REVISTA NEUROCIÊNCIAS

Increased levels of brain-derived neurotrophic factor in cultures of nervous cells treated with a selective serotonin reuptake inhibitor

Níveis aumentados do fator neurotrófico derivado do cérebro em culturas de células nervosas tratadas com um inibidor seletivo da recaptação de serotonina

Niveles aumentados del factor neurotrófico derivado del cerebro en cultivos de células nerviosas tratadas con un inhibidor selectivo de la recaptación de serotonina

Hotair Phellipe Martins Fernandes[1], Sara Domingues Soares e Silva[1], Adriano Junio Moreira de Souza[2]

1.Acadêmico(a) do curso de Medicina, Centro Universitário Tocantinense Presidente Antônio Carlos (UNITPAC). Araguaína-TO, Brazil.
2.Professor, Doutor, Universidade Federal do Tocantins (UFT). Miracema-TO, Brazil.

Resumo

Introdução. Os efeitos de antidepressivos como a fluoxetina na plasticidade cerebral estão associados ao aumento da transcrição e sinalização de fatores tróficos como o fator neurotrófico derivado do cérebro (BDNF), o que aumenta os processos sinápticos e neurogênicos. **Objetivo.** Avaliar se o tratamento com fluoxetina poderia aumentar os níveis de BDNF em células da retina. **Método.** Para conduzir o experimento, as retinas foram dissecadas de ratos neonatos e mantidas em meio de cultura. Em seguida, as culturas designadas para o grupo experimental foram tratadas com 1μM de fluoxetina e mantidas por 2, 12, 24, 48 ou 72 horas in vitro. As culturas designadas para o grupo controle foram submetidas ao mesmo protocolo, porém não receberam o tratamento com fluoxetina. Os níveis de BDNF foram determinados por análise de Western blot e os dados foram analisados pelo teste t de *Student* para comparar os dois grupos (controle e experimental). **Resultados.** Os resultados mostraram que as culturas tratadas com fluoxetina por 12, 24 e 48 horas tiveram níveis significantemente mais elevados de BDNF do que o grupo controle. **Conclusão.** Os achados sugerem que o tratamento com fluoxetina pode ter efeitos terapêuticos potenciais em células da retina, aumentando os níveis de BDNF. Isso acrescenta à evidência existente sobre o papel do BDNF na mediação dos efeitos terapêuticos de antidepressivos e aponta o potencial para pesquisas adicionais sobre o uso de antidepressivos no tratamento de doenças retinianas.
Unitermos. Fator Neurotrófico Derivado do Encéfalo; Fluoxetina; Células da Retina; Técnicas de Cultura de Células; Western Blotting; Agentes Antidepressivos

Abstract

Introduction. The effects of antidepressants such as fluoxetine on brain plasticity are associated with increased transcription and signaling of trophic factors such as brain-derived neurotrophic factor (BDNF), which enhances synaptic and neurogenic processes. **Objective.** To evaluate whether treatment with fluoxetine could increase BDNF levels in retinal cells. **Method.** To conduct the experiment, retinas were dissected from neonatal rats and cultured in complete culture medium. Next, the cultures designated for the experimental group were treated with 1μM of fluoxetine and maintained for 2, 12, 24, 48, or 72 hours in vitro. The cultures designated for the control group underwent the same protocol, but did not receive the fluoxetine treatment. The levels of BDNF were determined by Western blot analysis, and the data were analyzed by Student's t-test to compare the two groups (control and experimental). **Results.** The results showed that cultures treated with fluoxetine for 12, 24, and 48 hours had

significantly higher levels of BDNF than the control group. **Conclusion.** The findings suggest that treatment with fluoxetine may have potential therapeutic effects on retinal cells by increasing BDNF levels. This adds to the existing evidence on the role of BDNF in mediating the therapeutic effects of antidepressants and highlights the potential for further research on the use of antidepressants in the treatment of retinal diseases.
Keywords. Brain-Derived Neurotrophic Factor; Fluoxetine; Retinal Cells; Cell Culture Techniques; Western Blotting; Antidepressive Agents

