Amino Acids
https://doi.org/10.1007/s00726-023-03241-0

ORIGINAL ARTICLE

How does neurokinin 3 receptor agonism affect pathological and cognitive impairments in an Alzheimer's disease-like rat model?

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Received: 22 August 2022 / Accepted: 20 January 2023

Abstract

Alzheimer's disease (AD) is accepted as a form of progressive dementia. Cholinergic systems are commonly affected in AD. Neurokinin 3 receptor (NK3R) is involved in learning memory-related processes. It is known that the activation of NK3R affects the release of many neurotransmitters. The aim of this project was to investigate the effects of NK3R agonist senktide administration on neurobehavioral mechanisms in the experimental AD-like rat model. 50 male Wistar albino rats were divided into Control (C), AD, Control + NK3R agonist (CS), AD + NK3R agonist (ADS), AD + NK3Ragonist + antagonist groups (ADSO). We designed AD-like model by intrahippocampal administration of Aβ1-42. After NK3R agonist + antagonist injections, open field (OF), Morris water maze (MWM) tests were applied. Cholinergic mechanism analysis from hippocampus-cortex tissues was performed by ELISA and catecholamine analysis from brain stem tissue were performed by HPLC method. The transitions from edge to center, rearing, grooming parameters were found to be reduced in final values of OF. While the group–time interaction was significant in the OF test findings, there was no significant difference between the groups. In MWM test, ADS group showed a learning level close to control group and animals in AD and ADSO groups could not learn target quadrant in MWM test. The brain stem NA and DA concentrations were not statistically significant. Hippocampal AChE-ChAT levels were supported by positive effects of senktide on learning via the cholinergic mechanisms. As a result, NK3R agonists were found to be effective in improving cognitive functions in rats with AD pathology. In the experimental AD model, positive effects of NK3R on learning memory may be mediated by cholinergic mechanisms.

Keywords Alzheimer's disease · Neurokinin 3 receptor · Learning and memory · Cholinergic mechanism · Catecholaminergic mechanism

Introduction

Alzheimer's disease (AD), which is characterized by memory loss, cognitive dysfunction, and mood changes, constitutes the majority of dementia patients (Loera-Valencia et al. 2018). AD is the most common cause of dementia and it is estimated that more than 50 million people worldwide have dementia. This number is expected to exceed 150 million by 2050 (Alzheimer's Disease International 2019). AD is an age-related neurodegenerative disease with characteristic clinical and pathological features (Chu 2012). The symptoms of the disease begin with a loss of working memory and in later stages, there is a decrease in mental performance and learning ability. This disease is associated with amyloid β peptide (Aβ) plaque formation, hyperphosphorylation of tau protein, neurofibrillary tangles (NT), loss of neuronal synapses, inflammation, oxidative damage, apoptosis and

Handling editor: Ka Wan Li.

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Published online: 06 February 2023

necrosis of brain cells (Kumar et al. 2015). The accumulation of Aβ peptide in the form of amyloid plaques is the main cause of the neurodegenerative process of AD (Selkoe and Hardy 2016). Age, genetics, head trauma, cognitive capacity (intelligence, education), physical activity, obesity, alcohol, smoking, stroke, diabetes, hypertension, and hypercholesterolemia are potential risk factors for AD (Ballard et al. 2011). Acetylcholinesterase enzyme (AChE) inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists are used for the treatment of AD. These drugs do not prevent neuronal loss and brain atrophy (Alzheimer's Association 2020). There are also degenerative changes in various neurotransmitters in AD pathology (Chu 2012). It has been reported that the reduction in ACh synthesis is due to reduced choline acetyltransferase (ChAT) enzyme and damage in cholinergic neurons projecting to the cortex. It has been reported that cholinergic loss in AD is associated with various behavioral and psychiatric symptoms, such as depression, agitation, anxiety, and psychosis observed in patients. In addition to cholinergic dysfunction, irregularities and loss of neurons in catecholaminergic neurotransmission are thought to be effective in the occurrence of these symptoms (Mesulam 2000a). The mechanism underlying memory dysfunction in old age and AD is thought to be cholinergic hypofunction (Bartus 2000). A significant cell loss in basal forebrain neurons and a decrease in ChAT activity were detected in AD patients (Mesulam 2000b). Treatment protocols for AD aim to increase the activity of the cholinergic neurotransmitter system by inhibiting AChE (Pinz et al. 2018). Although the cholinergic system plays an important role in dementia and cognitive symptoms of normal aging, it should not be overlooked that changes in other neurotransmitter systems, such as noradrenaline (NA), dopamine (DA), and serotonin, may also cause these pathologies (Garibotto et al. 2013).

