

SURAT KETERANGAN

Nomor: 2667/UNUSA-LPPM/Adm.I/XII/2022

Lembaga Penelitian dan Pengabdian Kepada Masyarakat (LPPM) Universitas Nahdlatul Ulama Surabaya menerangkan telah selesai melakukan pemeriksaan duplikasi dengan membandingkan artikel-artikel lain menggunakan perangkat lunak **Turnitin** pada tanggal 27 November 2022.

Judul : Expression of Brain-Derived Neurotrophic Factor in the Brain of Depressed Mice: Systematic Literature Review

Penulis : Nur Azizah, Lysa V, Hafid A, Hotimah

No. Pemeriksaan : 2022.12.09.1084

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Website : lppm.unusa.ac.id

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Expression of Brain-Derived Neurotrophic Factor in the Brain of Depressed Mice: Systematic Literature Review

by HotimahHotimah

Submission date: 27-Nov-2022 01:37PM (UTC+0700)

Submission ID: 1963932680

File name: Art._Brain_Derrived.pdf (1.12M)

Word count: 2599

Character count: 14103



Literature Review

Brain-derived neurotrophic factor after long term stress exposure of depressed mice: systematic literature review

NurAzizah AS^{1*}, Lysa Veterini², Hafid Algristian³, Hotimah Masdan Salim⁴

1,2,3,4) Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Indonesia

ARTICLE INFO

Submitted : 06st November 2020

Accepted : 01st July 2021

Published : 25st July 2021

Keywords:

Chronic depression, Stress, Brain-Derived Neurotrophic Factor, BDNF

***Correspondence:**

dr.nurazizah@unusa.ac.id

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ABSTRACT

BDNF plays an important role in the management of chronic depression. Adequate levels of BDNF trigger the formation of new synapses in the brain, thereby improving symptoms of depression, which is a mechanism known as neuroplasticity. BDNF has a central role in brain cell development due to its ability to protect brain cells from a wide variety of pathological conditions. BDNF also affects the number of glial cells and indicates a good nerve synapse function. At some point, long-term exposure to stress, which causes chronic depression, actually stops BDNF from working itself, resulting in decreased neuroplasticity of the brain. This paper aims to analyze long-term stress exposure on BDNF levels in depressed mice. This *systematic literature review* uses the PubMed and Google Scholar databases for the period 2015-2020. A total of 322 articles at the beginning of identification, and those that met the inclusion criteria in this study were six articles. Data extraction results showed that the depression condition caused by various stressors resulted in BDNF levels in the hippocampus decreased significantly by $p \leq 0.005$. Based on the literature study, the BDFN levels in the brain in depressive conditions



INTRODUCTION

Depression is a mental condition that causes individuals to experience cognitive deficits, both temporary and permanent (Jeon and Kim, 2016). Short exposure to stress can lead to acute depression where there is a reversible cognitive deficit, while long-term stress exposure can lead to chronic depression characterized by permanent cognitive deficits. Despite being declared remission from depression, some individuals still show sequelae in the form of anhedonia, and this anhedonia is closely related to permanent cognitive deficits to suicidal ideation (Winer *et al.*, 2014; Hawes *et al.*, 2018).

Several theories regarding the pathogenesis of depression are the monoamine hypothesis, neuroendocrine mechanisms, neuroimmunity, cytokines, and neuroplasticity (Jeon and Kim, 2016; Yang *et al.*, 2020). The monoamine hypothesis states that low serotonin (5-HT₂) neurotransmitters cause depression in the postsynaptic cleft. This condition is reversible, but in chronic stress, there is the pruning of nerve cell dendrites so that the depression condition becomes irreversible. Recent findings in experimental animals show that serotonergic preparations are needed to increase serotonin levels in the synaptic cleft so that it triggers the formation of new dendrites to make depression conditions reversible (Massart, Mongeau, and Lanfumey, 2012). The results differ when these preparations cause resistance in cases of chronic depression (Diaz *et al.*, 2016) and increase the risk of uncomfortable and even fatal side effects in long-term use.

