RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2025.78608
J Ankara Univ Fac Med

BASIC MEDICAL SCIENCES / TEMEL TIP BİLİMLERİ

In Vivo Effects of Exogenous BDNF Administration on AMPA Receptors in the Dentate Gyrus of Female Rats

Eksojen BDNF Uygulamasının Dişi Sıçan Dentat Girus AMPA Reseptörüne *In Vivo* Etkisi

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Abstract

Objectives: Previous *in vitro* studies have demonstrated that brain-derived neurotrophic factor (BDNF) modulates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) expression and the trafficking of synaptic AMPA receptors (AMPARs). However, it is not known whether exogenous BDNF administration similarly regulates AMPAR trafficking *in vivo*. This study aims to elucidate this unknown aspect. Additionally, considering that BDNF and estrogen have similar effects in the brain and may interact through their cellular mechanisms, the study also aims to explore the outcomes of BDNF administration in female rats.

Material and Methods: For this purpose, female Long-Evans rats in the same estrus phase were divided into the experimental group (EG, n=5) and the control group (CG, n=5). In the EG, recombinant BDNF protein (4 µg/day) was administered to the right hippocampus via osmotic minipumps for 7 days, while the CG received phosphate-buffered saline (PBS) (4 µg/day) under the same conditions. The Morris Water Maze (MWM) test was employed for assessing learning and memory. AMPAR levels in the left and right hippocampi were examined using immunohistochemical methods, and the intensity patterns were evaluated using H-scoring.

Results: Although no significant behavioral differences were observed between the groups in the MWM task ($p>0.05$), H-scoring results revealed that BDNF treatment significantly increased GluR1 subunit immunoreactivity in the right hippocampus of the EG compared to the CG ($p\leq0.001$). Notably, GluR1 H-score levels were also significantly increased in the left hemisphere, which was not directly infused with BDNF.

Conclusion: This study demonstrates that long-term BDNF administration increases AMPAR levels in the hippocampus *in vivo*. While this increase has behavioral implications for spatial learning and memory in female rats, further research is needed to explore the full extent of these effects.

Keywords: AMPA receptor, long-term potentiation, brain-derived neurotrophic factor, learning and memory, synaptic plasticity

Öz

Amaç: Önceki *in vitro* araştırmalar, beyin türevli nörotrofik faktörün (BDNF) α-amino-3-hidroksi-5-metil-4-izoksazol propiyonik asit (AMPA) ekspresyonunu ve sinaptik AMPA reseptörlerinin (AMPAR) trafiğini modüle ettiğini göstermiştir. Ancak, eksojen BDNF uygulamasının *in vivo* ortamda AMPAR trafiğini benzer şekilde düzenleyip düzenlemediği bilinmemektedir. Bu çalışma, bu bilinmeyeni aydınlatmayı amaçlamaktadır. Ayrıca BDNF ve östrojenin beyinde benzer etkilere sebep olması ve hücresel mekanizmalarının birbirini etkilemesi nedeniyle dişilere BDNF uygulamanın sonuçlarını gözlemlemek de hedeflenmiştir.

Gereç ve Yöntem: Aynı estrus evresindeki dişi Long-Evans sıçanları, deney grubu (DG, n=5) kontrol grubu (KG, n=5) olarak ayrılmıştır. DG sağ hipokampüslerine BDNF proteini (4 µg/gün), KG grubu sıçanlarınkine ise PBS (4 µg/gün), ozmotik minipompalar vasıtasıyla intrahipokampal olarak

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Received/Geliş Tarihi: 16.07.2024 **Accepted/Kabul Tarihi:** 28.02.2025 **Epub:** 12.05.2025
Cite this article as/Atıf: Sırmatel Bakrıyanık B, Özer Ç, Seymen CM. *In vivo* effects of exogenous BDNF administration on AMPA receptors in the dentate gyrus of female rats. J Ankara Univ Fac Med.

7 gün boyunca verilmiştir. Öğrenme ve bellek görevleri için Morris Su Labirenti (MWM) testi uygulanmıştır. Sol ve sağ hipokampal AMPAR seviyeleri immünohistokimyasal yöntemle incelenmiş ve yoğunluk deseni H-skorlama yapılarak değerlendirilmiştir.

Bulgular: Genel olarak davranış deneyi açısından gruplar arasında anlamlı farklılık gözlenmemesine rağmen H-skor sonuçlarına göre BDNF uygulaması DG'nin sağ ($p \leq 0,001$) hipokampusunda KG'ye kıyasla AMPAR alt birimi GluR1'in immünoreaktivitesini önemli ölçüde artırmıştır. Hatta GluR1 H-skor seviyeleri infüzyon alanı olmayan sol hemisferde de anlamlı olarak yükselmiştir ($p \leq 0,001$).

Sonuç: Bu çalışma, uzun süreli BDNF uygulamasının hipokampusta AMPAR seviyelerini artırdığını *in vivo* olarak göstermektedir. Bu artışın dişi sıçanlardaki mekansal öğrenme ve bellek sürecine davranışsal yansımaları olmakla birlikte anlamlı etkileri için ileri araştırmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: AMPA reseptörü, uzun-dönem potansiyasyon, beyin türevli nörotrofik faktör, öğrenme ve bellek, sinaptik plastisite

Introduction

Memory is a complex cognitive function based on synaptic plasticity. Long-term potentiation (LTP), a fundamental mechanism of synaptic plasticity, has been widely explored in hippocampal neurons, where it plays a crucial role in memory formation (1,2). LTP progresses through three distinct stages: Short-term potentiation, early-LTP (E-LTP), and late-LTP (L-LTP). The first two phases involve modifications of pre-existing proteins, whereas L-LTP depends on the activation of transcription factors and protein synthesis, allowing sustained synaptic strengthening (3,4). Glutamatergic excitatory synapses and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are essential for LTP in the hippocampus. Specifically, the regulation of AMPAR synaptic trafficking is crucial for the long-term encoding of synaptic activity (5,6). AMPARs are tetrameric receptors composed of combinations of four distinct subunits: GluR1, GluR2, GluR3, and GluR4. In the adult hippocampus, there are two dominant types of AMPARs: Those formed by "GluR1 and GluR2" and those formed by "GluR3 and GluR2" (5). Among these subunits, the GluR1 subunit is particularly important for synaptic plasticity and LTP (7,8).

As essential modulators of synaptic plasticity, neurotrophins are a group of secreted proteins involved in neuronal survival and function (9,10). Brain-derived neurotrophic factor (BDNF) is essential for neuron differentiation and survival, and unlike other members of this family, it has a broad distribution in the adult brain and hippocampal subregions. BDNF plays a crucial role in regulating activity-dependent changes in synaptic structure and function. BDNF is postulated to potentiate synaptic responsiveness to tetanic stimulation, thereby facilitating E-LTP induction, while orchestrating synaptic vesicle dynamics through modulation of synaptic protein phosphorylation and spatial redistribution (11). Genetic and pharmacological interrogations of BDNF and its cognate receptor Tyrosine kinase B (TrkB) underscore its indispensable role in both the initiation and sustenance of L-LTP (12,13). In hippocampal slices derived from BDNF-deficient mice, the perturbation in LTP induction was ameliorated upon reinstatement of *BDNF* gene expression (14). These findings suggest that endogenous BDNF is necessary for various stages of LTP. Moreover, to better understand its functions and mechanisms in this process, and how it alters extrinsic modulation of synaptic plasticity and memory, numerous studies have explored exogenous BDNF applications (15-20). Studies focusing on its effects on AMPARs have shown that BDNF modulates AMPAR expression by regulating specific phosphorylation pathways (21,22). Similarly, studies in hippocampal and cortical neuron cultures have revealed that BDNF increases AMPAR levels in both the membrane (23,24) and synaptic pools (25,26). It was also noted that BDNF treatment in cortical cultures was found to significantly elevate AMPAR levels, particularly enriching GluR1- and GluR2-bearing subunits (27). These findings set the stage for further exploration of how AMPAR modulation by BDNF contributes to synaptic plasticity and memory formation.

Among these, the activation of estrogen receptor beta (ERβ), a nuclear receptor for the hormone estrogen, has been implicated in the regulation of AMPAR GluR1 subunit expression (28). Estrogen is known to modulate cognitive processes and hippocampal structure and function through interactions with ERα, ERβ nuclear receptors, and ER-like proteins on the plasma membrane (29,30). Selective ERβ agonists, when administered *in vivo*, have been demonstrated to increase the expression of AMPAR GluR1 and postsynaptic density protein 95 (PSD-95), a postsynaptic scaffolding protein critical for the regulation of AMPAR trafficking. Furthermore, ERβ activation has been reported to alter synaptic architecture by influencing dendritic branching, dendritic spine morphology, and density in the hippocampal CA1 and dentate gyrus regions (28). It was found that spatial memory performance was significantly enhanced by both the activation of ERβ and the administration of estradiol. Furthermore, functional overlap was revealed in the examination of the effects of estrogen and BDNF on the central nervous system, indicating that the influence of estrogen on the hippocampus is analogous to that of BDNF (31). There is evidence suggesting that estradiol can induce BDNF expression (32,33), and estrogen receptors have been found to colocalize with neurons expressing BDNF and TrkB (34). However, some studies report that elevated endogenous estrogen levels correlate with reduced BDNF mRNA levels (35,36), while others observe no significant effect of estrogen (37). Consequently, the mechanisms that contribute to the inconsistent findings concerning estrogen-BDNF interactions remain unclear, highlighting the necessity for additional research.