Resumen

Introducción. Los efectos de los antidepresivos como la fluoxetina en la plasticidad cerebral están asociados con el aumento de la transcripción y señalización de factores tróficos como el factor neurotrófico derivado del cerebro (BDNF), lo que aumenta los procesos sinápticos y neurogénicos. **Objetivo.** El objetivo del estudio fue evaluar si el tratamiento con fluoxetina podría aumentar los niveles de BDNF en células de la retina. **Método.** Para llevar a cabo el experimento, las retinas fueron diseccionadas de ratones neonatos y cultivadas en medio de cultivo completo. A continuación, las culturas designadas para el grupo experimental fueron tratadas con 1μM de fluoxetina y mantenidas por 2, 12, 24, 48 o 72 horas in vitro. Las culturas designadas para el grupo control fueron sometidas al mismo protocolo, pero no recibieron el tratamiento con fluoxetina. Los niveles de BDNF fueron determinados por análisis de Western blot y los datos fueron analizados mediante la prueba t de Student para comparar los dos grupos (control y experimental). **Resultados.** Los resultados mostraron que los cultivos tratados con fluoxetina durante 12, 24 y 48 horas tuvieron niveles significativamente más altos de BDNF que el grupo control. **Conclusión.** Los hallazgos sugieren que el tratamiento con fluoxetina puede tener efectos terapéuticos potenciales en células de la retina, aumentando los niveles de BDNF. Esto se suma a la evidencia existente sobre el papel del BDNF en la mediación de los efectos terapéuticos de los antidepresivos y destaca el potencial para investigaciones adicionales sobre el uso de antidepresivos en el tratamiento de enfermedades retinianas.
Palabras clave. Factor Neurotrófico Derivado del Encéfalo; Fluoxetina; Células de la Retina; Técnicas de Cultivo de Células; Western Blotting; Agentes Antidepresivos

Research developed at Centro Universitário Tocantinense Presidente Antônio Carlos (UNITPAC). Araguaína-TO, Brazil.

Conflict of interest: no Received in: 02/25/2023 Accepted in: 05/17/2023

Corresponding address: Adriano JM Souza. R. São Salvador 85. Setor Oeste. Araguaína-TO, Brazil. CEP 77816-220. E-mail: junio.adrano@hotmail.com

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family. In addition to BDNF, the family of neurotrophins includes nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). BDNF was been implicated in various processes related to the growth and maintenance of neurons and synapses. It has been established that BDNF has an essential role in axonal guidance during development; BDNF can also promote the

growth of dendrites and dendritic spines, contributing to the long-term potentiation of synaptic strength[1].

BDNF transcription can be modulated by neuronal activity through the influx of Ca2+. In neurons, Ca2+ influx triggers phosphorylation of Cyclic AMP Response Element Binding Protein (CREB), which by binding to a critical Ca2+ response element (CRE) within the BDNF gene activates BDNF transcription[2]. Initially, BDNF is synthesized in its pro-form with 32 kiloDalton (kDa); in the extracellular space, pro-BDNF is proteolytically processed into its mature form of about 14 kDa[3]. The mature BDNF isoform binds with the high-affinity TrkB receptor[1]. Phosphorylated-TrkB activates several intracellular pathways: phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), phospholipase C-γ (PLC-γ), and guanosine triphosphate hydrolases (GTPases) of the Ras homolog (Rho) gene family. These activate signaling cascades with determined cellular roles such as the PI3K/Akt-related pathway, which exerts important antiapoptotic and prosurvival activity, the MAPK/Ras signaling cascade that regulates protein synthesis during neuronal differentiation, and the PLC-γ signaling that enhances synapse plasticity[4]. Compared to other neurotrophin family members, BDNF can regulate a death domain through a lower affinity for the pan-neurotrophin receptor p75NTR. As evidenced by studies, BDNF plays a critical role in diverse neuronal mechanisms[5,6].

BDNF has been implicated in various psychiatric disorders, such as schizophrenia, intellectual disability,

autism, and mood disorders[7-9]. Recent studies have highlighted the possibility that major depression is characterized by a reduction in overall brain plasticity and an increased susceptibility to stressful situations. These dysfunctions may result from reduced expression and function of key proteins involved in neuroplasticity, including BDNF. It has been hypothesized that the therapeutic effects of some antidepressants may be mediated by increasing the expression and signaling of BDNF[10]. There is ample evidence to suggest that BDNF plays a profound role in neural plasticity processes, and this plasticity has been linked to the therapeutic effects of antidepressants such as fluoxetine.