Continuously low levels of serotonin (5-HT₂) in the long run can activate the HPA (Hypothalamic Pituitary Adrenal) axis, which increases glucocorticoid (cortisol) then causes Ca²⁺ influx to increase. This activation of Ca²⁺ influx stimulates

the N-methyl D-aspartate (NMDA) receptor from glutamate through the 5-HT₂ receptor so that the Brain-Derived Neurotropic Factor (BDNF) levels will decrease (Tunisy, Maria Maramis, and Kusuma, 2010). Decreased BDNF will contribute to the pruning of nerve cell dendrites which makes depression irreversible even with extensive treatment.

BDNF is a linking variable between clinical depression and serotonin levels in the brain. BDNF has a central role in brain cell development due to its ability to protect brain cells from a wide variety of pathological conditions, including depression (Stadelmann *et al.*, 2002). In addition to nerve cells, BDNF affects the number of glial cells (Sanyal *et al.*, 2013), while the increase in the number of glial cells indicates a good nerve synapse function (Verkhatsky, 2010).

METHODS

This study used a review method with the PRISMA method. A literature search was using PubMed and Google Scholar in English. According to PICO, the search criteria are based on the inclusion criteria; namely, the population is experimental animals with intervention in the form of all methods that can make the animals become depressed, the comparison is control animals, and the desired result is BDNF.

The search results with keywords (Brain-Derived Neurotropic Factor OR BDNF) AND Stress AND Chronic Depression AND Experimental Study found 322 articles. After conducting a review that met the inclusion criteria, there were six articles.

DATA EXTRACTION

Several data were extracted from the six articles included in the inclusion criteria, including research title, researcher, year, research design, research sample, intervention, random, parameters studied, and research results.



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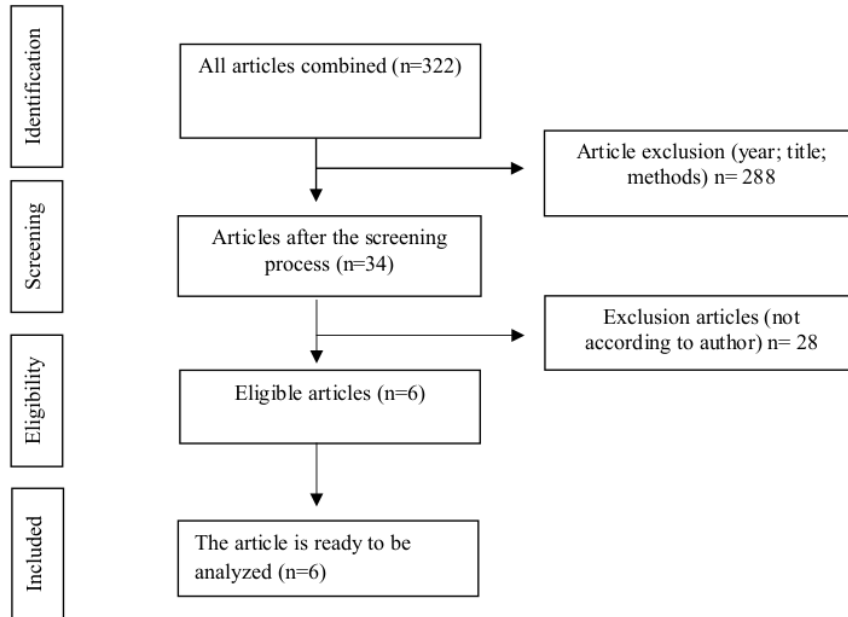


