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Neuregulin1β improves both spatial and associative learning and memory in Alzheimer model of rats possibly through signaling pathways other than Erk1/2

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ABSTRACT

Background: Neuregulin-1β (NRG1 β) is associated with various neurological disorders such as schizophrenia, depression and Parkinson's disease. However, its role in Alzheimer's (AD) has not been understood yet. Here, we have studied the effect of NRG1 β and extracellular-signal-regulated kinase (ERK) signaling on special and associative memories and emotional stress in AD model of rats.

Methods: Fifty six male Wistar rats were divided into eight groups of: Saline + Saline, Aβ + Saline, Aβ + NRG1β (5 μg/5 ul), Aβ + PBS, Aβ + NRG1β + PD98059 (PD, 5 μg/2 μl), Aβ + NRG1β + Saline and Saline + PD. AD model was induced by intracerebroventricular (ICV) injection of beta-amyloid protein (Aβ1-42, 4 μg/2 μl). The cognitive performances of rats were evaluated using Morris Water Maze (MWM) and Step through passive avoidance. Also locomotors activity and emotionality of animals were considered in an Open field test. Data were analyzed by one way Anova one way, repeated measure and T-test.

Results: Significant improvement was found in spatial learning and memory assessed by total time spent in target quadrant [$F(4, 32) = 12.4$, $p = 0.001$], escape latency [$F(4, 32) = 15.767$, $p = 0.001$] and distance moved [$F(4, 32) = 5.55$, $p = 0.002$], in Aβ + NRG1β compared with Aβ + Saline in MWM. Also Aβ + NRG1β showed long latencies to enter into the dark compartment [$F(4, 32) = 6.43$, $p = 0.001$], but short time spent [$F(4, 32) = 6.93$, $p = 0.001$] compared with control. Administration of an ERK inhibitor (PD98059, 5 μg, 15 min before NRG1β) didn't completely block learning memory restored by NRG1β in AD model ($p = 0.7$). No significant between groups differences was found in emotional stress characteristics in open field, except the grooming numbers which were higher in Saline + PD compared with Saline + Saline ($p = 0.02$).

Conclusion: Our findings indicate that NRG1β restores cognitive dysfunctions induced by amyloid β through signaling pathways possibly other than Erk1/2, with no significant change in anxiety, locomotion and vegetative activities.

1. Introduction

Alzheimer's disease is the most common age-related disorder (Association, 2013), with affected 50 million victims worldwide currently, and it is predicted to reach over 152 million in 2050 (Patterson, 2018). Despite tremendous literature on AD, the etiology of this disease remains unclear, with multiple complex hypotheses such as oxidative stress, metabolic disturbances, mitochondrial dysfunction, calcium deregulation, disturbed neurotransmission and finally increasing apoptotic pathways activities (Culmsee and Landshamer, 2006). In the brains of patients with AD, abnormally amyloid polypeptide (Aβ1-42) tends to be aggregated and accumulated in extracellular and disturbs the synaptic connections (Price et al., 2014). Therefore, AD is mostly characterized by the deposition of Aβ and extensive degeneration of synapses and neurons (Barage and Sonawane, 2015; Goedert and Spillantini, 2006).

Current therapies including memantine and donepezil do not show disease-modifying effects in patients and they only ameliorate disease symptoms for a distinct period (Wang et al., 2018). Thus, strategies to find new and effective treatments have got great importance for health care organizations.

Recently neurotrophic factors have got attention in different

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neurodegenerative diseases considering their potency to modulate neurotransmission (Su et al., 2014), synaptogenesis and neurogenesis (Poon et al., 2013). Among them, neuregulins are members of neurotrophic family encoded by four genes (*NRG1–4*), all of which act through ErbB tyrosine and activate different signaling pathways (Wang et al., 2018) responsible for growth and differentiation (Marchionni, 2014).

In addition, NRG1β-ErbB4 signaling has a key role in neurotransmission, synaptic plasticity, and synchronization of neuronal network activity in the cortex and hippocampus, which is of great importance for cognition, learning, and memory (Chen et al., 2011).

Neuregulin1β and its main receptor ErbB4 wildly found in the hippocampus, cerebral cortex, Piriform cortex, reticular nucleus of the thalamus, cholinergic nucleus of diencephalon, choroid plexus, cerebellar tentorium and ventral midbrain (Rösler et al., 2011).

Genetic association studies are providing growing evidences that NRG1β and ErbB genes are associated with complex brain disorders, such as schizophrenia, bipolar disorder, and depression. Also NRG1β has been reported to prevent brain injury following stroke (Xu et al., 2006), and to exert a neuro protective role in a mouse model of Parkinson's disease (Carlsson et al., 2011), traumatic brain injury (Cespedes et al., 2018) and AD (Jiang et al., 2016).