Fluoxetine, a potent and selective inhibitor of neuronal 5-HT reuptake (SSRIs), is commonly used to treat mood disorders. Fluoxetine administration has been shown to selectively upregulate BDNF mRNA levels within mesocorticolimbic pathway structures[11,12]. In particular, chronic treatment with fluoxetine leads to a significant increase in BDNF gene expression in the limbic and dopaminergic regions, including the ventral tegmental area, prefrontal cortex, and nucleus accumbens[11,12]. This upregulation of BDNF suggests that the function of the dopaminergic system in individuals with depression may be improved.

In addition to being present in limbic areas and dopaminergic pathways, serotoninergic and dopaminergic markers have also been described in mammalian retinas[13]. The retina, which belongs to the central nervous system

(CNS), is situated on the inner and posterior surface of the eyeball and is a layered structure comprising cell bodies and synapses[14]. Several neurotransmitters and cell signaling systems, previously identified in other CNS regions, are also found in the retina[14]. The retinal maturation process is completed postnatally, with dopamine neurons appearing early in development and becoming functional before the onset of vision[14]. In the retina, dopaminergic cell bodies are located among the layer of amacrine cells, at the border of the inner nuclear (INL) and inner plexiform layers (IPL)[15]. 5-HT has also been reported to be a neuroactive biogenic amine in the retina[13], with its activity shown to elevate BDNF transcription. Moreover, BDNF has been identified as trophic for dopaminergic neurons[16]. Although there are several data indicating the involvement of 5-HT in retinal physiology and pathophysiology[13], the signaling pathways activated by the binding of 5-HT receptors have been poorly investigated. These pathways, as in other central regions, may be related to an increase in BDNF.

Based on the aforementioned data, the aim of this study was to investigate the effect of fluoxetine on the modulation of BDNF levels in developing retinal cells. To this end, cultures of retinal cells from newborn rats were treated with fluoxetine, and BDNF levels were assessed at different time intervals to determine the optimal treatment duration for BDNF modulation. The results of this study may contribute to a better understanding of the potential use of fluoxetine on retinal cells.

METHOD

Ethical Aspects

The experimental procedures involving animals were approved by the Local Animal Care and Use Committee at the Fluminense Federal University, under the project numbers CEPA #00196-10. All possible efforts were made to ensure the well-being of the animals and to minimize the number of animals used and any suffering they may have experienced.

Experimental Procedures

Tissue Dissection and Culture Preparation

Neonatal rats at postnatal day one were euthanized via decapitation, and their retinas were extracted free from scleral tissue and pigmented epithelium in a calcium- and magnesium-free balanced salt solution (CMF) containing 100 ng/mL streptomycin and 100 U/mL penicillin. The tissue was then incubated in CMF containing 0.1% trypsin for approximately 16 min at 37°C, and trypsin activity was halted by adding culture medium (199 medium supplemented with 2 mM glutamine, 100 ng/mL streptomycin, and 100 U/mL penicillin) with 5% fetal calf serum (FCS). The tissue was subsequently resuspended in a complete culture medium and mechanically dissociated using a polished Pasteur pipette. Cells were then added to Petri dishes previously treated with poly-L-ornithine (50 µg/mL) at a plating density of 10^5 cells/cm^2. After plating, cultures were incubated in 1 mL of culture medium for 2-4

hours to allow cells to adhere to the substrate. Then, either 1 mL of culture médium (control group) or 1 mL of medium containing drugs (2 μM of fluoxetine) (experimental group) was added to each Petri dish. In our experimental procedure, drug treatment was maintained over the entire duration of the culture. Drugs were added only once, after 2-4 hours in culture. The cultures were then maintained for different time intervals (2, 12, 24, 48, or 72 hours) in vitro at 37°C in a humidified atmosphere of 5% CO2 and 95% air. For each time interval (2, 12, 24, 48, or 72 hours), samples were collected for Western blot analysis. A total of ten (10) animals were used in the experiments, 2 at each time interval. All efforts were made to minimize the number of experimental animals used and their suffering, and procedures using animals were approved by the local animal care and experimentation committee at Fluminense Federal University (CEPA projects #00196-10).