Although many findings suggest that exogenous BDNF administration modulates AMPAR trafficking, it remains uncertain whether similar stimulation occurs in an *in vivo* setting or what effects it has specifically on female rats. To address this gap, the first part of our study involved the infusion of recombinant BDNF protein into the right hippocampal dentate gyrus of female rats using osmotic minipumps, differing from traditional single injection studies. This approach aimed to observe the *in vivo* effects of unilateral intrahippocampal BDNF administration on the levels of AMPAR GluR1 subunits in the dentate gyrus. In the second part of the study, the Morris Water Maze (MWM) task, a well-established model for measuring spatial learning and memory (38), was employed to investigate the impact of the administered protein on spatial memory.

Materials and Methods

This study was supported by the Gazi University Scientific Research Projects Coordination Unit under grant number 01/2017-26 and approved by the Gazi University Local Ethics Committee for Animal Experiments (decision number: E.28661, date: 16.08.2017).

Animals

In this study, we utilized adult female Long-Evans rats (n=10), each weighing between 200-250 g. The animals were obtained from the Gazi University Laboratory Animals Breeding and Experimental Research Center (GUDAM). All procedures involving the rats were conducted in the designated laboratory settings of GUDAM. Prior to inclusion in the study, vaginal smears were collected to confirm that the animals were in the estrus phase, characterized by baseline levels of estrogen and progesterone (39). Throughout the experiment, vaginal smears were periodically monitored. The rats, all in the same phase, were randomly assigned to two groups: the control group (CG, n=5) and the experimental group (EG, n=5).

Each rat was housed individually in a controlled laboratory environment, ensuring unrestricted access to food and water. The housing conditions included a stable 12:12 hour light-dark cycle and a maintained temperature of 24±2 °C. Behavioral tests were conducted during the light phase. In the EG, recombinant BDNF protein (4 µg/day) was infused into the right hippocampal dentate gyrus via osmotic minipumps for 7 days. In contrast, phosphate-buffered saline (PBS) was infused into the CG rats. Aside from this difference, all other experimental procedures were applied equally to the animals in both groups (Figure 1a).

Implantation of Osmotic Minipumps and Stereotactic Surgery

One day prior to stereotactic surgery, osmotic minipumps (ALZET 1007D, Alza Scientific Products, Palo Alto, CA) were filled with recombinant human BDNF protein (140 µg dissolved in 0.1 M PBS; 4 µg/day per animal; ProSpec, Israel) for the EG and PBS (BioShop, Canada) for the CG. The pumps were then incubated overnight in sterile PBS and set to operate at a flow rate of 0.5 µl/hour for seven days. The appropriate BDNF dose was determined based on the study by Mamounas et al. (40).

Intrahippocampal surgery was performed using a stereotaxic apparatus (Stoelting, USA) under anesthesia with a combination of 10% Ketamine and 2% Xylazine (75-90 mg/kg Ketamine+5-8 mg/kg Xylazine, intramuscular). Infusion cannula (ALZET Brain Infusion Kit 2, Alza Scientific Products, Palo Alto, CA) were implanted into the right dentate gyrus. Based on the Paxinos and Watson (41) atlas, coordinates for the dentate gyrus were determined using bregma as the reference point: -3.8 mm AP, 2.0 mm ML, and -3.2 mm dorsoventral. Prior to initiating the experimental procedure, the accuracy of these coordinates was confirmed in a separate rat using a Hamilton syringe with methylene blue (Figure 1c). The infusion cannula were secured to the skull with dental cement, fixed with machine screws, and connected to the osmotic minipumps. The minipumps were placed in a subcutaneous pocket between the scapula bones

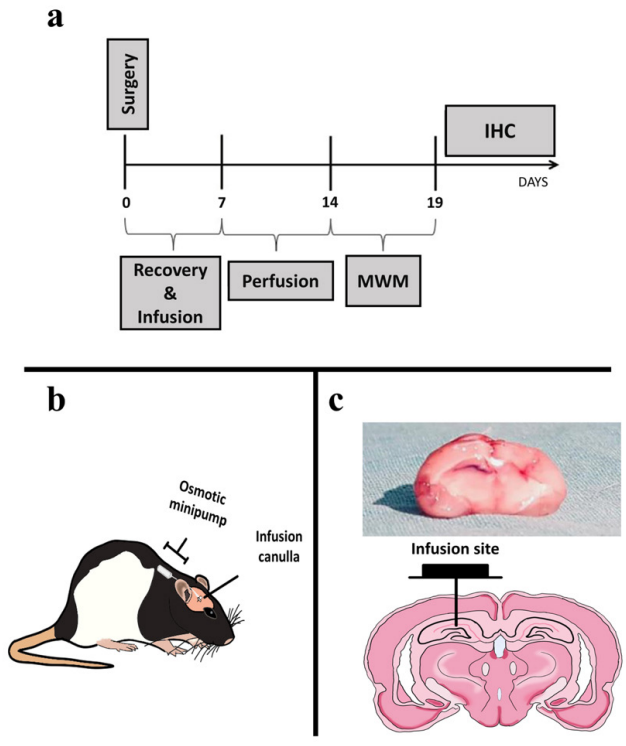

Figure 1: **A)** A diagram illustrating the overall experimental flow; day 0 symbolizes the start of the experiment, which is the day of the stereotactic surgery. **B)** A schematic representation of the osmotic minipump and infusion cannula implanted into the rat's body. **C)** Right dentate gyrus; infusion area for the EG with BDNF and for the CG with PBS.

MWM: Morris Water Maze, EG: Experimental group, CG: Control group, BDNF: Brain-derived neurotrophic factor, PBS: Phosphate buffered saline

(Figure 1b). After the seven-day infusion period, a seven-day perfusion phase followed to allow for the full diffusion of the administered substances into the hippocampus and to observe their long-term effects. During this perfusion phase, the incision sites were also allowed to heal. Upon completion of the perfusion, the MWM spatial memory task was initiated (Figure 1).

Spatial Memory Task: Morris Water Maze

Cognitive function, specifically learning and memory, was evaluated using the MWM paradigm (42). The maze consists of a tank filled with water, made opaque with black food coloring, measuring 140 cm in diameter and 45 cm in depth. The tank was placed in a room with standard lighting and surrounded by fixed visual cues. The space was conceptually segmented into four distinct quadrants, labeled as star, triangle, pentagon, and circle. A hidden platform was positioned in the center of the triangle quadrant, 2 cm below the water surface. The experiments were recorded using an appropriate camera and software system.

The task consists of two phases: The acquisition phase and the probe test. The acquisition phase spans four days, with four trials per day, aiming to teach the rats the location of the hidden platform using fixed visual cues. At the beginning of each trial, rats were introduced into the maze from varying starting locations and allowed up to 90 seconds to reach the platform. If a rat was unable to locate the platform within this timeframe, it was gently guided by the researcher. Once on the platform, each rat was allowed to remain for 30 seconds to explore the surroundings.

During the acquisition phase, key behavioral metrics, including escape latency, traveled distance, and swimming speed, were systematically recorded. After completing this phase, a probe test was administered, in which the platform was removed, allowing the rats to explore the maze freely for 90 seconds. Throughout this test, multiple parameters were assessed, including the duration spent in each quadrant, swimming speed, the frequency of crossings over the former platform location, time allocation between the center and periphery, and total distance traveled.

Immunohistochemical Analysis

After completing the MWM task, the animals were sacrificed via cardiac puncture under deep anesthesia. Following extraction, brain hemispheres were briefly rinsed with saline and immersed in 10% neutral formaldehyde for at least 72 hours to facilitate immunohistochemical (IHC) analysis. Paraffin-embedded tissue blocks were then prepared using standard histological techniques, from which 4 µm-thick cross-sections were obtained (43).