The changes in pyramidal neurons, in particular in the hippocampus, such as the CA3 region, may play a significant role in memory deficits that herald the onset of AD and the development of AD dementia. The hippocampal regions, including the CA3 region, are currently the main focus of research due to their involvement in learning and memory and participation in the development of similar neurodegenerative changes in dementia resulting from AD (Padurariu et al. 2012). A cognitive decline through primary neurodegeneration of various hippocampal regions, including the CA3 area, plays an important role in the development of AD. Therefore, bilateral damage to the hippocampal CA3 region results in impaired short-term memory, which results in the inability to form new memories (Pluta et al. 2020).

Animal models have been very useful in understanding the psychological and physiological basis of neurodegenerative diseases such as AD (Narwal et al. 2012). Aβ alone can be effective in the impairment of different types of memory, such as associative and reference memory. It is generally thought to be a peptide that has a direct role in memory disorders (Puzzo et al. 2014). The intrahippocampal injection of Aβ 1–42 represents one of the most useful animal models of AD. Since none of these available models fully represents the main pathological hallmarks of AD, stereotaxic Aβ 1–42 infusion provides researchers with an in vivo alternative paradigm. When performed by well-trained individuals, this model is the best-suited one for short-term studies focusing on the effects of Aβ 1–42 on a specific brain region or circuitry (Facchinetti et al. 2018). In previous studies, bilateral injections of the amyloid beta peptide into the CA3 hippocampal region have been reported to impair spatial learning and memory processes (Richardson et al. 2002; Zeng et al. 2017; More et al. 2018).

Tachykinins, which are widely distributed in CNS, play an important functional role in both physiological and pathological conditions (Severini et al. 2002). Tachykinins are thought to have neurotransmitter, neuromodulator, and neuroprotective roles (Nagano et al. 2006). In CNS, tachykinins, are found in regions that play a role, especially in the regulation of various peripheral autonomic functions (blood pressure, respiration, etc.), mood-related functions (anxiety, aggression and pain), and higher cerebral functions (learning memory) (Severini et al. 2002). Neurokinin B (NKB) acts as the most potent natural agonist for the neurokinin 3 receptor (NK3R). Some agonists selective for NK3R, such as senktide, have been obtained by modification of the primary structure of NKB (Linari et al. 2002). The physiological effects of NK3R in the mammalian central nervous system are not fully known. Various studies have shown that NK3R plays a role in schizophrenia, Parkinson's disease, epilepsy, depression, and memory processes (Siuciak et al. 2007). NK3R is commonly found in areas related to learning and memory, such as the frontal cortex, amygdala, medial septum, and hippocampus (Duarte et al. 2006). It has been shown that NK3R is expressed on cholinergic neurons (Chen et al. 2001) and such neurons in the septohippocampal cholinergic system are activated by NK3R agonists (Morozova et al. 2008). In addition, NK3R agonists cause ACh secretion (Arenas et al. 1991) and protect cholinergic neurons in the basal forebrain from neurotoxicity (Wenk et al. 1997). Senktide administration to the striatum and hippocampus has been associated with increased extracellular ACh in the amygdala and prefrontal cortex (Schable et al. 2011; De Souza Silva et al. 2013). The activation of the septohippocampal and frontal cortex cholinergic pathways supports the promnestic effects of agonism created by senktide and NKB by improving ACh and memory (De Souza Silva et al. 2000). Anxiolytic, antidepressant, and promnestic effects were observed in rats and mice with senktide administration (Ribeiro et al. 1999; Schable et al. 2010). Based on the systemic and peripheral administration of senktide, it can be

concluded that NK3R may have a modulating role in anxiety and learning processes and forebrain cholinergic systems may be likely to participate in these actions (Schable et al. 2012). NK3R expression in cholinergic neurons (Chen et al. 2001) and the activating effect of NK3R on ACh (Schable et al. 2011) suggest a possible role of NK3R in learning and memory through cholinergic modulation. Despite the positive effects on learning and memory in some animal models by activation of NK3R, there is still little evidence of neurodegenerative diseases (Zlomuzica et al. 2008; Chao et al. 2014, 2015).