Materials

Medium 199 fetal bovine serum was purchased from GIBCO (Gaithersburg—USA). Glutamine, streptomycin, penicillin, poly-L-ornithine were purchased from Sigma–Aldrich (St. Louis—USA). Glutaraldehyde was purchased from Mallinckrodt Baker (Phillipsburg—USA). BDNF was purchased from PeproTech (Rocky Hill, NJ, USA). Trypsin was purchased from Worthington (USA). The secondary antibodies used came from Santa Cruz Biotechnology (USA).

Western Blot analysis

BDNF levels were determined through Western blot analysis. To achieve this, retinal cell cultures were lysed in lysis buffer containing 2% sodium dodecyl sulfate and 0.5 M Tris pH 6.8. Following determination of protein concentration using the Bradford method (Bradford, 1976), samples were loaded onto a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (15%) and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were then incubated overnight with rabbit anti-BDNF antibody at a dilution of 1:200. After washing in TBS, the membranes were exposed to horseradish peroxidase-conjugated secondary anti-rabbit antibodies at a dilution of 1:20,000 for 60 minutes at room temperature. Detection was performed on X-ray film using an enhanced chemiluminescence kit (ECL kit). The density of protein bands was analyzed through densitometry with Scion Image, and the mean value for the control was set at 100%.