Figure 1. PRISMA *Flowchart*



Table 1. Data Extraction

No	Title	Researcher, year	Design	Sample	Intervention	Random	Parameters studied	Results
1.	Effect of combined chronic predictable and unpredictable stress on depression-like symptoms in mice	(Qiao <i>et al.</i> , 2020)	Experimental study	Male mice 8 weeks old	<ul style="list-style-type: none"> - Mice were placed in cages with wood shavings, temperature 22 ± 1 °C and artificial lighting from 7.00 am to 7.00 pm, fed laboratory standard chow, and distilled water ad libitum. - one week of mice were randomly divided into 4 groups (n = 10) = (I) normal, (II) CRS, (III) CUMS, and (IV) combined stress (CRS + CUMS) - The CRS group was placed in a tube of 8.30 until 14.30 for three weeks - CUMS mice were exposed to 7 different stressors for three weeks <ul style="list-style-type: none"> - 5 minutes of heat stress at 45 - 2 minutes cold stress at 10 - 2 minutes shaking back and forth - 24 hours 45 ° inclined cage and humid environment, - 24 hours of the shortage of food, - 24 hours of water shortages - 24 hours of reversal of day and night. 	Yes	<ul style="list-style-type: none"> - Stress stimulation with bodyweight - Stress stimulation with the level of glucocorticoid receptors (GR) - Stress stimulation with the neurotransmitter monoamine, BDNF, neuroendocrine in HPA. - Stress stimulation by oxidative stress. - Stress stimulation with gut microbiota. 	BDNF intervention in hippocampus (p <0.05)



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No	Title	Researcher, year	Design	Sample	Intervention	Random	Parameters studied	Results
2.	Decreased BDNF in female but not male rats after exposure to stress: a sex-sensitive rat model of stress?	(Weisbrod <i>et al.</i> , 2019)	Experimental study	Eighty male and female rats aged 51-55 days	<ul style="list-style-type: none"> - Each stimulation is arranged and carried out randomly three times per day. - Mice in the combined stress group were exposed to daily confinement for 6 hours (8.30-14.30) combined with unexpected mild stress from 7 different stressors for three weeks. - Mice are placed in cages with hardwood plinths, Rodent food is available (Harlan Teklad 4% Mouse / Rat Diet 7001), and water - Temperature is maintained at 23 ° C with 40% relative humidity with a 12-hour reverse light cycle (05.00-17.00 dark) - Male and female rats separated - Divided into three groups (no stress, CUMS, and SS) - CUMS, bright, fluorescent, and overhead lighting maintained for 20 minutes every day for 14 days. - On the first day, a stressor in the form of a cotton ball soaked in 10 mL of urine is placed in each rat cage in clear plastic (29x 18x12cm) for 20 minutes. - The next day the first 10 minutes of the cotton ball stressor, then the additional stressors vary (e.g., 	Yes	<ul style="list-style-type: none"> - Weight of male, female mice and treatment - BDNF control, CUMS, and SS 	<ul style="list-style-type: none"> - BDNF intervention in hippocampus <control - (p <0.001)



Next Table 1

No	Title	Researcher, year	Design	Sample	Intervention	Random	Parameters studied	Results
					<ul style="list-style-type: none"> - whistle blast, coin wobble, flashing light, and rocking cage) at 20-minute intervals - First stress on days 6 to 12 (seven consecutive days) and started again on day 14 to day 20 - SS was performed by restraining the head and immobilized rats in a ventilated Plexiglas tube. Forty electric shocks (2mA, duration 3 seconds, Floor Shocker Animal Test Case Box, Coulbourn Instruments, Holliston, MA) were delivered to the tail at random intervals of 150-210 seconds for three consecutive days on days 18 to 20 at CUMS days 12-14 - The first day the animals arrived were taken randomly and put in a cage - The second day was numbered - On the fourth day, five were allowed to move and evaluated the condition of the mice - On the 22nd day was euthanized with carbon monoxide and decapitated. Blood from the brain stem was immediately taken - The blood was then centrifuged at 4 ° and 3,600rpm for 10 min; the serum aliquots were put into an Eppendorf 			