Following deparaffinization and rehydration, antigen retrieval was achieved using heat in the presence of EDTA (pH: 8.0). To suppress endogenous peroxidase activity, tissue sections were incubated with a 3% hydrogen peroxide solution, followed by serum blocking (Lot: 1754084A, LifeTech, Waltham, MA) for 15 minutes to enhance epitope stability. Subsequently, samples were exposed to a primary antibody specific for anti-glutamate AMPAR 1 (ab31232, Abcam, Cambridge, UK; 1:200 dilution in PBS) at room temperature for 90 minutes. After thorough rinsing with PBS (pH: 7.4), sections were treated with a biotinylated secondary antibody and streptavidin-peroxidase complex (Lot: 1754084A, LifeTech, Waltham, MA) for 10 minutes. The antibody-antigen interaction sites were visualized using 3,3'-diaminobenzidine (DAB; Lot: 38703, DAB Chromogen/Substrate Kit, ScyTek). Finally, slides underwent counterstaining with Harris' Hematoxylin, sequential dehydration in ethanol, clearance with xylene, and permanent mounting with balsam.

Staining intensity and density of anti-glutamate receptor 1 (AMPA Subtype) were analyzed in the dentate gyrus of the right and left hemispheres using a Leica DM4000B light microscope equipped with a DFC280 camera and LAS software (Leica, Wetzlar, GermanyA semi-quantitative evaluation system was employed to categorize staining intensity on a graded scale: 0 (absence of staining), 1 (faint staining), 2 (mild to moderate staining), 3 (moderate staining), 4 (moderate to intense staining), and 5 (pronounced staining). Immunoreactivity scores were independently assessed by two blinded evaluators, who were unaware of the experimental conditions. The H-score was computed using the formula H-score = $\Sigma Pi\ (i + 1)$, where "i" represents the staining intensity (ranging from 0 to 5), and "Pi" corresponds to the proportion of cells exhibiting each intensity level. Scoring was conducted independently by two investigators.

Statistical Analysis

In this study, MP4 format videos obtained from the MWM task were converted into PNG format images for frame-by-frame analysis. For this purpose, the open-source multimedia processing software FFmpeg was utilized (44). The positions of the rats in the converted PNG images were labeled using the open-source labeling software "LabelMe" (45). The data obtained from the labeled images were analyzed using a custom code written in the Python language (46). To evaluate performance during the acquisition phase of the MWM task, the following metrics were measured: Escape latency, time spent in the center, center distance (the distance the animal traveled in the central area of the maze), peripheral distance, mean cumulative distance (the mean total distance traveled by the animal until reaching the platform), and mean speed. For the probe test phase, the time spent in each quadrant and the number of crossings at the

location of the hidden platform, which served as an indicator of the learning process, were recorded.

The data were analyzed using the Jeffrey's Amazing Statistics Program (47). Repeated measures analysis of variance (ANOVA) was utilized for the evaluation of MWM behavioral data and IHC H-score data. Multiple comparisons were assessed using the post-hoc Tukey test or the Holm test. An independent samples t-test was employed solely for group comparisons. Descriptive statistics were presented as means and standard deviations, with differences considered statistically significant at $p<0.05$.

Results

MWM Task Results

In the acquisition phase of the MWM experiment, the effects of the group, trial, and day variables on escape latencies (the time taken to find the hidden platform) were analyzed using repeated measures ANOVA. The main effect of days on escape latencies was found to be significant [F (3,12): 7.965, p=0.003, η^2=0.238], while the interaction between day and group was not significant [F (3,12): 0.789, p=0.523, η^2=0.456]. Similarly, although the main effect of trials was significant [F (3,12): 7.583, p=0.004, η^2=0.083], the interaction between trial and group was not significant [F (3,12): 3.184, p=0.063, η^2=0.035; Figure 2a, Table 1]. Regarding the time spent in the center, neither the main effects of days [F (3,12): 0.489, p=0.696, η^2=0.016] nor trials [F (3,12): 0.246, p=0.862, η^2=0.007] were found to be significant, nor was the interaction between day and group [F (3,12): 1.464, p=0.274, η^2=0.049; Figure 2b]. All effects related to distances traveled in the center were similarly not significant (Figure 2c). For distances traveled in the periphery, both day [F (3,12): 6.658, p=0.007, η^2=0.217] and trial main effects [F (3,12): 5.940, p=0.010, η^2=0.041] were significant; however, group interactions were not significant [$F_{day*group}$ (3,12): 0.383, p=0.767, η^2=0.012; $F_{trial*group}$ (3,12): 0.471, p=0.708, η^2=0.003; Figure 2d]. The main effects of days and trials on mean cumulative distance were significant [F (3,12): 6.415, p=0.008, η^2=0.131; F (3,12): 5.555, p=0.013, η^2=0.042], while group interactions remained non-significant [$F_{day*group}$ (3,12): 1.000, p=0.426, η^2=0.020; $F_{trial*group}$ (3,12): 1.188, p=0.356, η^2=0.009; Figure 2e]. When examining the effects on mean swimming speed, only the main effect of days was found to be significant [F (3,12): 4.210, p=0.030, η^2=0.107], with the Holm test indicating that the speed on day 4 was statistically significantly lower than on Day 1 (p=0.004; Figure 4a, Table 1).

During the probe phase of the MWM task, the duration spent in each quadrant did not exhibit a statistically meaningful variation between the two groups [$F_{quadrant*group}$ (3, 12): 1.262, p=0.31, η^2=0.092; Figure 3a, Table 2]. Similarly, while the EG demonstrated a greater frequency of crossings at the

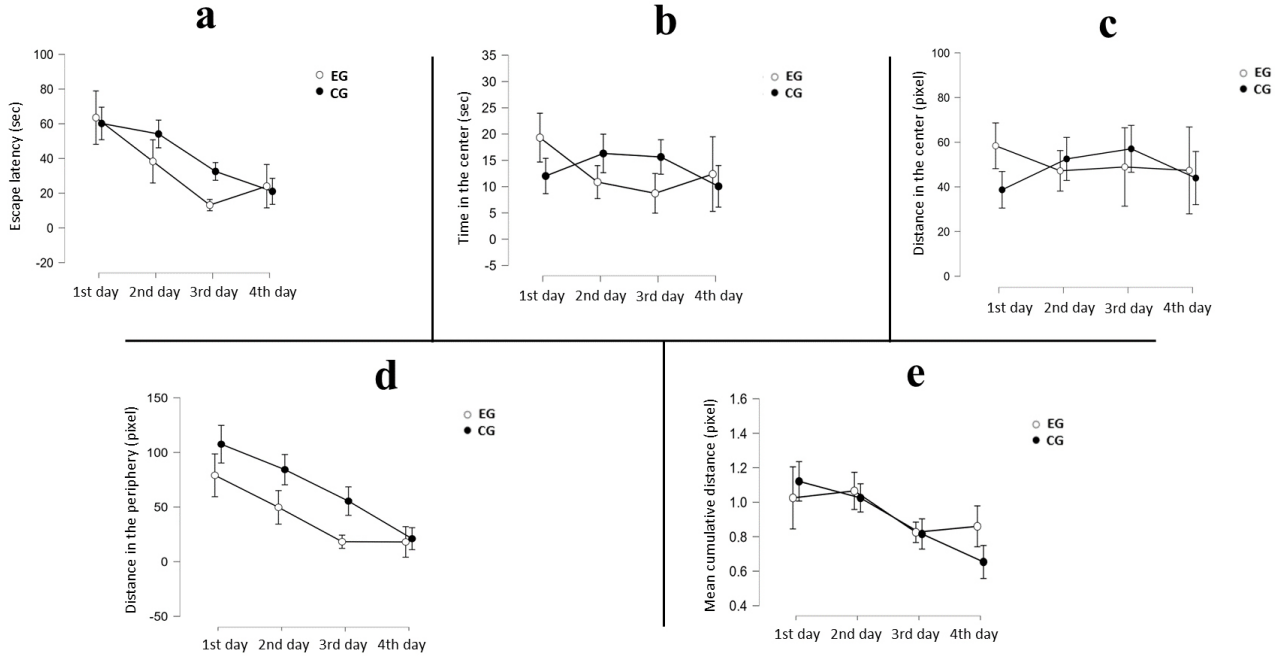

Figure 2: Daily findings of the Morris Water Maze task during the acquisition phase for the groups; **a)** shows mean escape latency times, **b)** mean time spent in the center of the tank, **c)** mean distances traveled in the center of the tank, **d)** mean distances traveled in the periphery of the tank, and **e)** mean cumulative distance traveled

EG: Experimental group, CG: Control group

former platform location, this trend did not reach statistical significance[t(8): 1.968, p=0.085, Cohen's d=1.244; Figure 3b, Table 3]. The two groups showed comparable results in terms of time allocation within the center [t(8): 0.673, p=0.520, Cohen's d=0.647; Figure 3c, Table 3], distance covered in the central region [t(8): 1.280, p=0.236, Cohen's d=0.682; Figure 3d, Table 3], distance traveled along the periphery [t(8): -1.520, p=0.167, Cohen's d=-0.961; Figure 3e, Table 3], and overall cumulative distance [t(8): 0.613, p=0.557, Cohen's d=0.388; Figure 3f, Table 3]. However, akin to the acquisition phase, a statistically robust difference was detected between the groups in terms of mean swimming speed [t(8): -3.466, p=0.008, Cohen's d=-2.192; Figure 4b, Table 3], with the EG exhibiting a notably lower swimming speed than the CG.