This study hypothesized that learning and memory processes would be positively affected by NK3R agonism in experimental Alzheimer's disease-like model. For this purpose, NK3R agonist and antagonist were injected in experimental Alzheimer's model rats created by intrahippocampal amyloid beta injection. Cholinergic and catecholaminergic mechanisms which play role in the pathophysiology of AD were investigated.

Materials and methods

Experimental protocol

The protocols of animal experiments were approved by the Local Ethics Committee of Application and Research Center of Experimental Medicine, Necmettin Erbakan University, No. 2016-050, on 30.09.2016. In the study, 50 male Wistar albino rats were used. In previous studies, rats from ages 12 to 18 months were preferred because they could simulate the main features of sporadic AD. It has been reported that active progression of AD-like pathology and changes in behavioral parameters can be better observed in rats of this age group (Galeano et al. 2014; Bitencourt et al 2017; Stefanova et al. 2019). Therefore, 12-month-old rats were preferred in the study to create an Alzheimer's disease-like model with acute amyloid beta pathology.

The animals were cared for at room temperature (22 ± 1 °C) with a 12-h light/dark period under standard laboratory conditions. They were fed ad libitum. Rats were divided into 5 groups: (1) Control group (C, $n = 10$), (2) Alzheimer's disease-like group (AD, $n = 10$), (3) Alzheimer's disease-like + NK3R agonist (Senktide) administered group (ADS, $n = 10$), (4) Control + NK3R agonist administered group (CS, $n = 10$), (5) Alzheimer's disease-like + NK3R agonist + NK3R antagonist (Osanetant) administered group (ADSO, $n = 10$) (Fig. 1).

Intrahippocampal Aβ injections and drug administration

Aβ 1–42 (Sigma-Aldrich, USA) was used to construct the experimental AD-like rat model. The Aβ peptide was