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No	Title	Researcher, year	Design	Sample	Intervention	Random	Parameters studied	Results
3.	BDNF prevents central oxidative damage in a chronic unpredictable mild stress model: the possible role of PRDX-1 in anhedonic behavior	(Scotton <i>et al.</i> , 2019)	Experimental study	Thirty-five male Wistar mice (45 days old, 220-250g).	<p>Results checked ELISA</p> <p>Rats were housed alone in standard polycarbonate rat cages under standard environmental conditions, a 12 hour light / dark cycle (lights on between 7.00 am and 19.00 am), controlled temperature ($22 \pm 1^\circ\text{C}$), and food and water available.</p> <p>SPT After two weeks of exposure to water and 1% sucrose solution in eight basic tests, which are carried out twice a week.</p> <p>After 12 hours of lack of food and water, two bottles, one with a 1% sucrose solution and the other with water for animals for 1 hour. The bottles are weighed before and after the test to evaluate sucrose intake.</p> <p>All analyzes were performed half an hour after the start of the dark cycle. Based on the preference level of sucrose in the final baseline test, animals with an unstable and/or low basal sucrose preference (below 60%) were excluded. The remaining animals were divided into paired control (n = 7) and the CUMS group (n = 16).</p>	No data	<ul style="list-style-type: none"> - Oxidative damage - Total antioxidant capacity - BDNF Level 	BDNF Intervention in hippocampus > control (p = 0.001)



Next Table 1

No	Title	Researcher, year	Design	Sample	Intervention	Random	Parameters studied	Results
					<ul style="list-style-type: none"> - whistle blast, coin wobble, flashing light, and rocking cage) at 20-minute intervals - First stress on days 6 to 12 (seven consecutive days) and started again on day 14 to day 20 - SS was performed by restraining the head and immobilized rats in a ventilated Plexiglas tube. Forty electric shocks (2mA, duration 3 seconds, Floor Shocker Animal Test Case Box, Coulbourn Instruments, Holliston, MA) were delivered to the tail at random intervals of 150-210 seconds for three consecutive days on days 18 to 20 at CUMS days 12-14 - The first day the animals arrived were taken randomly and put in a cage - The second day was numbered - On the fourth day, five were allowed to move and evaluated the condition of the mice - On the 22nd day was euthanized with carbon monoxide and decapitated. Blood from the brain stem was immediately taken - The blood was then centrifuged at 4 ° and 3,600rpm for 10 min; the serum aliquots were put into an Eppendorf 			



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No	Title	Researcher, year	Design	Sample	Intervention	Random	Parameters studied	Results
3.	BDNF prevents central oxidative damage in a chronic unpredictable mild stress model: the possible role of PRDX-1 in anhedonic behavior	(Scotton <i>et al.</i> , 2019)	Experimental study	Thirty-five male Wistar mice (45 days old, 220-250g).	<p>tube (200 IL each) and stored in the 80 C freezer.</p> <p>Results checked ELISA</p> <ul style="list-style-type: none"> Rats were housed alone in standard polycarbonate rat cages under standard environmental conditions, a 12 hour light / dark cycle (lights on between 7.00 am and 19.00 am), controlled temperature (22 ± 1°C), and food and water available. SPT After two weeks of exposure to water and 1% sucrose solution in eight basic tests, which are carried out twice a week. <ul style="list-style-type: none"> After 12 hours of lack of food and water, two bottles, one with a 1% sucrose solution and the other with water for animals for 1 hour. The bottles are weighed before and after the test to evaluate sucrose intake. All analyzes were performed half an hour after the start of the dark cycle. Based on the preference level of sucrose in the final baseline test, animals with an unstable and/or low basal sucrose preference (below 60%) were excluded. The remaining animals were divided into paired control (n = 7) and the CUMS group (n = 16). 	No data	<ul style="list-style-type: none"> Oxidative damage Total antioxidant capacity BDNF Level 	BDNF Intervention in hippocampus> control (p = 0.001)