Immunohistochemistry Findings

The molecular, granular cell, and polymorphic layers of the dentate gyrus region were observed in the dentate gyrus examinations conducted on the right and left hemispheres of the CG (Figures 5a and 6a). In high magnification examinations of this group, immunoreactivity for the "AMPAR GluR1 subunit" was predominantly observed in a small number of granular neurons within the granular cell layer and in some neurons of the polymorphic layer. It was determined that this immunoreactivity

Table 1: The results of the ANOVA analysis conducted to evaluate the performance of both groups on various behavioral measures during the acquisition phase of the Morris Water Maze task

		Repeated-measures ANOVA					
	Mean ± Standard deviation	Sum of squares	*df*	Mean square*	*F*	p	η^2
Escape latency							
Main effect of days (sec)	36.89±15.73 sec	23566.641	3.000	7855.547	7.965	0.003***	0.238
Days x Groups interaction		2334.570	3.000	778.190	0.789	0.523	0.024
Main effect of trials (sec)	37.6±19.49 sec	8213.693	3.000	2737.898	7.583	0.004***	0.083
Trials x Groups interaction		3448.380	3.000	1149.460	3.184	0.063	0.035
Time spent in the center							
Main effect of days (sec)	14.01±2.32 sec	238.026	3.000	79.675	0.489	0.696	0.016
Days x Groups interaction		715.227	3.000	238.409	1.464	0.274	0.049
Main effect of trials (sec)	14.08±5.35 sec	95.965	3.000	31.988	0.246	0.862	0.007
Trials x Groups interaction		1023.655	3.000	341.218	2.629	0.098	0.070
Distance traveled in the center							
Main effect of days (px)	50.32±2.17 px	588.929	3.000	196.310	0.138	0.935	0.005
Days x Groups interaction		2511.396	3.000	837.132	0.598	0.634	0.020
Main effect of trials (px)	50.62±15.02 px	424.988	3.000	141.663	0.162	0.920	0.003
Trials x Groups interaction		8527.323	3.000	2842.441	3.255	0.060	0.067
Distance traveled in the periphery							
Main effect of days (px)	47.89±28.39 px	68103.016	3.000	22701.005	6.658	0.007***	0.217
Days x Groups interaction		3913.324	3.000	1304.441	0.383	0.767	0.012
Main effect of trials (px)	49.49±35.85 px	12834.172	3.000	4278.057	5.940	0.010**	0.041
Trials x Groups interaction		1016.941	3.000	338.980	0.471	0.708	0.003
Cumulative distance							
Main effect of days (px)	0.92±0.15 px	1.611	3.000	0.537	6.415	0.008***	0.131
Days x Groups interaction		0.251	3.000	0.084	1.000	0.426	0.020
Main effect of trials (px)	0.93±0.22 px	0.515	3.000	0.172	5.555	0.013**	0.042
Trials x Groups interaction		0.110	3.000	0.037	1.188	0.356	0.009
Swimming speed							
Main effect of days (px/sec)	1.01±0.21 px/sec	4.092	3.000	1.364	4.210	0.030**	0.107
Days x Groups interaction		0.661	3.000	0.220	0.681	0.581	0.017
Main effect of trials (px/sec)	1.03±0.31 px/sec	0.488	3.000	0.163	1.944	0.176	0.013
Trials x Groups interaction		0.451	3.000	0.150	1.799	0.201	0.012

Significance levels are indicated as p<0.05 and p<0.01. *Type III mean square, **p<0.05, ***p<0.01
sec: Second, px: Pixel, ANOVA: Analysis of variance

was present in the cell membrane and the cytoplasm (Figures 5b and 6b).

In the dentate gyrus examinations of the left hemisphere of the EG, moderate immunoreactivity was observed, particularly in neurons of the granular and some polymorphic layers. It was determined that this immunoreactivity was present in the cell membrane and the cytoplasm. Neurons showing immunoreactivity for the AMPAR GluR1 subunit demonstrated a range of effects from moderate to severe in the polymorphic cell layers compared to the neurons in the granular cell layer (Figures 7a and b).

The most intense immunoreactivity for the AMPAR GluR1 subunit was observed in the dentate gyrus of the right hemisphere of the EG. In the examinations conducted in this region, it was noted that the immunoreactivity for the AMPAR GluR1 subunit in the cell membrane and cytoplasm of neurons in both the granular and polymorphic cell layers varied from severe to very severe. The most significant finding was that

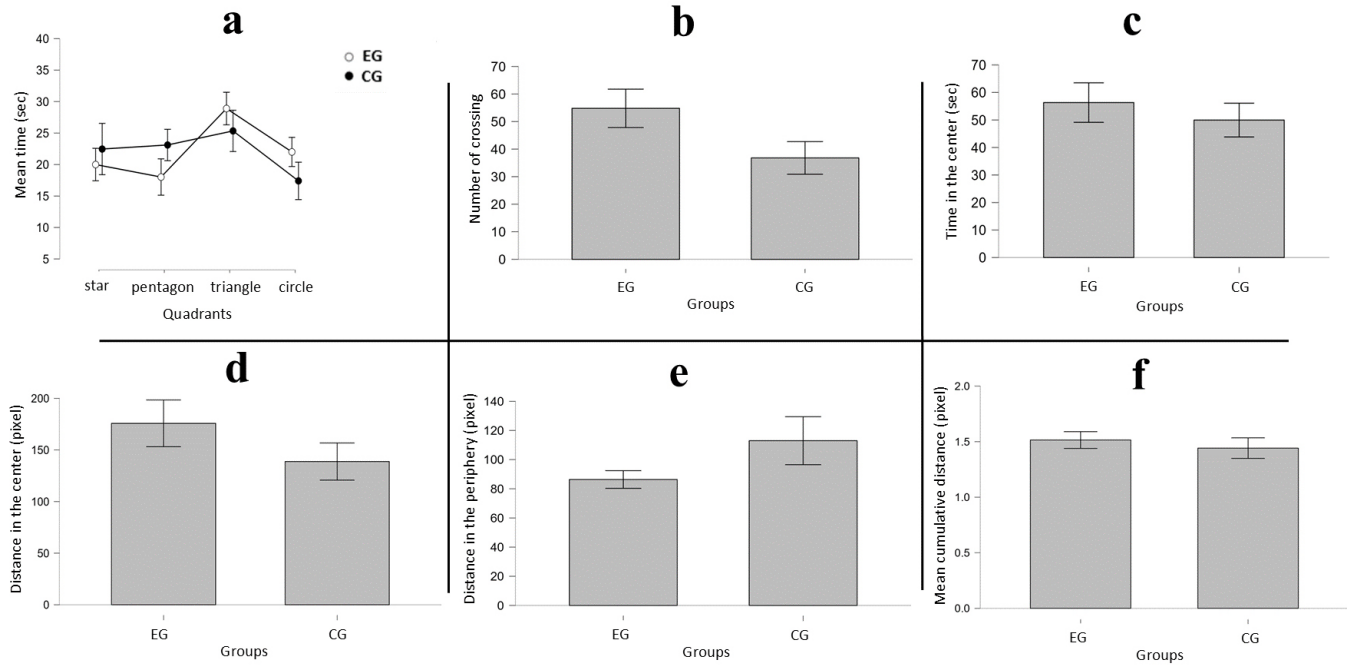

Figure 3: Findings from the testing phase of the Morris Water Maze task for the groups; **a)** mean time spent in quadrants, **b)** mean number of crossings at the location of the previously hidden platform throughout the test, **c)** mean time spent in the center of the tank, **d)** mean distance covered in the center of the tank, **e)** mean distance covered in the periphery of the tank, and **f)** mean cumulative distance covered.
EG: Experimental group, CG: Control group

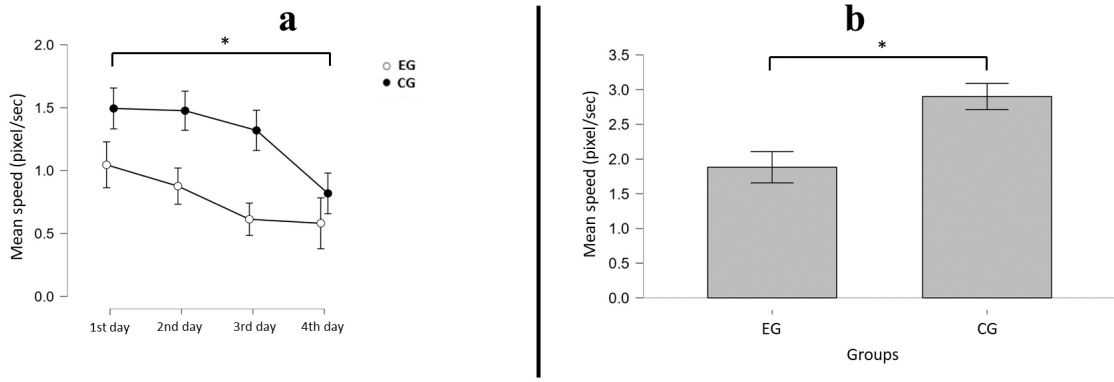

Figure 4: The mean swimming speeds of the groups are shown during, **a)** the days of the acquisition phase and **B)** the probe test phase of the Morris Water Maze task. The significant difference observed between the days, as determined by the post-hoc Holm test, was found to be due to the speed difference between the 1st and 4th days of the acquisition phase (p=0.04). *Indicates significant comparisons at p<0.05 level

the number of cells exhibiting immunoreactivity in the right hemisphere of this group was greater than that of the other group. Additionally, unlike the CG, particularly intense immunoreactivity was observed in the neuronal extensions extending towards the molecular layer (ML) of the dentate gyrus in this group (Figures 8a and b).