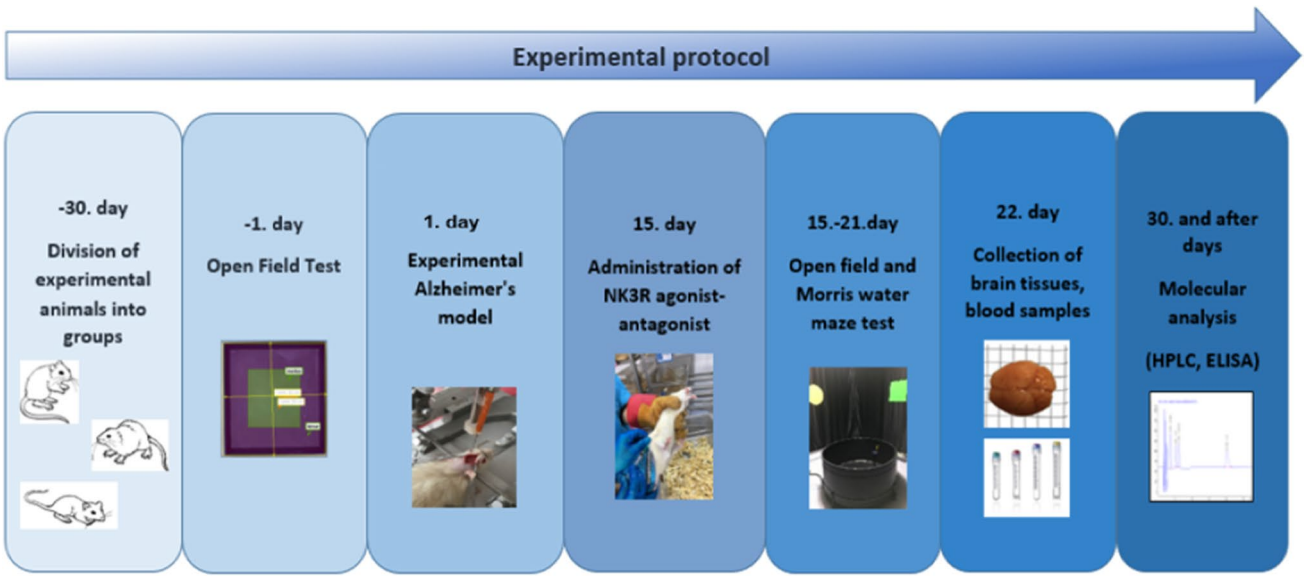

Fig. 1 Experimental protocol. Animals were divided into 5 groups 30 days before starting the experiments. For the evaluation of locomotor activities, OF test was performed before the experiment. On the 1st day of the experiment, Alzheimer's disease model was created by intrahippocampal Aβ injections. Behavioral experiments were started on the 15th day. NK3R agonist and antagonist injections were administered daily before the behavioral experiments. The OF test was performed on the 15th day. The habituation stage of the MWM test was on the 16th day, MWM training trials were on the 17-18-19-20th days, and the probe test was applied on the 21 day. On the 22nd day, the animals were decapitated and brain tissues were collected. Finally, HPLC analyses from brain stem tissue and ELISA analyses from hippocampus and cortex tissues were performed

dissolved in saline. It was incubated at 37 °C for 72 h for the formation of fibrillary structures. NK3R agonist (Senktide) ([succinyl-Asp6-N-Me-Phe8] SP6-11; Bachem, Germany) was dissolved with 5% dimethylsulfoxide in phosphate-buffered physiological saline (PBS). NK3R antagonist (Osanetant) (SR142801, Sigma Aldrich, USA) was dissolved in 0.01% Tween 80 (Sigma-Aldrich, Steinheim, Germany). Senktide and Osanetant were prepared in a volume of 1 mg/kg body weight. Senktide was administered subcutaneously at a dose of 0.2 mg/kg and osanetant was administered intraperitoneally at a dose of 6 mg/kg. Injections were administered daily, 30 min before the behavioral experiments. In the antagonist (ADSO) group, osanetant was injected 1 min before senktide injection. Control animals were injected with solvent. Doses were determined taking into account previous studies that showed a healing effect in learning and memory tests (Schable et al. 2011, 2012; De Souza Silva et al. 2013; Zlomuzica et al. 2008; Chao et al. 2014, 2015).

For anesthesia, a combination of ketamine (100 mg/kg) and xylazine (5 mg/kg) was administered. After the administration of the anesthetic agent, animals were placed in a stereotaxic device for intrahippocampal injection. After the scalp of the rats was cut from the midline, Bregma, the reference point, was identified. The Aβ peptide was bilaterally injected (2.2 nmol/10 µl) into the CA3 region of the hippocampus (AP: − 4.36 mm, ML: − 4.4 mm, and DV: − 8.0 mm, Paxinos and Watson 2009) in AD-like model groups (AD, ADS, and ADSO) (Richardson et al. 2002; Zeng et al. 2017; More et al. 2018). Rats in C and CS groups were injected with the same amount of saline as. On the 15th day, when the behavioral and memory experiments started, the animals were injected with the NK3R agonist senktide and the NK3R antagonist until the end of the experiment.

OF and MWM tests were performed to evaluate the locomotor activity, anxiety-like behaviors, and spatial memories of animals. OF test and MWM were carried out between 09:00 and 12:00 am. Animals were brought to the room where the test would be performed at least one hour before the test and adaptation to the environment was ensured. After the injections and behavioral tests were completed, the animals were decapitated under anesthesia, and the hippocampus, cortex and brainstem parts of the brain tissue on dry ice were separated according to the Paxinos and Watson rat brain atlas and placed in tubes. All tissues were kept at – 80 °C until analysis.

Open field test (OF)

We performed OF test at the beginning and end of the experiment to evaluate the locomotor activities, and some behaviors, such as anxiety and depression of animals. OF test apparatus used was made of 80 × 80 × 30cm square black plexiglass material. We recorded all test applications with video recording system associated with the special software program (Ethovision Video Monitoring System XT11, The Netherlands). With the software program, two regions are determined. The middle area of 40 × 40 cm was accepted as the central region and the remaining area as the edge region in the test apparatus. The parameters of the distance moved (cm), speed (cm/s), movement (%) and the number of transitions from the edge to the center 5 min were calculated. We scored rearing and grooming behaviors of animals manually. Test apparatus was cleaned with ethyl alcohol solution after each animal (Sahin et al. 2018, 2020).