On the other hand, NRG1β production is thought to be regulated by neuronal activity, in a way that, an optimal level of NRG1β/ERBB signaling is required for normal brain functions (Mei and Nave, 2014). Both transgenic mice overexpressing neuronal *Nrg1* and *Nrg1* heterozygous mouse mutants exhibited impaired working memory, contextual fear conditioning, and social interaction(Yin et al., 2013).

In addition, disruption of NRG1β signaling is closely linked to the pathogenesis of AD (Ryu et al., 2017) and pretreatment with this neuropeptide prevents Aβ-induced impairment of long-term potentiation in hippocampal slices (Min et al., 2011). Also NRG1β-ErbB4 has been known to activate multiple signal transduction pathways such as PI3K–Akt, ERK1/2, FAK, Rac1, cdc42, and calcineurin to serve various physiological responses. Among them, ERK1/2 has been implicated in the activation of transcription processes during LTM formation (Sherrin et al., 2011).

Given the alteration of NRG1β signaling in patients with neurodegenerative diseases, and potency of NRG1β in neurotransmission, neuroprotection in both spine and central nervous system (Kilic et al., 2006; Mei and Nave, 2014), we hypothesized that NRG1β may alleviate cognition deficit induced by Aβ injection.

ERK signaling pathway is best known for its physiological regulatory roles in nucleus and variety of organelles including the endoplasmic reticulum, endosomes, golgi and mitochondria (Cook et al., 2017). Here we assumed that NRG-1 might play significant neuroprotective role in AD via ERK signaling. To approach this, we used a selective cell-permeable inhibitor of ERK1/2, PD098059 (Alessi et al., 1995) and assessed short and long term memories in Morris water maze (MWM) and Step through passive avoidance. Also emotional and locomotor reactions of animals to all drugs were evaluated by open field test.

2. Material and methods

2.1. Animals

Fifty six adult male Wistar rats weighing 200–220 g were kept in a room with 12/12 h light/dark cycle (lights on 7:00 h) and temperature of 22 ± 2 °C and fed standard-pellet rat chow and tap water ad libitum. The following groups were included in the study (8 animals per each group): Saline + Saline, Aβ + Saline, Aβ + NRG1β, Aβ + PBS, Saline + PD98059, Aβ + NRG1β + PD98059, Aβ + NRG1β + Saline. The study was approved by ethical committee of Guilan University of Medical Sciences, Rasht, Iran, IR.GUMS.REC.1396.264.

2.2. Surgery

Animals were handled and adapted to the experiment room and experimenter for 10 days. Then were deeply anesthetized with (75 mg/kg of ketamine (TRITTAU, Germany) and 5 mg/kg of xylazine (SciENcelab, Hoston), and placed in stereotaxic apparatus (Stoelting, USA) for bilaterally implantation of cannula into the ventricle according to the coordination's of: AP = −0.8 mm, DV = −3.6 mm, ML = ± 1.6 mm (During et al., 2003) (Paxinos and Watson).

2.3. Drugs & treatments

One week after the recovery period, Aβ1-42 (Sigma-Aldrich, USA, 4 μg/2 μl) dissolved in 0.9% saline and was injected in a volume of 2 μl into the right lateral cerebral ventricle using Hamilton micro syringe pump for 3 min. NRG1β (R&D Systems, Inc., Catalog number: 396-HB, 5 μg/5 ul) was dissolved in PBS and was injected through the right lateral ventricle one week after Aβ1-42 injection, and continued until the day of 14th which behavioral tests were performed.

Antagonist of ERK1/2, PD98059 (Sigma-Aldrich, USA, 5 μg/2 μl, ICV), was dissolved in Saline and DMSO 5% and injected 15 min before NRG1β in the group of AD + NRG1β + PD98059. All controls group received the same volume of related vehicles (saline + DMSO) in same time and status.

3. Behavioral test

3.1. Morris water maze

Spatial learning and memory of animals were assessed using Morris water maze (MWM) task. The apparatus consisted of a circular water tank (148 cm diameter and 60 cm high) with a rectangular platform (10 cm) at a fixed position in the target quadrant, 1.5 cm below the water level. The water temperature maintained at 26 °C. The protocol used for MWM included 4 blocks of working memory and one reference memory or probe test. Each block consisted of 4 trials and each trial lasted for 90 s with an interval of 30 min. Animals individually performed MWM task in order to find the hidden platform based on special cues on the walls. Also, visible test was carried out after the probe test to examine the animal's vision. Total time spent in the target quadrant (TTS), escape latency time, distance to reach the platform, and velocity of swimming were recorded using camera and “Ethovision 12 Noldus” tracking system (Netherland) based on our previous protocol (Babaei et al., 2017).

3.2. Step through passive avoidance learning

Long-term memory was evaluated by the step-through passive avoidance apparatus for 3 day after the last injection of NRG1β and PD. The apparatus consisted of two compartments of dark and light boxes separating with a guillotine door. The floor of the dark compartment consisted of a stainless steel shock grid.