The distribution and intensity of AMPAR GluR1 subunit IHC staining in the dentate gyrus of both groups' right and left hemispheres were evaluated using the H-scoring method (Table 4). When comparing the H-scores of the EG in the right and left hemispheres with those of the CG, a significant increase was observed in both hemispheres [right hippocampus: t(6): 112.715, p≤0.001, Cohen's d=71.287; left hippocampus: t(6): 68.259, p≤0.001, Cohen's d=43.171, Figure 9, Table 5]. Additionally, the H-score of the EG left hemisphere was found to be significantly lower than that of the right hemisphere [t(6): -45.163, p≤0.001, Cohen's d=−28.117].

Table 2: The results of the repeated measures ANOVA and post-hoc comparisons for the time spent in quadrants (star, pentagon, triangle and circle) during the probe trial phase of the Morris Water Maze task for both groups

			Repeated-measures ANOVA					
	Mean ± Standard deviation		Sum of squares	*df*	Mean square*	*F*	p	η^2
Main effect of quadrants	22.16±3.37 sec		340.520	3.000	113.507	2.617	0.074	0.190
Quadrants x Groups interaction			164.292	3.000	54.764	1.262	0.310	0.092
			Post-hoc comparisons					
				Mean difference	*SE*	*t*	Cohen's d	p (Holm)
Experimental group								
Quadrant duration	**Mean ± SD**	**Quadrant duration**	**Mean ± SD**					
Star (sec)	20.0±7.25 sec	Pentagon (sec) Triangle (sec) Circle (sec)	18.02±6.09 sec 28.9±7.51 sec 22.01±3.66 sec	1.982 -8.901 -2.005	3.675 3.675 3.675	0.539 -2.422 -0.546	0.314 -1.409 -0.318	1.000 0.161 1.000
Pentagon (sec)	18.02±6.09 sec	Triangle (sec) Circle (sec)	28.9±7.51 sec 22.01±3.66 sec	-10.883 -3.988	3.675 3.675	-2.961 -1.085	-1.723 -0.631	0.071 0.898
Triangle (sec)	28.9±7.51 sec	Circle (sec)	22.01±3.66 sec	6.895	3.675	1.876	1.092	0.341
Control group								
Star (sec)	22.47±7.74 sec	Pentagon (sec) Triangle (sec) Circle (sec)	23.11±5.18 sec 25.34±6.52 sec 17.41±5.82 sec	-0.644 -2.878 5.059	4.604 4.604 4.604	-0.140 -0.625 1.099	-0.101 -0.451 0.739	1.000 1.000 1.000
Pentagon (sec)	23.11±5.18 sec	Triangle (sec) Circle (sec)	25.34±6.52 sec 17.41±5.82 sec	-2.234 5.703	4.604 4.604	-0.485 1.239	-0.350 0.893	1.000 1.000
Triangle (sec)	25.34±6.52 sec	Circle (sec)	17.41±5.82 sec	7.937	4.604	1.724	1.243	0.662

Significance levels are indicated as p<0.05 and p<0.01. *Type III mean square
SD: Standard deviation, SE: Standard error, sec: Second, ANOVA: Analysis of variance

Table 3: The results of Levene's test for the homogeneity of variances between groups and the Independent Samples t-test for the mean differences between groups for various behavioral parameters during the probe test of the Morris Water Maze task

		Levene's test		Independent Samples t-test			
		F	p	t	*df*	p	SE effect size
	Mean ± SD						
Number of crossings (count)	45.8±16.61 count	0.477	0.509	1.968	8	0.085	0.745
Time spent in the center (sec)	53.14±14.48 sec	0.051	0.827	0.673	8	0.520	0.647
Distance traveled in the center (px)	157.27±47.31 px	0.662	0.439	1.280	8	0.236	0.682
Distance traveled in the periphery (px)	99.67±29.65 px	4.344	0.071	-1.520	8	0.167	0.702
Cumulative distance (px)	1.48±0.18 px	1.347	0.279	0.613	8	0.557	0.644
Swimming speed (px/sec)	2.39±0.69 px/sec	0.019	0.895	-3.466	8	0.008*	0.938**

Significance levels are indicated as p<0.01. *p<0.01, **The effect size is reported using Cohen's d
SD: Standard deviation

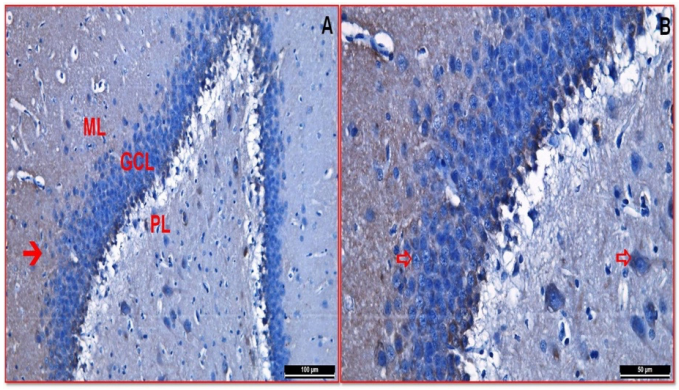

Figure 5: In the AMPAR GluR1 subunit staining of CG left hemisphere (A): →: Dentate Gyrus, ML: Molecular Layer, GCL: Granular Cell Layer, PL: Polymorphic Layer and (B): ⇨: Neurons with weak immunoreactivity
Immunoperoxidase-Hematoxylin; A: 200x, B: 400x, AMPAR: AMPA receptors, CG: Control group

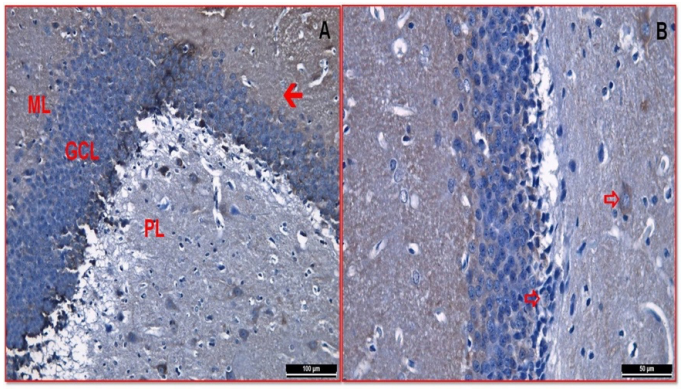

Figure 6: In the AMPAR GluR1 subunit staining of CG right hemisphere (A): →: Dentate Gyrus, ML: Molecular Layer, GCL: Granular Cell Layer, PL: Polymorphic Layer and (B): ⇨: Neurons with weak immunoreactivity
Immunoperoxidase-Hematoxylin; A: 200x, B: 400x, AMPAR: AMPA receptors, CG: Control group

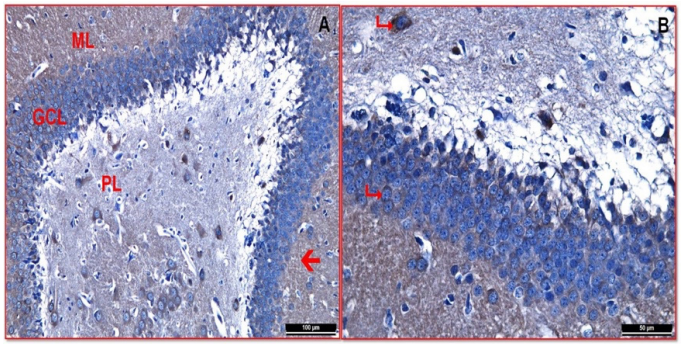

Figure 7: In the AMPAR GluR1 subunit staining of EG left hemisphere (A): →: Dentate Gyrus, ML: Molecular Layer, GCL: Granular Cell Layer, PL: Polymorphic Layer and (B): ↳: Neurons showing moderate immunoreactivity
Immunoperoxidase-Hematoxylin; A: 200x, B: 400x, AMPAR: AMPA receptors, EG: Experimental gorup