Morris water maze test (MWM)

MWM consists of a circular water tank with a diameter of 180 cm and a depth of 60 cm and a 10 × 10 cm hidden platform. The water tank was darkened with black paint to prevent the platform from being seen. Different shapes were hung on the walls of the room where the experiment was made, which could provide clues for the subjects. Attention was paid to making the experiment with the same team, wearing the same clothes during the experiment, being quiet in the room, and not creating an extra stimulus for the subjects. All rats were given a habituation swim the day before their experiment. On the day of the habituation, the platform was kept visible 1 cm above the water. The animals were floated for 60 s and the animals that could not find the platform were left on the platform and kept there for 20 s. In the training of the experiment for 4 days, the rats were left in the pool 4 times in different directions consecutively every day. Rats were expected to find the hidden platform within 60 s. After staying on the platform for about 15 s, they were removed from the pool. In the memory test (probe), the platform was removed from the pool and the rats swam for 60 s. We recorded all tests with a special software program (Ethovision Video Monitoring System XT11, The Netherlands). Distance moved, latency to the platform, the number of passes through the target quadrant, and time spent in the target quadrant parameters were analyzed (Vorhees and Williams 2006).

Histopathological analysis

Brain tissues taken from each group were first fixed in 10% formaldehyde fixation solution for 24 h. After the relevant determination was made, the brain tissues were washed under tap water. Brain tissues that were washed under tap water for 24 h were subjected to routine histological follow-up series. In the next step, brain tissues were embedded in paraffin blocks. Then, sections of 5–6 µm thickness were taken from these paraffin blocks. Brain sections were stained with hematoxylin–eosin (H&E) and toluidine blue to evaluate histological images and to show degeneration

in neurons. The preparations were then examined under a research microscope and photographed.

Enzyme-linked immunosorbent assay (ELISA) analysis

Rat hippocampus and cortex tissues were placed separately in tubes filled with cold phosphate buffer. Each hippocampus and cortex tissue sample of the rats in the same group was weighed. In the homogenization buffer containing the protease inhibitor cocktail, all homogenization steps are made by the cold chain. Homogenates were centrifuged at 10,000 g and + 4 °C for 15 min using Hettich Rotina 46R (Hettich Zentrifugen, Tuttlingen, Germany) brand-cooled centrifuge and portioned to − 80 °C. The levels of Aβ1-42 in the hippocampal tissue were measured by ELISA. The levels of Aβ1-42 in the hippocampus were determined as pg/mg of tissue protein. Rat Choline Acetyltransferase (Catalog number: MBS161266, Sensitivity: 0.01 ng/mL, %CV: < 10) and Rat Acetylcholinesterase (Catalog number: MBS725468, Sensitivity: 1 pg/mL, % CV: < 10) levels were measured using the double antibody sandwich ELISA method (MyBioSource, San Diego, CA, USA). Protein levels in rat hippocampus and cortex tissues were measured by a spectrophotometric method using Pierce bicinchoninic acid-BCA kits (Thermo Scientific, Illinois, USA). Parameter levels in rat hippocampus and cortex tissues were standardized by dividing the results into protein concentrations. In spectrophotometric measurements, results were calculated according to the absorbance concentration calibration charts using the Bio-rad Microplate absorbance readerx Mark system (Bio-rad Laboratories, California, USA).

High-performance liquid cromatography (HPLC) analysis

The brainstem tissues were weighed and mixed with 1 ml 0.1 M hydrochloric acid to determine the NA and DA monoamines. The homogenates were centrifuged at 14,000 rpm at + 4 °C for 15 min and their supernatants were separated. The supernatants were made ready for analysis in HPLC (Agilent Technologies 1260 brand; 250 × 4.6 mm C18 ODS analytical HPLC column) by passing them through a 0.2 µm microfilter. HPLC analysis interval was set to 5. The temperature of the column oven was fixed at 40 °C. Injections were carried out in 20 µl volume. Between sample injections, the injection unit of the device was washed with methanol (Solak et al. 2021).

Statistical analysis

Data analysis of the study was carried out with SAS University Edition 9.4 program. Descriptive statistics about variables are given. Arithmetic Mean ± standard error (AM ± SE) for numerical variables, frequency and percentage values for qualitative variables were given. One-way ANOVA and mixed-effects models with the experimental unit being the random effect were used to model the data. Tukey's HSD test or the least squares means comparisons were performed after finding a significant effect. The Tukey–Kramer correction was used when suitable in mixed effect models. $p < 0.05$ was considered significant. Standard error plots of the least square means of the relevant variables were drawn.