First, animals were habituated to the apparatus and room for 3 days. In training day animals were placed individually in light room, then after 20s, the guillotine door was opened, and the initial latency to entering the dark compartment was recorded. Immediately after the rat entered the dark compartment, the guillotine door was closed and an electric foot shock (0.5 mA, 100 Hz) was delivered to the floor grids for 5 s. Five seconds later, the rat was removed from the dark compartment and returned to its home cage. After 24 h, the retention latency was measured placing animal in the light part and recording the latency to enter to the dark part, with no foot shock delivery. (Jee et al., 2008).

A diagram summarizing the experimental procedure is provided in Fig. 1.

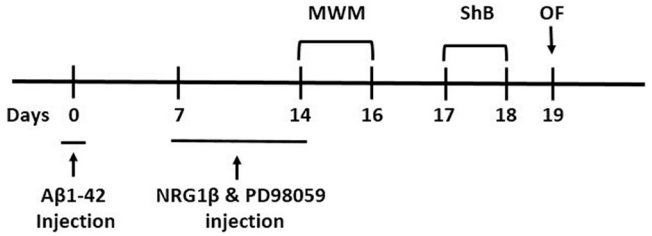

Fig. 1. Experimental design of ICV infusion of Aβ, NRG1β peptide, PD98059 and behavioral experiments.

3.2.1. Open field

The open field apparatus was constructed of white cylindrical wooden measured diameter of 72 cm with 36 cm height. The floor was divided into 3 concentric circles (1peripheral and 2 central) and 16 quadrants (18 × 18 cm squares) labeled by black lines. The open field was located in the test room and lit by a 60-watt red lamp for background lighting. Rats were carried to the test room and were gently placed into the open field and allowed to explore the apparatus for 5 min. After the 5 min test, rats were returned to their home cages and the open field was cleaned with 70% ethyl alcohol and permitted to dry between tests to avoid stress for next animal (Brown et al., 1999; Babaei et al., 2017). Variables were measured in open field included: first latency to move, first latency to enter to the center of the field, duration and number of grooming,freezing, rearing in central and peripheral, ambulation in central and peripheral, exploration, and finally two vegetative activities of urination and defecation.

3.3. Statistical analysis

Normality of variables was estimated by Shapiro and Kolmogorov-Smirnov tests. The data were evaluated statistically using Student's t test, with $p < 0.05$ indicating a significant difference between the control and experimental groups.

Also repeated measure and one-way ANOVA with Tukey's posttest were used for comparing between group differences and level of significance was $P < 0.05$ in all statistical evaluations. Data were analyzed in SPSS version 23, and expressed as means ± S.D.

4. Results

4.1. Morris water maze

To test whether exogenous NRG1 improves cognitive function in AD model, it was stereotaxically injected into the ventricle after 7 days of Aβ1-42 injection, and two weeks later performance in the Morris water maze was evaluated. Latency to find the escape platform, total time spent in the target quadrant (TTS), swimming speed and distance move toward platform were measured. The means of latency or distance across all four blocks were calculated.

4.1.1. Working memory

Repeated measure and one-way ANOVA analyses showed significant between groups differences in escape latency time in the acquisition phase in Block1: [$F(4, 15) = 6.68$, $p = 0.003$], Block2: [$F(4, 15) = 12.5$, $p = 0.001$], Block3: [$F(4, 15) = 4.7$, $p = 0.01$, Fig. 1].

The group of Aβ + NRG1β showed significant lower escape latency in Block1 ($p = 0.02$), B2 ($p = 0.001$) and Block 3 ($p = 0.03$) compared with Aβ + PBS, indicates that acquisition of NRG1 β receiving group improved significantly (Fig. 2).

As Fig. 1 shows, escape latency time was increased significantly in Saline + PD98059 compared with Saline + saline in Block 1 ($p = 0.02$) and Block2 ($p = 0.04$), which shows that PD98059 disrupted learning and memory in control rats successfully.

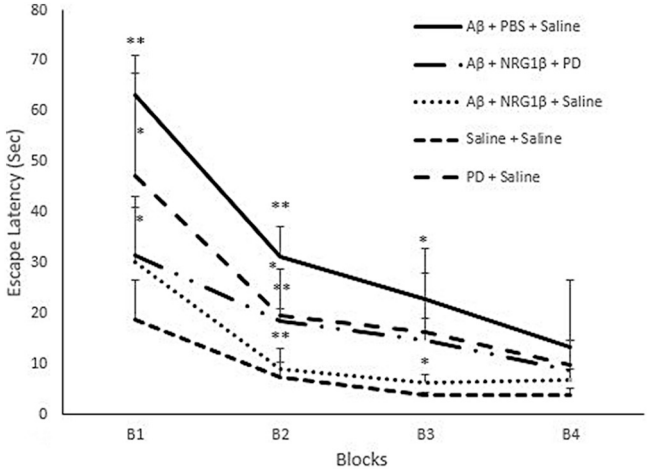

Fig. 2. Effect of NRG1β treatment on the escape latency time in 4 blocks in the acquisition phase of spatial memory in MWM. *P < 0.05, **P < 0.01, compared with their respected control group. Repeated measure ANOVA followed by Tukey's post-hoc tests were used and data are expressed as mean ± SD, n = 8 rats per group.