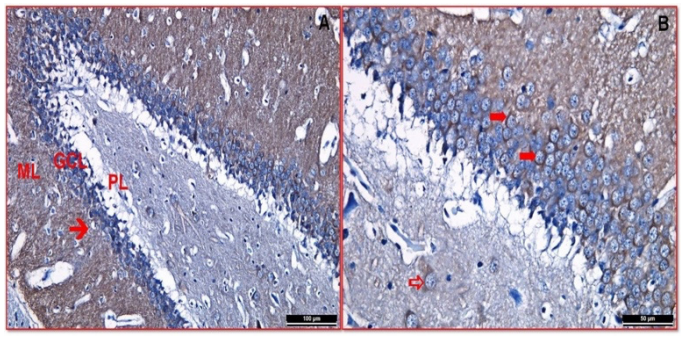

Figure 8: In the AMPAR GluR1 subunit staining of EG right hemisphere (A): →: Dentate Gyrus, ML: Molecular Layer, GCL: Granular Cell Layer, PL: Polymorphic Layer and (B): ➡: Neurons with severe immunoreactivity ⇨: Nerve cell extensions showing immunoreactivity
Immunoperoxidase-Hematoxylin; A: 200x, B: 400x, AMPAR: AMPA receptors, EG: Experimental gorup

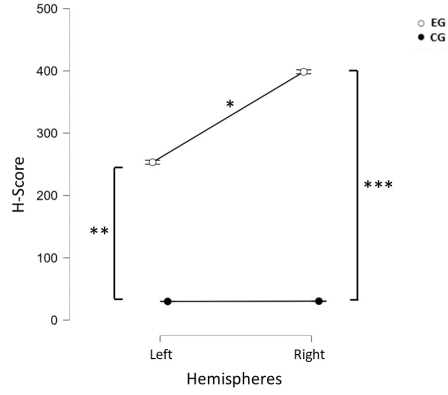

Figure 9: H-score values for the AMPA receptor GluR1 immunoreactivity in the left and right dentate gyrus of the groups. The H-scores were significantly higher in the BDNF-treated right hemisphere of the EG, while both hemisphere scores were found to be significantly elevated compared to the control group
*, **, ***p<0.001; EG: Experimental group

Discussion

Numerous studies investigating the effects of BDNF on neuronal activity and synaptic plasticity have revealed that this protein and its receptors play critical roles in learning and memory processes (3,48). Studies conducted with TrkB or BDNF mutant mice have shown that disruption of BDNF signaling severely negatively affects hippocampal E-LTP and L-LTP (13,14). However, it has also been found that these LTP defects can be reversed with the restoration of gene expression (14). These findings underscore the importance of BDNF in the LTP process. Neuron culture studies aimed at investigating the effect of BDNF on AMPARs served as the fundamental starting point for planning the current study. These studies have shown that BDNF

Table 4: H-score results based on the evaluation of AMPA receptor GluR1 subunit immunoreactivity in the dentate gyrus region obtained from slices of the left and right hemispheres of the groups

Dentate gyrus AMPA receptor GluR1 subunit	Left hemisphere			Right hemisphere		
	Percentage intensity (%)	Staining intensity	H-Score (AU)	Percentage intensity (%)	Staining intensity	H-Score (AU)
CG1	25	0	30.8	20	1	30.6
CG2	15	1	31.2	20	0	31
CG3	20	1	29.9	15	1	32.5
CG4	25	0	29.8	15	1	28.7
CG5	25	0	28	15	1	29
EG1	70	3	250.8	75	4	405
EG2	65	2	250.4	75	5	385.7
EG3	55	3	249.85	70	5	400.8
EG4	70	3	250	75	4	402.3
EG5	70	3	265.45	80	4	398.7

Staining intensity values were categorized on a graded scale: 0 (absence of staining), 1 (faint staining), 2 (mild to moderate staining), 3 (moderate staining), 4 (moderate to intense staining), and 5 (pronounced staining)
CG: Control group, EG: Experimental group, H-score: Histochemical scoring, AU: Arbitrary unit

Table 5: The ANOVA results of the differences in H-score values of the AMPA receptor GluR1 subunit between hemispheres for the experimental groups, as well as post-hoc comparisons between hemispheres and groups

			Repeated-measures ANOVA					
		Mean ± SD	Sum of squares	*df*	Mean square*	*F*	p-value	η^2
Main effect of hemispheres (AU)		111.51±82.36	26506.480	1	26506.480	1025.760	<0.001**	0.054
Hemispheres x Groups interaction			26201.560	1	26201.560	26201.560	<0.001**	0.053
			Post-hoc comparisons - Hemispheres x Groups interaction					
H-score (AU)	**Mean ± SD**	**H-score (AU)**	**Mean ± SD**	**Mean difference**	***SE***	***t***	**Cohen's d**	**p (Tukey)**
EG, left	253.3±6.8	CG, left EG, right CG, right	29.94±1.24 398.5±7.51 30.36±1.55	223.360 -145.200 222.940	3.266 3.215 2.266	68.387 -45.163 68.259	43.252 -28.117 43.171	<0.001** <0.001** <0.001**
CG, left	29.94±1.24	EG, right CG, right	398.5±7.51 30.36±1.55	-368.560 -0.420	3.266 3.215	-112.844 -0.131	-71.369 -0.081	<0.001** 0.999
EG, right	398.5±7.51	CG, right	30.36±1.55	368.140	3.266	112.715	71.287	<0.001**

Significance levels are indicated as p<0.001. *Type III mean square, **p<0.001
CG: Control group, EG: Experimental group, SD: Standard deviation, SE: Standard error, AU: Arbitrary unit, ANOVA: Analysis of variance

modulates AMPAR gene expression (21), increases membrane AMPAR levels (23,24), converts AMPAR-deficient silent synapses into AMPAR-containing synapses (22), enhances the synaptic transmission of AMPARs (25,26), and specifically increases the expression of AMPARs containing GluR1 and GluR2 (27). Moreover, it has been demonstrated that slow and continuous administration of BDNF strengthens the positive effects that support synaptic transmission (17). In this study, in line with the results of these *in vitro* studies in the literature, it was observed that recombinant BDNF protein administered via long-term infusion significantly increased the levels of the AMPAR GluR1 subunit in the right dentate gyrus region compared to the control group (p≤0.001; Figure 9). Additionally, the intense immunoreactivity of AMPAR GluR1 subunits observed in both the synaptic membrane and cytoplasm suggests that BDNF administration may have increased AMPAR levels not only at the membrane receptor level but also in the synaptic vesicle pool (Figure 8). This situation indicates that exogenous BDNF application could support E-LTP by increasing the amount of membrane AMPARs, like the effects observed in neuron cultures, and could even contribute to the formation and maintenance of L-LTP by triggering the production of new AMPARs. Therefore, the findings of our study support the observations from neuron culture studies (25,49). Furthermore, the mentioned neuron culture studies generally reveal the effects of BDNF on the hippocampal tissues of male animals. However, the existence

of certain correlations between the neural effects of BDNF and estrogen (31), the presence of common signaling transduction molecules and transcription factors in the mechanisms of action of both molecules (50-52), and studies indicating estrogen's control over BDNF synthesis in both sexes (34,53,54) raise the question of whether the hippocampal effects of exogenous BDNF application in non-ovariectomized females would be similar to those observed in males. Our findings indicate that the effect demonstrated in terms of AMPAR also operates similarly in female rats, yet the interaction between estrogen and BDNF and their common contributions, especially in LTP and learning and memory processes, contain many unanswered questions that need to be investigated.

One of the other interesting findings of our study is that in the EG rats, the AMPAR GluR1 subunit H-scores for the left dentate gyrus region, showed significantly higher immunoreactivity compared to controls ($p \leq 0.001$; Figure 9, Table 5). This increase in GluR1 subunit levels observed in this hemisphere, where BDNF was not directly administered, suggests that BDNF likely exerts a bilateral effect either through cerebrospinal fluid, local circulation, or a mechanism different from these pathways. A possible explanation for this mechanism could be the fibers originating from the mossy cells located in the polymorphic layer of the dentate gyrus, projecting to some cells in both the ipsilateral and contralateral MLs (55-57). These projections, referred to as associative/commissural projections, are believed to exist in the dentate gyrus of rodents but not in the primate brain (58,59). Moreover, there are studies indicating that these projections are excitatory due to their immunoreactivity to glutamate (60,61). However, the mechanisms underlying the effects of BDNF on these projections that influence both hemispheres need to be clarified in further studies.