Results

Open field test

The distance moved parameter showed a significant difference in time and group–time interaction ($p < 0.05$). The second parameters were found to be decreased according to the first distance moved parameters of the groups (Fig. 2a and Fig. 2b). There was no significant difference between the groups ($p = 0.4185$) (Table 1, Fig. 2aA). For the velocity, a significant difference was observed between the two measurements according to time ($p < 0.0001$). There was no significant difference between the groups ($p = 0.4185$). It was observed that the group–time interaction was statistically significant ($p < 0.05$). Second measurements were found to be decreased compared to the first velocity values of the groups. At the end of the experiment, the ADSO group had the lowest speed (Table 1, Fig. 2aB). The percentages of the movement were found significant between the two measurements according to time ($p < 0.0001$). Groups effect ($p = 0.7501$) and group–time interaction ($p = 0.0969$) were not statistically significant. It was observed that the second values decreased according to the first movement percentages of the groups. At the end of the experiment, the lowest percentage of movement was seen in the ADSO group (Table 1, Fig. 2aC).

In the OF test, the number of transitions from edge to center was calculated. A statistically significant difference was found between the first and second measurements according to time ($p = 0.0006$) ($p < 0.05$). No significant difference was observed between groups ($p = 0.8017$) and group–time interaction ($p = 0.8488$). The second measurement values were found to be decreased according to the parameter of the number of transitions from the first edge to the center of groups. The AD group had the highest number of edge-to-center transitions in the first measurement and decreased in the second measurement, while ADSO group had the lowest number of edge-to-center transitions at the end of the experiment (Table 1, Fig. 2aD).

