155/2022/5360277
    PMid:35465181
19. Ocampo AC, Squire LR, Clark RE. Hippocampal area CA1 and remote memory in rats. Learn Mem. 2017;24(11):563-8. https://doi.org/10.1101/lm.045781.117
    PMid:29038217
20. Bechara RG, Lyne R, Kelly ÁM. BDNF-stimulated intracellular signalling mechanisms underlie exercise-induced improvement in spatial memory in the male Wistar rat. Behav Brain Res. 2014;275:297-306. https://doi.org/10.1016/j.bbr.2013.11.015
    PMid:24269499
21. Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD. From acquisition to consolidation: On the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. Learn Mem. 2002;9(5):224-37. https://doi.org/10.1101/lm.51202
    PMid:12359832
22. Clark RE, Broadbent NJ, Squire LR. Hippocampus and remote spatial memory in rats. Hippocampus. 2005;15(2):260-72. https://doi.org/10.1002/hipo.20056
    PMid:15523608
23. O'Keefe J, Speakman A. Single unit activity in the rat hippocampus during a spatial memory task. Exp Brain Res. 1987;68:1-27. https://doi.org/10.1007/BF00255230