Next Table 1

No	Title	Researcher, year	Design	Sample	Intervention	Random	Parameters studied	Results
5.	The effects of chronic and acute physical and psychological Stress on Brain-Derived Neurotrophic Factor in Rats	(Ghooschki and Jahromi, 2018)	Experimental study	Ninety 3-month-old male Wistar rats weight 200 ± 40 gram	<p>phosphate buffer then 4% paraformaldehyde.</p> <p>The brain is removed, saturated with 30% sucrose, and frozen in isopentane. Sixteen µm thick sections were cut using a cryostat. This section evaluates BDNF and TPH proteins in ventral DRN both throughout the structure and in 5-HT cells located within the DRN (30 cells/part) using conventional immunofluorescence methods.</p>	Yes	Shows BDNF exercise emotional stress physical stress exercise and emotional stress	BDNF: chronic emotional stress intervention in hippocampus <control (p = 0.002)
					<p>Indoor rats with temperature 22 ± 1°C, light (from 07.00 to 19.00). Divided into six groups (each group of 8 rats): exercise (EX), emotional (ES), physical stress (PS), exercise combined with emotional stress (EXES), exercise combined with physical stress (EXPS), and control. EX mice with a treadmill for 1-2 sessions for acute and two weeks for chronic.</p> <p>In the first week, all rats were on the treadmill for 10 min. Each mouse ran for 23 minutes on the treadmill at low speed, then increased it to 5 m / min every 3 minutes until the rats were exhausted (unable to continue running).</p>			



Next Table 1

No	Title	Researcher, year	Design	Sample	Intervention	Random	Parameters studied	Results
					<ul style="list-style-type: none"> - The second day, all rats were given EX 50 minutes/day 5 days/week with an intensity of 60-75% maximum oxygen uptake. Each EX session begins with a warm-up for 10 minutes (gradual increase in speed) followed by 30 minutes of EX with 60-75% maximum oxygen intensity and 10 min speed reduced for cooling. - EX at 09.00 - 12.00 - The physical stress group of mice received 0.5 mA, 1-second leg sting every 30 seconds for 10 minutes, five times a week for one minute. - Emotional stress rats only looked at physical stress rats. - A blood sample (2 ml) was taken from the ventral caudal artery of light etherized mice immediately after the first treatment and 12 hours after the last treatment (after two weeks). Blood samples were centrifuged for 10 minutes (3500 rounds) and stored at -20 C. BDNF levels were determined using ELISA 	Yes	<ul style="list-style-type: none"> - Calorie Intake - Weight Loss - SPT - BDNF 	BDNF chronic stress intervention in hippocampus
6.	Chronic stress associated with a	(Macedo et al., 2015)	Experimental study	Thirty-two male Wistar rats, 60	Rats were placed in cages made of polypropylene and measuring 49 x 34 x 16 cm. on a 12 hour light / dark cycle (lights turn on at 7.00			



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Next Table 1

No	Title	Researcher, year	Design	Sample	Intervention	Random	Parameters studied	Results
	hypercaloric diet changes the hippocampal BDNF levels in male Wistar rats			days old and weighing 200–250 g	<ul style="list-style-type: none"> a.m. and turn off at 19.00), with a controlled temperature of $22 \pm 2^\circ\text{C}$ In 1 cage, there are four rats, after one week divided into four groups, namely control (C), hypercaloric diet (HD), a standard diet with restraint stress (S), a hypercaloric diet with restraint stress (SHD), Restraint stress using a plastic tube measuring 25×7 cm with adhesive tape outside one end of the open tube. The mice were exposed to stress for 1 hour each day in the morning (between 9 am and 12 pm), then returned to their cages. They performed 5 days a week for 12 weeks. Animals are weighed weekly, and their food intake is recorded every day. At 24 hours after the end of the stress session and after 12 hours of fasting, the rats were decapitated, and tissue samples were taken, frozen at -70°C. 			<control (p = 0,05)
					BDNF analysis by ELISA			



RESULTS AND DISCUSSION

Based on the six selected articles, all of them used experimental designs. The samples were mice whose ages were not the same; two articles showed the mice were three months old, while the others were eight weeks, 51-55 days, 45 days, and 60 days. In the division of the treatment and control groups in four articles using random and two articles, there is no information about how the groups are divided.