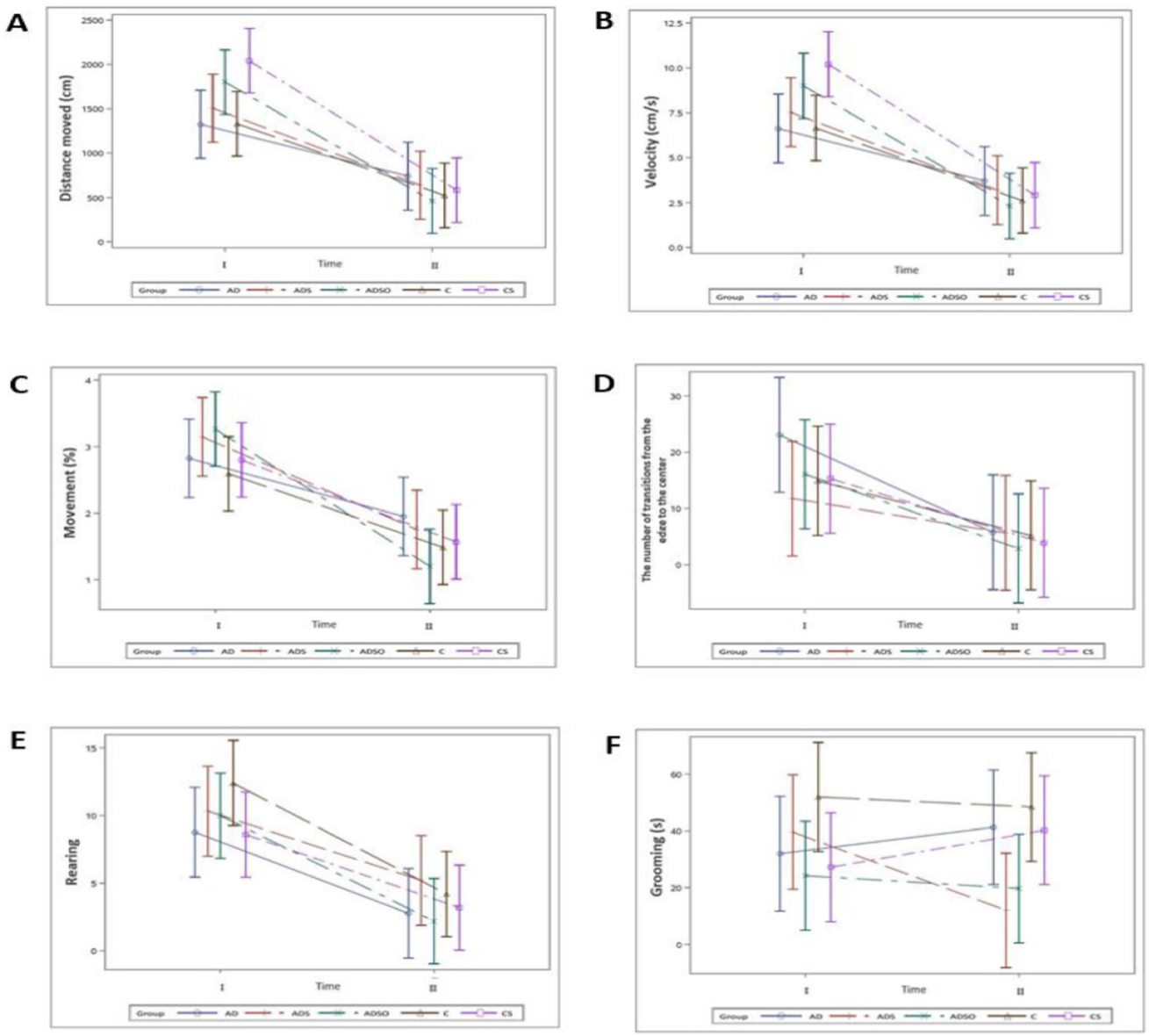

Fig. 2 **a** Distance moved, velocity, movement, the number transitions from edge to center, rearing, and grooming parameters in the OF test. **A** The distance moved parameter was not statistically significant between the groups ($p=0.4185$). **B** The velocity parameter was found to be significant in terms of time and group–time interaction ($p<0.05$). **C** The percentage of movement was not significant between the groups ($p=0.7501$). Distance moved, velocity and percentages of movement values were lower in all groups at the end of the experiment ($p<0.05$). **D** A statistically significant difference was found between the first and second measurement according to time of the number of transitions from the edge to the center ($p<0.05$). **E** There was a significant difference between the first and second values for rearing parameters of the animals over time ($p<0.0001$). **F** There was a statistically significant difference between the groups in the first and second measurements in the grooming parameters of the animals ($p<0.05$). *C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group. **b** Distance moved, velocity, movement, the number of transitions from the edge to the center, rearing and grooming parameters in the OF test (Graphs with individual data points). **A** The distance moved parameter was not statistically significant between the groups ($p=0.4185$). **B** The velocity parameter was found to be significant in terms of time and group–time interaction ($p<0.05$). **C** The percentage of movement was not significant between the groups ($p=0.7501$). Distance moved, velocity and percentages of movement values were lower in all groups at the end of the experiment ($p<0.05$). **D** A statistically significant difference was found between the first and second measurement according to time of the number of transitions from the edge to the center ($p<0.05$). **E** There was a significant difference between the first and second values for rearing parameters of the animals over time ($p<0.0001$). **F** There was a statistically significant difference between the groups in the first and second measurements in the grooming parameters of the animals ($p<0.05$). *C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group