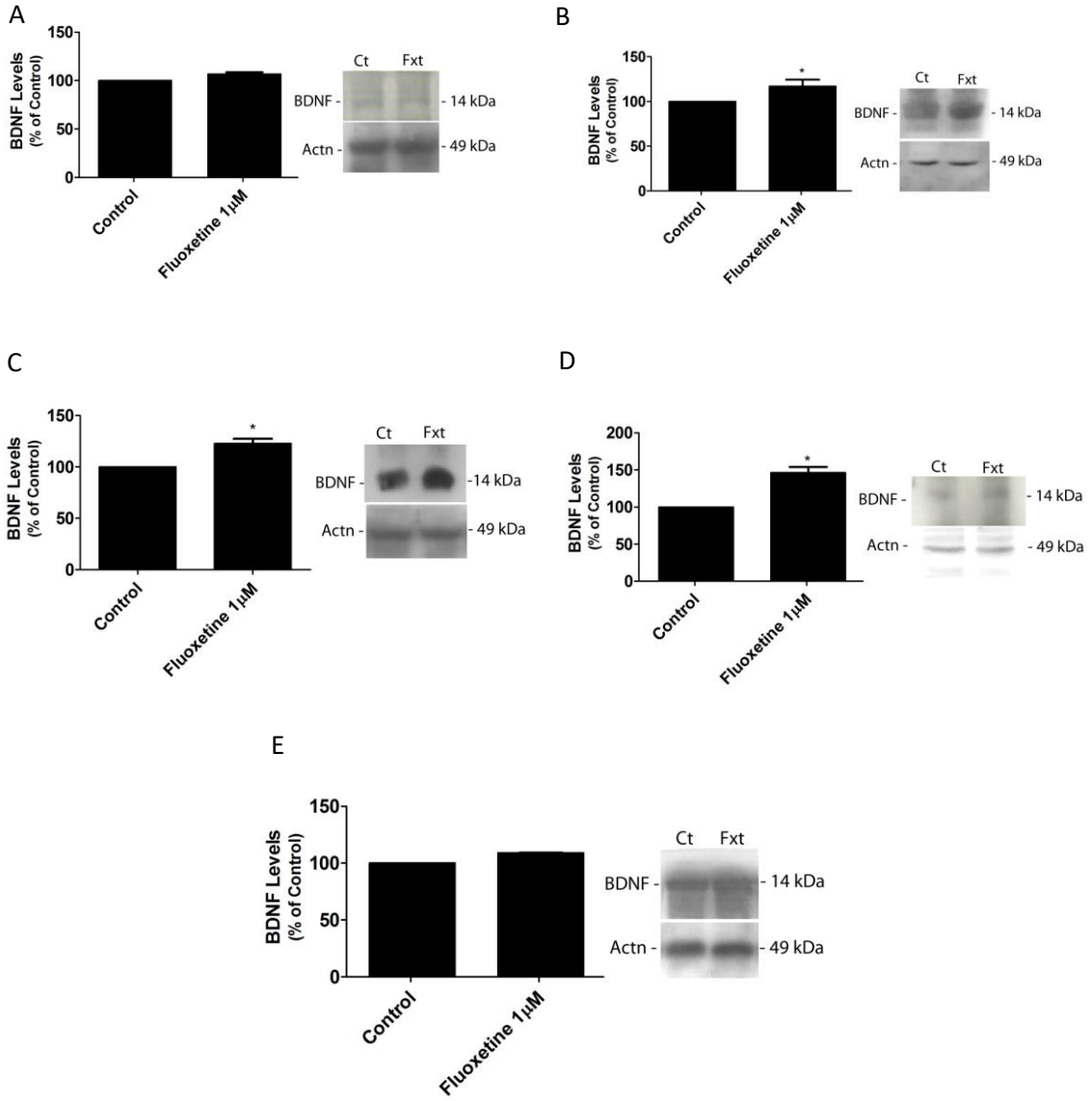
Statistical Analysis

Statistical analysis was performed by means of Student's t-test for unpaired data and expressed as mean±SEM. P values below 0.05 were considered statistically significant. The software GraphPad Prism version 5.01 was used for all analyses.

RESULTS

In this study, we investigated the effect of one (1) μM fluoxetine on BDNF levels in retinal cell cultures. Our results showed that there were no significant differences in BDNF levels between cultures treated with fluoxetine for 2 hours and the control ($n=3$, $p<0.2500$, $6.8\pm3.102\%$), as depicted in Figure 1A. However, we observed a significant increase in BDNF levels ($n=3$, $p<0.0186$, $11\pm1.510\%$) in cultures treated with fluoxetine for 12 hours (Figure 1B). This increase was maintained in cultures treated with fluoxetine for 24 hours ($n=4$, $p<0.0167$, $22.7\pm4.676\%$), as shown in Figure 1C, and even after 48 hours of treatment ($n=4$, $p<0.0092$, $46.2\pm7.697\%$), as illustrated in Figure 1D. However, when the cultures were maintained for 72 hours, we did not observe significant differences in BDNF levels ($n=3$, $p<0.2500$, $8.7\pm0.3215\%$) between fluoxetine-treated cultures and the control (Figure 1E). Therefore, our findings suggest that one μM fluoxetine can increase BDNF levels in retinal cell cultures, and this effect is maintained for up to 48 hours but not beyond that time point.

Figure 1. Depicts the levels of BDNF in retinal cell cultures treated with fluoxetine. **A**. shows that after treatment with fluoxetine for 2 hours. **B**. indicates that treatment with fluoxetine for 12 hours. **C**. shows that treatment with fluoxetine for 24 hours. **D**. demonstrates that treatment with fluoxetine for 48 hours. **E**. demonstrates that treatment with fluoxetine for 72 hours. In all figures, actin was used as a loading protein. Data are reported as the mean±SEM.



DISCUSSION

This study discovered elevated levels of brain-derived neurotrophic factor (BDNF) in cultures of newborn rat retinal cells treated with fluoxetine, a selective 5-HT reuptake inhibitor (SSRI). The observed increse on BDNF levels in our cultures, maintained for 12 (increse of 11%), 24 (increse of 22.7%), and 48 (increse of 46.2%) hours, may be attributed

to the increased extracellular serotonin induced by fluoxetine. On postnatal day 2 (the age of the retinas used in our study), various retinal cells exited the mitotic cycle, including retinal ganglion cells (RGCs), rods, cones, and amacrine cells (30). Serotonin (5-HT) is synthesized and released into the retina at this stage, where it performs neuromodulatory functions. The retina expresses several types of serotonin receptors, including the 5-HT1A receptor (5-HT1AR), which has been detected in photoreceptors, bipolar, amacrine, and ganglion cells in rats[13]. In developing tissues, activation of 5-HT1AR and TrK-B (the high-affinity BDNF receptor) is associated with serotonergic differentiation[17]. In embryonic raphe cultures, 5-HT1A receptor agonists increase BDNF mRNA levels[18]. In our cultures, these receptors may be more active due to the increase in extracellular serotonin induced by fluoxetine. This increase in neural activity may explain the upregulation of BDNF levels in retinal cells. The effect of fluoxetine on BDNF may be related to numerous physiological effects on retinal tissue.