4.1.2. Reference memory

One way ANOVA revealed significant between-group differences in total time spent in the target quadrant (TTS) during the probe test which platform has been removed [$F(4, 32) = 12.4$, $p = 0.001$]. TTS was significantly decreased in Aβ group compared with control ($p = 0.001$), and was restored in Aβ + NRG1β compared with Aβ + PBS ($p = 0.001$), indicating improvement in memory retrieval in Alzheimer's rats. The group of Saline + PD98059 showed deficits in memory retrieval compared with control group representing lower TTS ($p = 0.001$, Fig. 3).

In addition, there was a significant between group's differences in escape latency in probe test analyzed by one way ANOVA, [$F(4, 32) = 15.767$, $p = 0.001$], in a way that it was significantly increased in Aβ treating group compared with control ($p = 0.001$), which confirms deficits in spatial memory retrieval induced by amyloid beta injection, (Fig. 4). However, escape latency was decreased in Aβ + NRG1β compared with Aβ receiving group ($p = 0.001$), showing facilitating effects of NRG1 on reference memory. Group of Saline + PD98059 which received the same dose of PD98059 similar to the

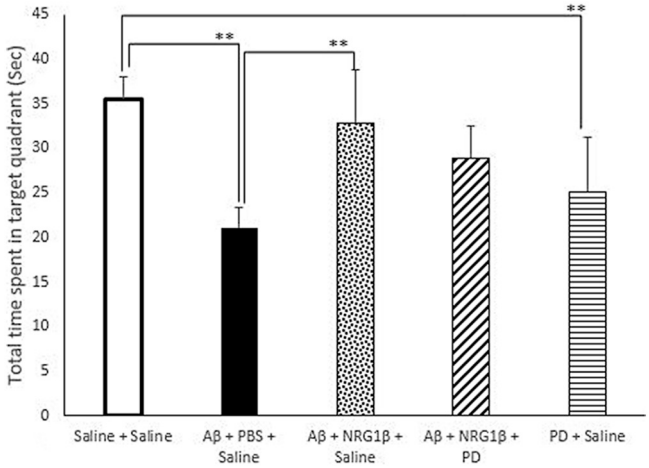

Fig. 3. Effect of NRG1β treatment on Aβ - induced memory impairment in rats: Total time spent in the target quadrant in probe trial of spatial memory. *P < 0.05, **P < 0.01, compared with control group. One-way ANOVA followed by Tukey's post-hoc tests were used to calculate p values. Data values are expressed as mean ± SD, n = 8 rats per group.

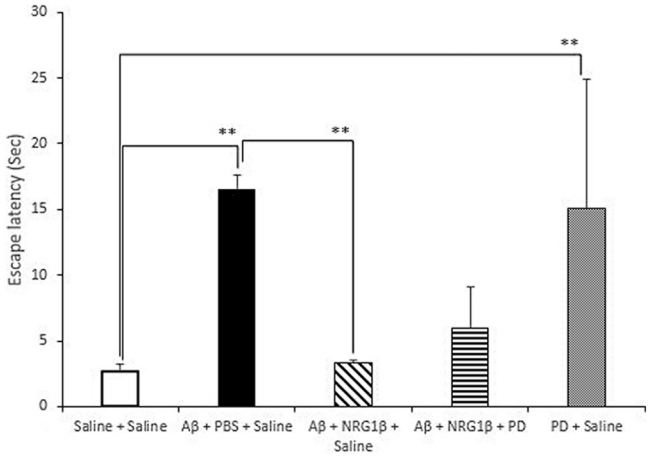

Fig. 4. Effect of NRG1β treatment on Aβ induced memory impairment in rats: escape latency time in probe trial of spatial memory. *P < 0.05, **P < 0.01, compared with control group. One-way ANOVA followed by Tukey's post-hoc tests were used to calculate p values. Data values are expressed as mean ± SD, n = 8 rats per group.

Aβ + NRG1β + PD98059, represented shorter latency escape compared with saline control group (p = 0.001, Fig. 4). However, no significant change was found in escape latency of Aβ + NRG1β + PD98059 compared with Aβ + NRG1β + Saline, which represents that NRG1 β might restores both working and reference memories at least partly via mechanisms other than ERK (Fig. 4).