Disruptions in BDNF-TrkB signaling have been associated with declines in performance on various learning and memory tasks, such as the radial arm maze (62), contextual fear conditioning (28), and the MWM. Notably, BDNF mutant mice exhibit significant impairments in spatial learning and memory functions (63-65). In our study, the MWM task, used to test spatial reference memory, utilized a circular pool filled with water in a specific room, where spatial fixed cues were placed at levels visible to the rats on the walls of the room. Although the neuroanatomical basis for this is not yet fully elucidated, there is evidence that rats possess a cognitive map representing the spatial characteristics of the testing environment in their hippocampus (66-68), and it is suggested that rats use these spatial cues in forming this representational map. There are few studies investigating the effects of exogenous BDNF application in the MWM task. For example, Cirulli et al. (69) found that intraventricular (ICV) BDNF injections did not affect MWM performance. Similarly, Fischer et al. (70) demonstrated that ICV BDNF application did not alter the MWM performance of aged rats with cholinergic neuron damage. These findings suggest that the method and/or duration of BDNF application may be responsible for these outcomes. TrkB receptors are expressed in both the ventricular ependymal layer and throughout the brain, so ICV BDNF application may have reduced the likelihood of reaching target tissues (71). In another study by Cirulli et al. (72) BDNF was administered as a single injection into the right hippocampus of rats, and after one day of training in the MWM, they evaluated the rats' short-term spatial memory performance. However, they again found no significant difference compared to the control group. This finding suggests that BDNF may play a more critical role in regulating long-term memory than in short-term memory. Therefore, in the present study, we aimed to directly apply BDNF to the right hippocampus through long-term infusion and to test long-term memory in the MWM task. According to our findings, although there was no significant difference between the BDNF group and the control group in the parameters assessed during the acquisition and testing phases of the MWM task (Figures 2-4, Tables 1-3), the EG rats exhibited a relatively better performance trend in the observed parameters. The lack of statistical significance in this trend may be due to the small sample size in the groups for reasons beyond our control; thus, future studies with a larger sample size could yield more consistent results. The fact that the mean swimming speeds of the animals were significantly higher at the beginning of the acquisition phase but slowed down toward the end may reflect a behavioral pattern associated with increased spatial learning. Similarly, the significantly slower swimming of EG rats compared to controls during the testing phase may also be interpreted as a reflecting difference. Another factor contributing to the perception of relatively better spatial memory performance is that the mean number of crossings at the escape platform location for EG rats [mean: 54 $\pm$ standard deviation (SD): 15] was relatively higher compared to controls (mean: 34 $\pm$ SD: 13). However, due to the absence of significant differences in overall performance results, it can be stated that the distinct molecular effects of BDNF application on AMPAR GluR1 subunit levels did not manifest as significant differences at the behavioral level. Nevertheless, some studies indicate that there may be differences in the performance strategies of rats in the MWM task, which could affect the results when evaluating measurement parameters that are considered standard for the task (73). The lack of significant differences in our behavioral findings between the groups may be due to the fact that the spatial memory strategies used by the animals in this study were not specifically assessed. Similarly, we believe it would be beneficial for future studies to evaluate the performance of animals from this perspective.

Conclusion

In conclusion, it was found that the chronically administered BDNF protein significantly increased the immunoreactivity of

the AMPAR GluR1 subunit in the right hemisphere of the EG group. Furthermore, this immunoreactivity was observed not only in the cell membrane but also in the cytoplasm due to the transmembrane nature of the receptor. Interestingly, a moderate effect was observed in the left hemisphere, where BDNF was not directly administered.

Ethics

Ethics Committee Approval: This study was approved by the Gazi University Local Ethics Committee for Animal Experiments (decision no: E.28661, date: 16.08.2017).

Informed Consent: In this study, we utilized adult female Long-Evans rats (n=10), each weighing between 200-250 g. The animals were obtained from the Gazi University Laboratory Animals Breeding and Experimental Research Center (GUDAM). All procedures involving the rats were conducted in the designated laboratory settings of GUDAM.

Acknowledgments

We would like to thank the expert and staff members at the Gazi University Laboratory Animal Breeding and Experimental Research Center. We also extend our gratitude to young researcher Batuhan Sözer for his contributions in preparing Python code for data analysis from the video recordings of the MWM task, which facilitated a detailed examination of this aspect of the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.S.B., Concept: B.S.B., Ç.Ö., Design: B.S.B., Ç.Ö., Data Collection and/or Processing: B.S.B., C.M.S., Analysis and/or Interpretation: B.S.B., Ç.Ö., C.M.S., Literature Search: B.S.B., Ç.Ö., Writing: B.S.B., C.M.S.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: This study is supported by the Gazi University Scientific Research Projects Coordination Unit with the decision number 01/2017-26.

References

1. Kandel ER, Schwards JH, Jessel TM, et al. Principles of neural science. 5th ed. New York, USA: McGrow Hill; 2013.
2. Lynch MA. Long-term potentiation and memory. Physiol Rev. 2004;84:87-136.
3. Leal G, Afonso PM, Salazar IL, et al. Regulation of hippocampal synaptic plasticity by BDNF. Brain Res. 2015;1621:82-101.
4. Lu Y, Christian K, Lu B. BDNF: A key regulator for protein synthesis-dependent LTP and long-term memory? Neurobiol Learn Mem. 2008;89:312-323.
5. Anggono V, Huganir RL. Regulation of AMPA receptor trafficking and synaptic plasticity. Curr Opin Neurobiol. 2012;22:461-469.
6. Bramham CR, Messaoudi E. BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. Prog Neurobiol. 2005;76:99-125.
7. Jensen V, Kaiser KMM, Borchardt T, et al. A juvenile form of postsynaptic hippocampal long-term potentiation in mice deficienct for the AMPA receptor subunit GluR-A. J Physiol. 2003;553:843-856.
8. Lee HK, Takamiya K, He K, et al. Specific roles of AMPA receptor subunit GluR1 (GluA1) phosphorylation sites in regulating synaptic plasticity in the CA1 region of hippocampus. J Neurophysiol. 2010;103:479-489.
9. Lessmann V. Neurotrophin-dependent modulation of glutamatergic synaptic transmission in the mammalian CNS. Gen Pharmacol. 1998;31:667-674.
10. Lewin GR, Barde Y-A. Physiology of the neurotrophins. Annu Rev Neurosci. 1996;19:289-317.
11. Lewin GR, Carter BR. Neurotrophic factors. In: Lewin GR, Carter BR, authors. Handbook of experimental pharmacology. 49th vol. Berlin, Germany: Springer-Verlag; 2014. s.3-251.
12. Patterson SL, Pittenger C, Morozov A, et al. Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. Neuron. 2001;32:123-140.
13. Minichiello L, Korte M, Wolfer D, et al. Essential role for TrkB receptors in hippocampus-mediated learning below a certain threshold before behavioral defects become apparent. neuron. 1999;24:401-414.
14. Korte M, Griesbeck O, Gravel C, et al. Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. Proc Natl Acad Sci U S A. 1996;93:12547-12552.
15. Figurov A, Pozzo-Miller LD, Olafsson P, et al. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature. 1996;381:706-709.
16. Kang HJ, Schuman EM. Neurotrophin-induced modulation of synaptic transmission in the adult hippocampus. J Physiol - Paris. 1995;89:11-22.
17. Ji Y, Lu Y, Yang F, et al. Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. Nat Neurosci. 2010;13:302-309.
18. Xu B, Michalski B, Racine RJ, et al. The effects of brain-derived neurotrophic factor (BDNF) administration on kindling induction, Trk expression and seizure-related morphological changes. Neuroscience. 2004;126:521-531.
19. Scharfman H, Goodman J, Macleod A, et al. Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. Exp Neurol. 2005;192:348-356.
20. Ye Y, Wang G, Wang H, et al. Brain-derived neurotrophic factor (BDNF) infusion restored astrocytic plasticity in the hippocampus of a rat model of depression. Neurosci Lett. 2011;503:15-19.
21. Narisawa-Saito M, Silva AJ, Yamaguchi T, et al. Growth factor-mediated Fyn signaling regulates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor expression in rodent neocortical neurons. Proc Natl Acad Sci USA. 1999;96:2461-2466.
22. Itami C, Kimura F, Kohno T, et al. Brain-derived neurotrophic factor-dependent unmasking of "silent" synapses in the developing mouse barrel cortex. Proc Natl Acad Sci U S A. 2003;100:13069-13074.
23. Narisawa-Saito M, Iwakura Y, Kawamura M, et al. Brain-derived neurotrophic factor regulates surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptors by enhancing the N-ethylmaleimide-sensitive factor/GLuR2 interaction in developing neocortical neurons. J Biol Chem. 2002;277:40910.
24. Jourdi H, Iwakura Y, Narisawa-Saito M, et al. Brain-derived neurotrophic factor signal enhances and maintains the expression of AMPA receptor-associated PDZ proteins in developing cortical neurons. Dev Biol. 2003;263:216-230.
25. Caldeira MV, Melo CV, Pereira DB, et al. Brain-derived neurotrophic factor regulates the expression and synaptic delivery of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunits in hippocampal neurons. J Biol Chem. 2007;282:12619-12628. Erratum in: J Biol Chem. 2007;282:27556.
26. Li W, Keifer J. BDNF-induced synaptic delivery of AMPAR subunits is differentially dependent on NMDA receptors and requires ERK. Neurobiol Learn Mem. 2009;91:243-249.
27. Jourdi H, Kabbaj M. Acute BDNF treatment upregulates GluR1-SAP97 and GluR2-GRIP1 interactions: implications for sustained AMPA receptor expression. PLoS One. 2013;8:e57124.