In the retina, several developmental processes depend on tissue electrical activity. To investigate the consequences of reduced electrical activity, the effect of electrical blocking with TTX (to block sodium channels and action potentials) or low Ca/high Mg (to secure transmitter release and synaptic activity) in individual neurons in vitro was studied[19]. In animal cell cultures, RGCs from postnatal days 2-10 treated with 1 μM TTX or 0.2 mM Ca/20 mM Mg resulted in the death

of approximately 50% of RGCs, which exhibited spontaneous electrical activity, but did not affect inactive RGCs[19]. These data indicate that the electrical activity of developing tissue affects cell survival. On the other hand, increased electrical activity in the developing retina induces survival and growth[20]. This survival and growth were related to an increase in BDNF expression. Likewise, the increase in BDNF levels observed in our cultures may be related to increased electrical activity induced by serotonergic signaling.

We also observed that the increase in BDNF levels in vitro occurs between 2 and 12 hours of treatment with fluoxetine. This data indicates that BDNF transcription occurs after two hours of in vitro treatment with fluoxetine. In other central regions, it has been observed (in vivo) that injection of fluoxetine twice daily for 14 days leads to a significant increase in BDNF levels in the dentate gyrus of the hippocampus 24 hours after the last injection[21]. It has also been reported that a single administration of the selective 5-HT6 agonist LY-586713 (1mg/kg, s.c.) increases BDNF expression in regions of the hippocampus within 24 hours[22]. In addition, fluoxetine treatment has been described to exert effects on BDNF levels via 5-HT1AR[21]. This indicates that an increase in serotonergic activity can elevate BDNF levels, which is time-dependent.

In our experiments, we observed that after 72 hours, there were no significant differences in BDNF levels between control and fluoxetine-treated cultures. This result may be related to the cell death process in cultures. The ganglion

cells are axotomized when the animal's eye is dissected. After losing the axon, the cells trigger a death program, resulting in a short survival period[23]. However, increased BDNF levels are a condition that can prolong the survival of axotomized cells[24]. Future work can be carried out to analyze whether treated cultures show a superior survival profile.

The increase in BDNF levels that we observed in our cultures may agree with many works describing the effects of antidepressants on growth, plasticity, and neural survival. Fluoxetine increases BDNF expression, which is decisive for improving depressive conditions[25]. In vivo studies have shown that treatment with fluoxetine for seven consecutive days is related to increased mRNA levels for BDNF in the CA3 region of the hippocampus and the dentate gyrus of the hippocampus of rats[26]. Increased BDNF levels in these regions have been correlated with increased hippocampal plasticity[27]. Administration of the antidepressant fluoxetine in female rats for five consecutive days has been described to induce a robust increase in synaptic density in pyramidal cells in the CA1 region of the hippocampus, with similar changes appearing in the CA3 area after two weeks of treatment[27]. Other researchers have reported similar results.

The effects described in central regions by fluoxetine treatment were related to the increase in BDNF transcription and signaling[25]. Many studies indicate that the antidepressant effects of treatment with drugs such as fluoxetine are mediated by increased neuronal plasticity. Added to this, it has been observed that with the

administration of a TrkB antagonist, the antidepressant effects of drugs such as fluoxetine are nullified[28].

Assuming this evidence, we can suggest that, similar to what occurs in other central regions, treatment with SSRIs can also increase BDNF levels in retinal cells. In an animal model of chronic diabetic retinopathy, fluoxetine had an anti-apoptotic effect with upregulation of BDNF expression in the retina of rats with STZ-induced diabetes[29]. Another study indicates that chronic administration of fluoxetine restores the plasticity of ocular dominance in adulthood and promotes the recovery of visual functions in adult amblyopic animals, tested electrophysiologically and behaviorally[30]. These effects were accompanied by reduced intracortical inhibition and increased expression of BDNF in the visual cortex.