Also there was a significant between group differences in distance move toward platform in probe test [F (4, 32) =5.55, p = 0.002] so that, Aβ and PD receiving groups showed longer distances in comparison to its counterparts (p = 0.01), and Aβ + NRG1β showed shorter path (p = 0.01) same as Saline control group. No significant difference was found between Aβ + NRG1β + PD98059 and Aβ + NRG1β. Finding of the variable of distance moved also confirmed poor memory in Aβ + PBS and Saline +PD receiving groups, in comparison to NRG1 β treated groups (Fig. 6).

Furthermore, our data demonstrated that there was no significant difference in swimming speed [F (4, 32) =2.023, p = 0.1], therefore, latency time, distance to find the platform and total time spent in the target quadrant (TTS) were used as indicators of memory retrieval, but not locomotion and anxiety (Fig. 5).

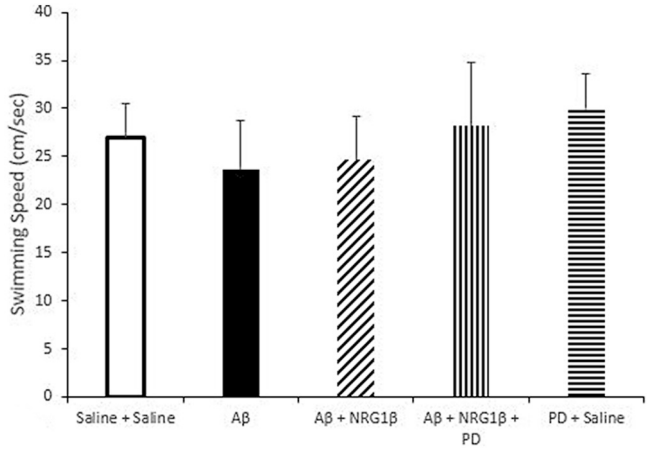

Fig. 5. Comparison of swimming speed in MWM among groups. One-way ANOVA followed by Tukey's post-hoc tests were used to calculate p values. Data values are expressed as mean ± SD, n = 8 rats per group.

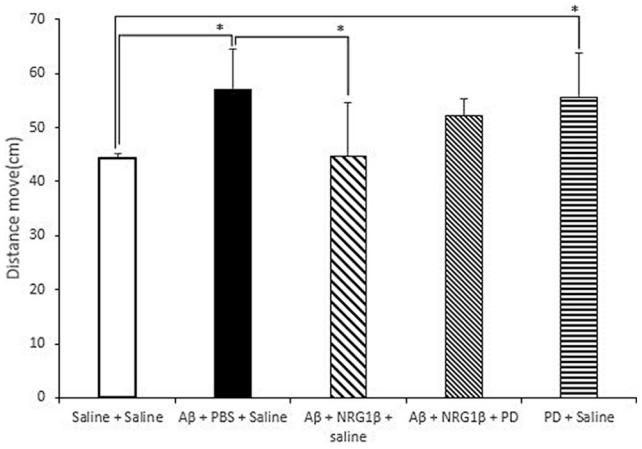

Fig. 6. Effect of NRG1β treatment on the distance move in MWM. *P < 0.05, **P < 0.01, compared with their respected control group. One-way ANOVA followed by Tukey's post-hoc tests were used to calculate p values. Data values are expressed as mean ± SD, n = 8 rats per group.

4.3. Step-through passive avoidance task

There was a significant between groups differences in the latency to enter to the dark compartment and total spent in dark compatment as well [F (4, 32) =6.43, p = 0.001]. Aβ + Saline exhibited a shorter latency to enter to the dark room (p = 0.002), but longer total time spent there (p = 0.01), which represents learning and memory impairment in Aβ group.

Aβ + NRG1β group showed significant latency to enter to the dark room (p = 0.001) and short TTS (p = 0.003) which represents learning and memory improvement in A β + NRG1β group (Fig. 7).

Also first latency to the dark room significantly was decreased (p = 0.009; Fig. 8) and TTS was increased in Saline + PD98059 group compared with control group (p = 0.03, Fig. 9). Less time for latency to enter to the dark compartment reflects learning and memory impairment in this group.

No significant change was found in neither of first latency nor total time spent in dark room between Aβ + NRG1β + PD98059 compared with Aβ + NRG1β + Saline, which confirm that NRG1 β might facilitates associative learning and memory possibly via mechanisms other than ERK signaling (p > 0.05, Figs. 8, 9).

4.4. Open field

To assess locomotor activities and emotionality of animals, first latency to move, grooming (duration and number), freezing, number of ambulation in peripheral and central, exploration, number of rearing in peripheral and also central of the arena, urination and defecation in the open-field test were measured.

Data analyzed by ANOVA showed significant difference, only between Aβ and NRG1β in first latency move to the center [F (4, 31) =16.75, p = 0.001], and Aβ showed more latency. Interestingly a significant between group differences was found in duration of grooming [F (4, 31) =4.876, p = 0.008, Fig. 10], and also numbers of grooming [F (4, 31) =3.395, p = 0.01], so that, PD + Saline represented more duration of grooming in comparison to intact animals.