28. Liu F, Day M, Muñiz LC, et al. Activation of estrogen receptor-beta regulates hippocampal synaptic plasticity and improves memory. Nat Neurosci. 2008;11:334-343.
29. Woolley CS. Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus. Horm Behav. 1998;34:140-8.
30. Levin ER. Cellular functions of plasma membrane estrogen receptors. Steroids. 2002;67:471-475.
31. Scharfman HE, Maclusky NJ. Similarities between actions of estrogen and BDNF in the hippocampus: coincidence or clue? Trends Neurosci. 2005;28:79-85.
32. Zhou J, Zhang H, Cohen R, et al. Effects of estrogen treatment on expression of brain-derived neurotrophic factor and cAMP response element-binding protein expression and phosphorylation in rat amygdaloid and hippocampal structures. Neuroendocrinology. 2005;81:294-310.
33. Allen A, McCarson K. Estrogen increases nociception-evoked brain-derived neurotrophic factor gene expression in the female rat. Neuroendocrinology. 2005;81:193-199.
34. Sohrabji F, Lewis DK. Estrogen-BDNF interactions: implications for neurodegenerative diseases. Front Neuroendocrinol. 2006;27:404-14.
35. Bimonte-Nelson H, Nelson M, Granholm AG. Progesterone counteracts estrogen-induced increases in neurotrophins in the aged female rat brain. Neuroreport. 2004;15:2659-2663.
36. Gibbs R. Levels of trkA and BDNF mRNA, but not NGF mRNA, Fluctuate across the estrous cycle and increase in response to acute hormone replacement. Brain Res. 1998;787:259-268.
37. Cavus I, Duman R, Influence of estradiol, stress, and 5-HT2A agonist treatment on brain-derived neurotrophic factor expression in female rats. Biol. Psychiatry. 2003;54:59-69.
38. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006;1:848-858.
39. Lebron-Milad K, Milad MR. Sex differences, gonadal hormones and the fear extinction network: implications for anxiety disorders. Biol Mood Anxiety Disord. 2012;7:2-3.
40. Mamounas LA, Altar CA, Blue ME, et al. BDNF promotes the regenerative sprouting, but not survival, of injured serotonergic axons in the adult rat brain. J Neurosci. 2000;20:771-782.
41. Paxinos, G, Watson C. The rat brain in stereotaxic coordinates. 4th ed. San Diego, CA, USA: Academic Press Inc; 1998.
42. D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Res Rev. 2001;36:60-90.
43. Ünal SG, Take G, Erdoğan D, et al. The effect of di-n-butyl phthalate on testis and the potential protective effects of resveratrol. Toxicol Ind Health. 2016;32:777-790.
44. Tomar, S. Converting video formats with FFmpeg. Linux Journal. 2006;146:10.
45. Wada, K. Image polygonal annotation with Python (polygon, rectangle, circle, line, point and image-level flag annotation). 2018. Available from: https://github.com/wkentaro/labelme
46. van Rossum, Guido. Python Reference Manual. Department of Computer Science [CS]. Centrum voor Wiskunde en Informatica (CWI). Amsterdam; 1995.
47. JASP Team, JASP [Computer software]. Version 0.18.3; 2024. Available from: https://jasp-stats.org/
48. Leal G, Comprido D, Duarte CB BDNF-induced local protein synthesis and synaptic plasticity. Neuropharmacology. 2014;76:639-656.
49. Fortin DA, Srivastava T, Dwarakanath D, et al. Brain-derived neurotrophic factor activation of CaM-kinase kinase via transient receptor potential canonical channels induces the translation and synaptic incorporation of GluA1-containing calcium-permeable AMPA receptors. J Neurosci. 2012;32:8127-8137.
50. Bi R, Broutman G, Foy MR, et al. The tyrosine kinase and mitogen-activated protein kinase pathways mediate multiple effects of estrogen in hippocampus. Proc Natl Acad Sci U S A. 2000;97:3602-3607.
51. Blanquet PR, Mariani J, Derer P. A calcium/calmodulin kinase pathway connects brain-derived neurotrophic factor to the cyclic AMPresponsive transcription factor in the rat hippocampus. Neuroscience. 2003;118:477-490.
52. McEwen BS. Invited review: Estrogens effects on the brain: multiple sites and molecular mechanisms. J. Appl. Physiol. 2001;91:2785-2801.
53. Blurton-Jones M. Kuan PN, Tuszynski MH. Anatomical evidence for transsynaptic influences of estrogen on brain-derived neurotrophic factor expression. J Comp Neurol. 2004;468:347-360.
54. Luine V, Frankfurt M. Interactions between estradiol, BDNF and dendritic spines in promoting memory. Neuroscience. 2013;3:34-45.
55. Laurberg S, Sorensen KE. Associational and commissural collaterals of neurons in the hippocampal formation (hilus fasciae dentate and subfield CA3). Brain Res. 1981;212:287-300.
56. Amaral DG, Scharfman HE, Lavenex P. The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). Prog Brain Res. 2007;163:3-22.
57. Matteo Egger, Wenshu Luo, Natalia Cruz-Ochoa, et al. Commissural dentate granule cell projections and their rapid formation in the adult brain. PNAS Nexus. 2023;2:pgad088.
58. Buckmaster PS, Strowbridge BW, Kunkel DD, et al. Mossy cell axonal projections to the dentate gyrus molecular layer in the rat hippocampal slice. Hippocampus. 1992;2:349-362.
59. Buckmaster PS, Wenzel HJ, Kunkel DD, et al. Axon arbors and synaptic connections of hippocampal mossy cells in the rat in vivo. J Comp Neurol. 1996;366:271-292.
60. Soriano E, Frotscher M. Mossy cells of the rat fascia dentata are glutamate-immunoreactive. Hippocampus. 1994;4:65-69.
61. Scharfman HE. Electrophysiological evidence that dentate hilar mossy cells are excitatory and innervate both granule cells and interneurons. J Neurophysiol. 1995;74:179-194.
62. Mizuno M, Yamada K, Olariu A, et al. Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. J Neurosci. 2000;20:7116-7121.
63. Linnarsson S, Björklund A, Ernfors P. Learning deficit in BDNF mutant mice. Eur J Neurosci. 1997;9:2581-2587.
64. Gorski JA, Balogh SA, Wehner JM, et al. Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice. Neuroscience. 2003;121:341-354.
65. Heldt SA, Stanek L, Chhatwal JP, et al. Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. Mol Psychiatry. 2007;12:656-670.
66. Deshmukh SS, Knierim JJ. Hippocampus. Wiley Interdiscip Rev Cogn Sci. 2012;3:231-251.
67. Eichenbaum H. The Hippocampus as a Cognitive Map ... of Social Space. Neuron. 2015;1:9-11.
68. Best PJ, White AM, Minai A. Spatial processing in the brain: the activity of hippocampal place cells. Annu Rev Neurosci. 2001;24:459-486.
69. Cirulli F, Berry A, Alleva E. Intracerebroventricular administration of brain-derived neurotrophic factor in adult rats affects analgesia and spontaneous behaviour but not memory retention in a Morris Water Maze task. Neurosci Lett. 2000;287:207-210.
70. Fischer W, Sirevaag A, Wiegand SJ, et al. Reversal of spatial memory impairments in aged rats by nerve growth factor and neurotrophins 3 and 4/5 but not by brain-derived neurotrophic factor. Proc Natl Acad Sci USA. 1994;91:8607-8611.
71. Yan Q, Matheson C, Sun J, et al. Distribution of intracerebral ventricularly administered neurotrophins in rat brain and its correlation with trk receptor expression. Experimental Neurology. 1994;127:23-36.
72. Cirulli F, Berry A, Chiarotti F, et al. Intrahippocampal administration of BDNF in adult rats affects short-term behavioral plasticity in the Morris water maze and performance in the elevated plus-maze. Hippocampus. 2004;14:802-807.
73. Daniel JM, Lee CD. Estrogen replacement in ovariectomized rats affects strategy selection in the Morris water maze. Neurobiol Learn Mem. 2004;82:142-149.