CONCLUSION

In conclusion, this study found that treatment with fluoxetine, a selective serotonin reuptake inhibitor (SSRI), increased levels of BDNF in cultures of newborn rat retina cells. The increase in BDNF levels may be related to the increase in extracellular serotonin induced by fluoxetine, which can activate 5-HT1AR and TrK-B receptors and lead to an increase in neural activity. The increase in BDNF levels observed in vitro may be related to the increase in electrical activity induced by the serotonergic signal. Furthermore, the increase in BDNF levels may prolong the survival of axotomized cells, indicating a potential therapeutic effect of fluoxetine. These findings suggest that, similar to what

occurs in other central regions, treatment with SSRIs may also increase BDNF levels in retina cells, which is crucial for improving depressive conditions and increasing neuronal plasticity. Future studies may explore the potential therapeutic benefits of fluoxetine in retinal diseases.

REFERENCES

1.Skaper SD. The neurotrophin family of neurotrophic factors: an overview. Neurotrophic factors: Springer; 2012; p.1-12. https://doi.org/10.1007/978-1-61779-536-7_1

2.Yan X, Liu J, Zhang Z, Li W, Sun S, Zhao J, *et al*. Low-level laser irradiation modulates brain-derived neurotrophic factor mRNA transcription through calcium-dependent activation of the ERK/CREB pathway. Lasers Med Sci 2017;32:169-80. https://doi.org/10.1007/s10103-016-2099-0

3.Yang J, Siao C-J, Nagappan G, Marinic T, Jing D, McGrath K, *et al*. Neuronal release of proBDNF. Nature Neurosci 2009;12:113. https://doi.org/10.1038/nn.2244

4.Kowiański P, Lietzau G, Czuba E, Waśkow M, Steliga A, Moryś J. BDNF: a key factor with multipotent impact on brain signaling and synaptic plasticity. Cell Mol Neurobiol 2018;38:579-93. https://doi.org/10.1007/s10571-017-0510-4

5.Woo NH, Teng HK, Siao C-J, Chiaruttini C, Pang PT, Milner TA, *et al*. Activation of p75 NTR by proBDNF facilitates hippocampal long-term depression. Nature Neurosci 2005;8:1069. https://doi.org/10.1038/nn1510

6.Greene LA, Kaplan DR. Early events in neurotrophin signalling via Trk and p75 receptors. Curr Opin Neurobiol 1995;5:579-87. https://doi.org/10.1016/0959-4388(95)80062-X

7.Nagahara AH, Tuszynski MH. Potential therapeutic uses of BDNF in neurological and psychiatric disorders. Nature Rev Drug Discovery 2011;10:209. https://doi.org/10.1038/nrd3366

8.Ikegame T, Bundo M, Murata Y, Kasai K, Kato T, Iwamoto K. DNA methylation of the BDNF gene and its relevance to psychiatric disorders. J Hum Gen 2013;58:434. https://doi.org/10.1038/jhg.2013.65

9.Nishimura K, Nakamura K, Anitha A, Yamada K, Tsujii M, Iwayama Y, *et al*. Genetic analyses of the brain-derived neurotrophic factor (BDNF) gene in autism. Biochem Biophys Res Comm 2007;356:200-6. https://doi.org/10.1016/j.bbrc.2007.02.135

10.Björkholm C, Monteggia LM. BDNF–a key transducer of antidepressant effects. Neuropharmacology 2016;102:72-9. https://doi.org/10.1016/j.neuropharm.2015.10.034