No significant change was found in first latency to move in peripheral [F (4, 31) =0.924, p = 0.4] and freezing [F (4, 31) =2.011, p = 0.1] as well, Fig. 10.

However, no significant change was found in peripheral ambulation (p) [F (4, 31) =0.95, p = 0.4], central ambulation [F (4, 31) =2.61, p = 0.054] exploration [F (4, 31) =2.3, p = 0.07 Fig. 11], peripheral rearing [F (4, 31) =1.23, p = 0.3], urination [F (4, 31) =1.63, p = 0.1], defecation [F (4, 31) =2.36, p = 0.07] and central rearing

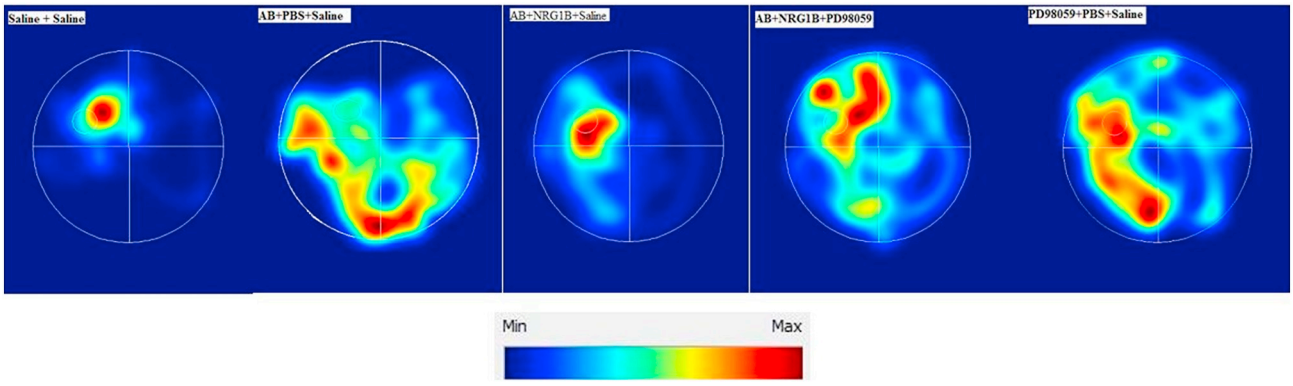

Fig. 7. Effect of NRG1β treatment on Aβ (ICV) induced memory impairment in rats: Heat maps showing the representatives Morris water maze tracking of groups during retrieval trial, increasing color intensity (arbitrary scale) represents increased time spent.

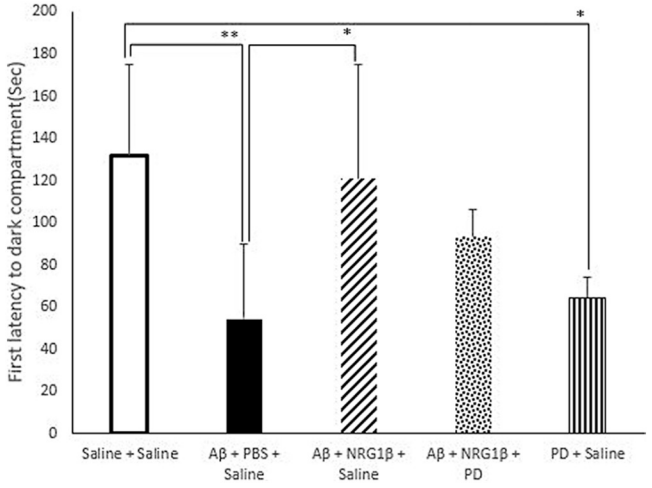

Fig. 8. Effect of NRG1β treatment on the latency to enter into the dark compartment in the step-through passive avoidance task. *P < 0.05, **P < 0.01, compared with their respected control group. One-way ANOVA followed by Tukey's post-hoc tests were used to calculate p values. Data values are expressed as mean ± SD, n = 8 rats per group.

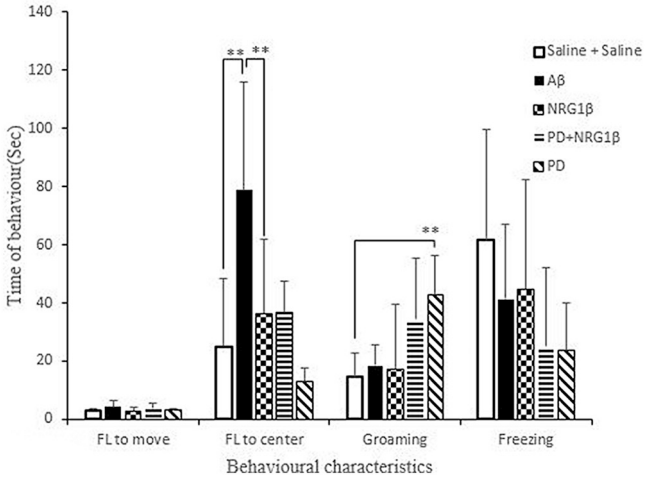

Fig. 10. Effect of NRG1β treatment on the first latency to move, first latency to center, groaming and freezing time in open field test. *P < 0.05, **P < 0.01, compared with their respected control group. One-way ANOVA followed by Tukey's post-hoc tests were used to calculate p values. Data values are expressed as mean ± SD, n = 8 rats per group.