Almost all of the lighting obtained in the study used artificial light. Only the study of Shishkina *et al.* (2018) uses natural lighting. Lighting is made from 07.00 to 19.00, but in the research of Weisbrod *et al.* (2019), lighting applies 12 hours backward, which is dark at 05.00-17.00. The lighting in this study is almost all the same, namely bright conditions in the morning. The intervention was given in the morning, where the time in the morning is the time to rest the mice because they are nocturnal animals. This condition creates stressors for mice in addition to the stressors given. In the study of Weisbrod *et al.* (2019), the lighting is given a different time, namely, at night; this is to show the disruption of circadian rhythms as well as being a stressor.

Various kinds of intervention methods were used to get stressed mice. Some studies have the same form of treatment and are added to other forms of treatment. The intervention method is that rats are placed in a tube, given stress stimuli in the form of heat stress, cold stress, shaken back and forth, tilted cage and humid environment, lack of food and water, hypercaloric diet, day and night reversal, giving cotton balls soaked in urine are placed in the cage, the whistle blew, the lights blinked, the rats restrain their heads and bodies immobilized in the tube, electric shocks, pinching the tails, giving sucrose solutions, swimming, treadmill, and only seeing the group of mice that were treated. The intervention method

used in each study was different; there was a single intervention or a combination of other interventions. This can represent a picture of stressors that occur both mild, moderate, and severe (Weisbrod *et al.*, 2019). Mental stress can lead to depression (Qiao *et al.*, 2020).

The duration of treatment in each study was almost the same, namely for two weeks except in Scotton *et al.* (2019) in mice with CUMS for six weeks, and in the Ghooshchi and Jahromi (2018) study, there was an EX (exercise) group who received 1-2 sessions of recording to show acute conditions and two weeks for chronic. The duration of the intervention showed the duration of the stressor given to the mice, and almost all studies lasted two weeks which could indicate chronic conditions. A meta-analysis showed that the sensitivity of mice varied between species, with the Wistar mouse species showing a more optimal distress response at exposure at the third week, while at exposure beyond that time, these mice tended to be non-responsive, which would have been expected there is an adaptation or even fatigue (Antoniuk *et al.*, 2019).

In this study, besides measuring the ratio of BDNF levels in stressed mice to control mice, it also measured stress by weight, stress with receptor levels glucocorticoids (GR), and stress with monoamine neurotransmitters, neuroendocrine in HPA, stress with oxidative stress, stress with gut macrobiotic, antioxidant capacity, tryptophan hydroxylase levels, and calorie intake.

From the study results, five studies were showing a significant reduction in BDNF levels in stressed mice compared to controls, and one study showed an increase in BDNF in stressed mice, namely Scotton *et al.* (2019) ($p = 0.001$). Research by Weisbrod *et al.* (2019) showed that the BDNF results in male and female rats with CUMS stressors were higher than controls, but with SS stress, BDNF levels in female rats decreased significantly ($p < 0.001$).



The study examined in this article demonstrated decreased BDNF levels in stressed mice, although not all studies have shown decreased BDNF results. In chronically stressed mice, there was a decrease in BDNF levels because chronic stress would affect the HPA axis, so that high glucocorticoid levels caused Ca²⁺ influx to increase. This activation Ca²⁺ influx stimulates the NMDA receptor from glutamate through the 5-HT₂ receptor to decrease the BDNF levels (Tunisya, Maria Maramis, and Kusuma, 2010). The research of Scotton et al. (2019) obtained hypertrophy of the adrenal glands due to stress which should have increased glucocorticoid levels and decreased BDNF, but in fact, there was an increase in BDNF, which was probably a compensatory response to maintaining hippocampal homeostasis.

CONCLUSION

Chronic stressors persistently affect the HPA axis which can lead to decreased BDNF levels in the hippocampus.

ACKNOWLEDGEMENT

We would like to thank LPPM UNUSA and RISTEKBRIN for providing the opportunity to carry out research.

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