11.Molteni R, Calabrese F, Bedogni F, Tongiorgi E, Fumagalli F, Racagni G, *et al*. Chronic treatment with fluoxetine up-regulates cellular BDNF mRNA expression in rat dopaminergic regions. Inter J Neuropsychopharmacol 2006;9:307-17. https://doi.org/10.1017/S1461145705005766
12.Mayberg HS, Brannan SK, Tekell JL, Silva JA, Mahurin RK, McGinnis S, *et al*. Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. Biolog Psychiatr 2000;48:830-43. https://doi.org/10.1016/S0006-3223(00)01036-2
13.Masson J. Serotonin in retina. Biochimie 2019;161:51-5. https://doi.org/10.1016/S0006-3223(00)01036-2
14.Braekevelt CR, Hollenberg MJ. The development of the retina of the albino rat. Am J Anat 1970;127:281-301. https://doi.org/10.1002/aja.1001270305
15.Witkovsky P. Dopamine and retinal function. Doc ophthalmol 2004;108:17-39. https://doi.org/10.1023/B:DOOP.0000019487.88486.0a
16.Baquet ZC, Bickford PC, Jones KR. Brain-derived neurotrophic factor is required for the establishment of the proper number of dopaminergic neurons in the substantia nigra pars compacta. J Neurosci 2005;25:6251-9. https://doi.org/10.1523/JNEUROSCI.4601-04.2005
17.Galter D, Unsicker K. Sequential activation of the 5-HT1A serotonin receptor and TrkB induces the serotonergic neuronal phenotype. Mol Cell Neurosci 2000;15:446-55. https://doi.org/10.1006/mcne.2000.0841
18.Schreiber R, Newman-Tancredi A. Improving cognition in schizophrenia with antipsychotics that elicit neurogenesis through 5-HT1A receptor activation. Neurobiol Learning Mem 2014;110:72-80. https://doi.org/10.1016/j.nlm.2013.12.015
19.Lipton SA. Blockade of electrical activity promotes the death of mammalian retinal ganglion cells in culture. Proc Nat Acad Sci 1986;83:9774-8. https://doi.org/10.1073/pnas.83.24.977
20.Galli-Resta L, Ensini M, Fusco E, Gravina A, Margheritti B. Afferent spontaneous electrical activity promotes the survival of target cells in the developing retinotectal system of the rat. J Neurosci 1993;13:243-50. https://doi.org/10.1523/JNEUROSCI.13-01-00243.1993
21.Zetterström TS, Coppell AA, Khundakar AA. The role of 5-hydroxytryptamine receptor subtypes in the regulation of brain-derived neurotrophic factor gene expression. J Pharm Pharmacol 2014;66:53-61. https://doi.org/10.1111/jphp.12153
22.De Foubert G, Carney S, Robinson C, Destexhe E, Tomlinson R, Hicks C, *et al*. Fluoxetine-induced change in rat brain expression of brain-derived neurotrophic factor varies depending on length of treatment. Neuroscience 2004;128:597-604. https://doi.org/10.1016/j.neuroscience.2004.06.054
23.Garcia-Valenzulela E, Gorczyca W, Darzynkiewicz Z, Sharma S. Apoptosis in adult retinal ganglion cells after axotomy. J Neurobiol 1994;25:431-8. https://doi.org/10.1002/neu.480250408

24.Nakazawa T, Tamai M, Mori N. Brain-derived neurotrophic factor prevents axotomized retinal ganglion cell death through MAPK and PI3K signaling pathways. Investig Ophthalmol Vis Sci 2002;43:3319-26. https://pubmed.ncbi.nlm.nih.gov/12356841/
25.Duman CH, Duman RS. Spine synapse remodeling in the pathophysiology and treatment of depression. Neurosci Lett 2015;601:20-9. https://doi.org/10.1016/j.neulet.2015.01.022
26.Pilar-Cuéllar F, Vidal R, Pazos A. Subchronic treatment with fluoxetine and ketanserin increases hippocampal brain-derived neurotrophic factor, β-catenin and antidepressant-like effects. Brit J Pharmacol 2012;165:1046-57. https://doi.org/10.1111/j.1476-5381.2011.01516.x
27.Hajszan T, MacLusky NJ, Leranth C. Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. Eur J Neurosci 2005;21:1299-303. https://doi.org/10.1111/j.1460-9568.2005.03968.x
28.Ma M, Ren Q, Yang C, Zhang J-c, Yao W, Dong C, *et al*. Adjunctive treatment of brexpiprazole with fluoxetine shows a rapid antidepressant effect in social defeat stress model: role of BDNF-TrkB signaling. Scientif Rep 2016;6:39209. https://doi.org/10.1038/srep39209
29.Kim ST, Chung YY, Hwang H-I, Shin H-K, Choi R, Jun YH. Differential Expression of BDNF and BIM in Streptozotocin-induced Diabetic Rat Retina After Fluoxetine Injection. In Vivo 2021;35:1461-6. https://doi.org/10.21873/invivo.12398
30.Vetencourt JFM, Sale A, Viegi A, Baroncelli L, De Pasquale R, O'Leary OF, *et al*. The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science 2008;320:385-8. https://doi.org/10.1126/science.1150516