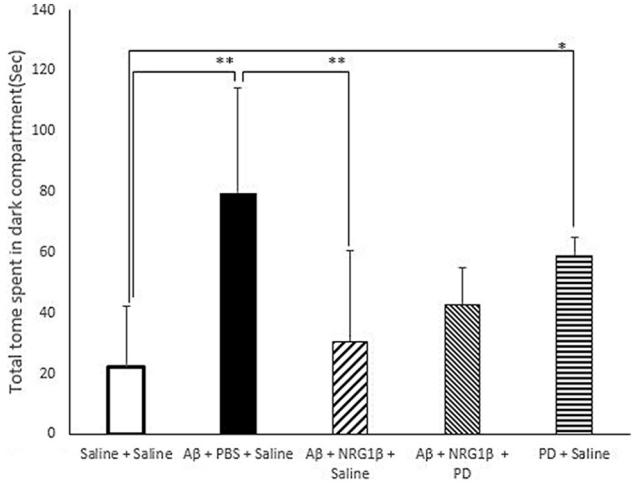

Fig. 9. Effect of NRG1β treatment on the total time spent in dark compartment in the step-through passive avoidance task. *P < 0.05, **P < 0.01, compared with their respected control group. One-way ANOVA followed by Tukey's post-hoc tests were used to calculate p values. Data values are expressed as mean ± SD, n = 8 rats per group.

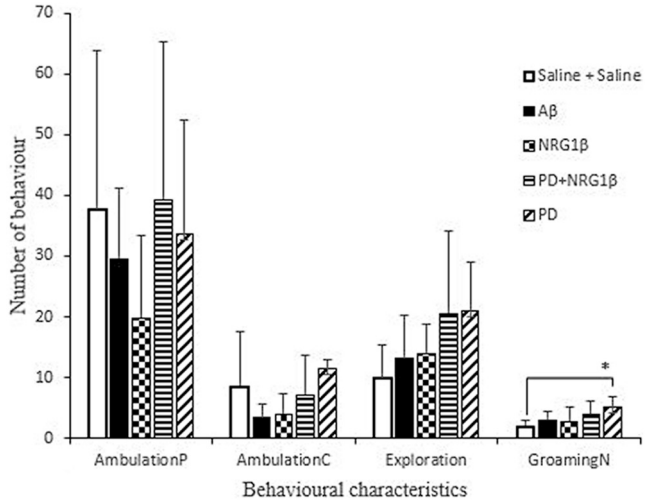

Fig. 11. Effect of NRG1β treatment on the ambulation peripheral, ambulation central, groaming and exploration number in open field test. *P < 0.05, **P < 0.01, compared with their respected control group. One-way ANOVA followed by Tukey's post-hoc tests were used to calculate p values. Data values are expressed as mean ± SD, n = 8 rats per group.

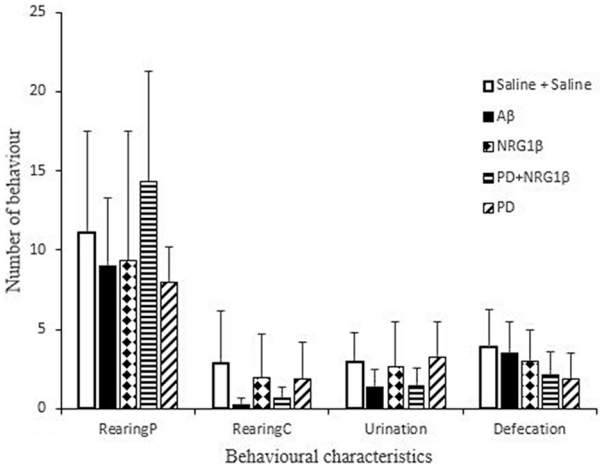

Fig. 12. Effect of NRG1β treatment on the rearing peripheral, rearing central, urination and defecation number in open field test. One-way ANOVA followed by Tukey's post-hoc tests were used to calculate p values. Data values are expressed as mean ± SD, n = 8 rats per group.

(p = 0.1), Fig. 12.

5. Discussion

Our study showed that intracerebroventricular (ICV) injection of Aβ, significantly impaired acquisition and retrieval of both spatial and associative memories in Morris water maze and step through passive avoidance. In addition, cognition deficits induced by Aβ were successfully restored with NRG1β, and this restoration was not completely reversed after inhibiting ERK1/2 signaling pathway.

Aβ has been known to exert a wide range of toxic mechanisms such as mitochondrial toxicity, synaptic dysfunction, calcium imbalance, and oxidative stress. Besides direct multiple toxic effects of Aβ, it also binds to different receptors such as insulin, RAGE, NMDA, nicotinic receptors, and leads in neuronal damage (Carrillo-Mora et al., 2014).

On the other hand, in the second set of experiments, we described, NRG1β administration 7 days after Aβ significantly restored both working and reference memories in MWM and also associative memory in step through passive avoidance paradigm. NRG1β has been found to bind to ErbB receptor and activates ErbB kinase domain, and results in phosphorylation of tyrosine residues (Liu et al., 2007), which consequently serves as docking sites for phosphopeptide-binding adaptor proteins such as ERK, PI3K–Akt, FAK, Rac1, cdc42, calcineurin, STAT3, GFAP pathways and leads in activation of further target proteins involving in memory formation (Yarden and Sliwkowski, 2001).

In the third set of experiments, ERK1/2 antagonists of PD98059 didn't completely affect memory restored by NRG1β. Although, it reduced NRG1β response insignificantly, this might represent its partial inhibitory effect. This finding suggests that NRG1β most likely exert its neuroprotective effect via different mechanisms. This finding is in agreement with the recent report by Vithayathil et al. (2017), indicating that chronic impairment of ERK1/2 signaling in glutamatergic neurons of the forebrain does not affect spatial memory retention (Vithayathil, Pucilowska et al.). However, it is in contradictory with the finding of Rong et al., 2015, who showed that NRG1β exerts neuroprotective effect via activating ERK1/2 (Rong et al., 2015).Contradictory results between Rong and our studies might be explained by difference in method. Rong and colleagues used organophosphorus pesticides as a model to reduce cognitive ability and treated animals with NRG1β one day after poisoning. They found that NRG1β improved cognitive function and increased phosphorylation level of ERK1/2 in hippocampal neurons, while we injected Aβ to establish sporadic AD, and treated animals with NRG1 β, 7 days after Aβ developed cognition deficits. Amyloid β exerts its cognition deficit via different complex ways such as change in flux of different ion channels such as Ca, NMDA and K, mitochondrial ROS, inflammatory pathways, tau phosphorylation, and apoptosis compared with organophosphorus which induces neural damage and reduces ERK phosphorylation(Rong et al., 2015).

The NRG1β-ErbB4 signaling in the brain is highly unusual because of the broad complex network of signaling proteins with multiple expression domains in adult nervous system. For example, it activates multiple signal transduction pathways such as PI3K–Akt, FAK, Rac1, cdc42, and calcineurin to serve various physiological responses (Cui et al., 2013). In addition, NRG1β has been implicated in the synthesis of receptors for NMDA, glutamate, GABA-A, and acetylcholine in cultured neurons or slices(Mei and Nave, 2014). Also NRG1β enhances entorhinal-hippocampal synaptic transmission (Roysommuti et al., 2003), GABA release in the prefrontal cortex and modulates hippocampal gamma oscillations (Fisahn et al., 2008) to synchronize neuronal networks during learning and memory. NRG1 and its receptor ErbB4 tyrosine kinase, have been known to be critical for maintaining GABAergic activity in amygdala and inhibition of either of receptor ErbB4 or neutralizing neuropeptide, both leads in reduction in GABAergic transmission and inhibits tone-cued fear conditioning(Lu et al., 2014). Also, NRG1β might directly elevate beta-amyloid degrading enzyme (NEP) and reduce the amount of amyloid beta (Xu et al., 2016). Therefore, the outcome of NRG1β signaling depends on interactions with different cross-talks within neurons. Blocking one pathway might compensate physiological responses reinforcing parallel signaling. One limitation of the present study is lack of molecular evidences on level of cytosolic pERK2 and activation of non- canonical pathways of NRG1β. Further investigations are needed to determine precise mechanisms of memory facilitation by NRG1β in AD model.

Moreover, the results of our study in open field test revealed that neither NRG1β nor ERK inhibitor did influence on locomotor, emotional and anxiolytic factors. However, injection of Aβ caused more latency to enter to the center of the arena which is one indicator of predisposition to emotional stress (Babaei et al., 2001). Our finding on NRG1β is in line with Li et al. (2014), but in contradictory with those studies on mice lacking one copy of the *Nrg1* gene which exhibited hyperactivity in open field (Stefansson et al., 2002). This contradiction might be related to chronic manipulation in NRG1 β signaling in their study.

Taken all together, the present study revealed that NRG1β improves cognition deficits in AD possibly through signaling pathways other than Erk1/2.

Declaration of Competing Interest

None.

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