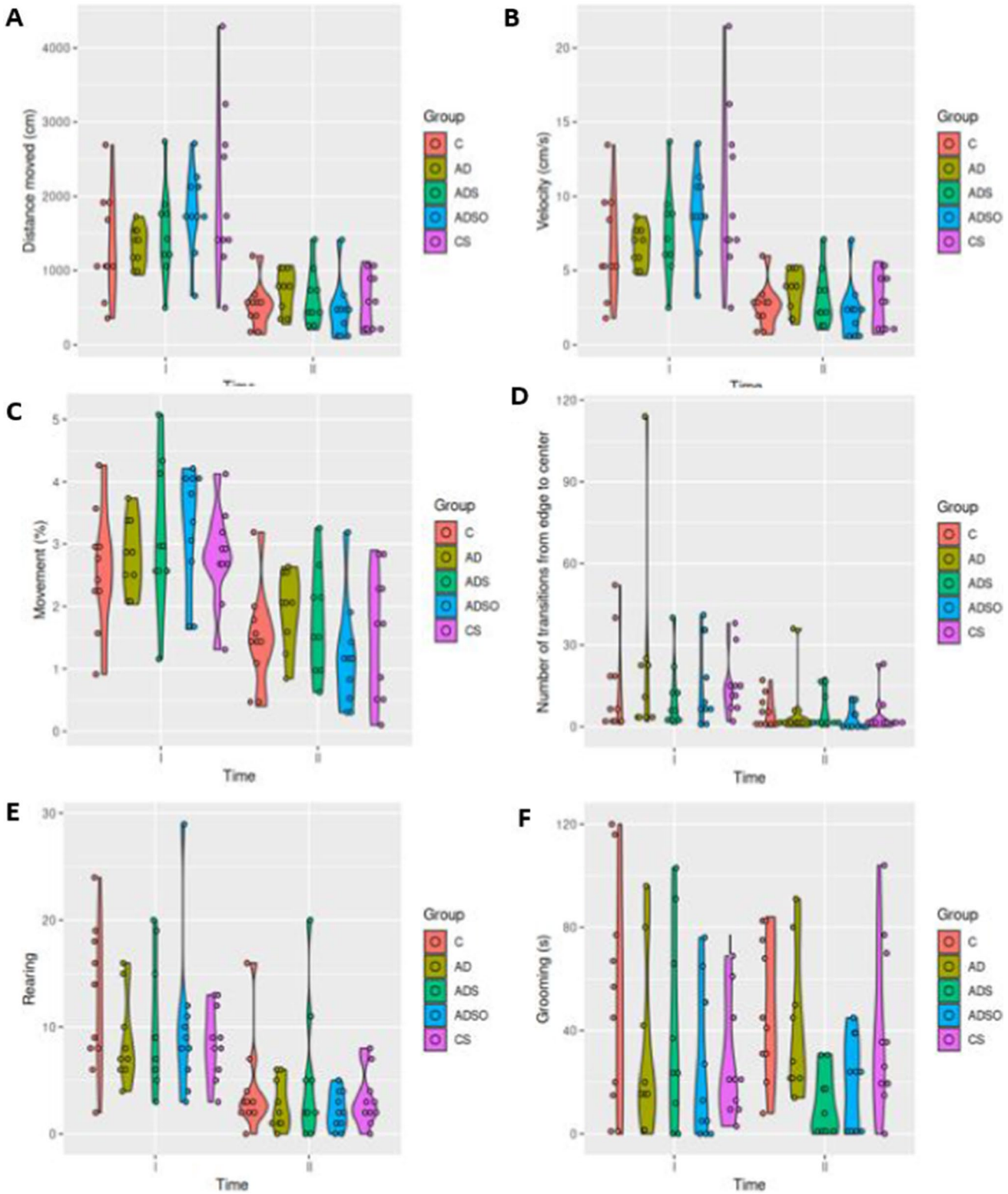

Fig. 2 (continued)

Table 1 The distance moved (cm), speed (cm/s), movement (%), the number of transitions from the edge to the center, rearing and grooming parameters in the OF test (*C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group)

	AM ± SE	C	AD	ADS	ADSO	CS
Distance moved (cm)	1	1330.8 ± 180.4	1325.6 ± 190.2	1506.2 ± 190.2	1801.8 ± 180.4	2041.4 ± 180.4
	2	522.9 ± 180.48	740.8 ± 190.24	638.5 ± 190.24	460.9 ± 180.48	582.2 ± 180.48
Velocity parameter (cm/s)	1	6.65 ± 0.90	6.62 ± 0.95	7.53 ± 0.95	9.00 ± ,0.90	10.20 ± 0.90
	2	2.61 ± 0.90	3.70 ± 0.95	3.19 ± 0.95	2.30 ± 0.90	2.91 ± 0.90
Movement parameter (%)	1	2.59 ± 0.27	2.82 ± 0.29	3.14 ± 0.29	3.26 ± 0.27	2.79 ± 0.27
	2	1.48 ± 0.27	1.95 ± 0.29	1.75 ± 0.29	1.20 ± 0.27	1.56 ± 0.27
The number of transitions from the edge to the center	1	14.90 ± 4.80	23.11 ± 5.06	11.77 ± 5.06	16.10 ± 4.80	15.30 ± 4.80
	2	5.20 ± 4.80	5.77 ± 5.06	5.66 ± 5.06	2.90 ± 4.80	3.90 ± 4.80
Rearing	1	12.40 ± 1.56	8.77 ± 1.64	10.33 ± 1.64	10.00 ± 1.56	8.60 ± 1.56
	2	4.20 ± 1.56	2.77 ± 1.64	5.22 ± 1.64	2.20 ± 1.56	3.20 ± 1.56
Grooming (s)	1	51.90 ± 9.48	32.00 ± 9.99	39.55 ± 9.99	24.20 ± 9.48	27.20 ± 9.48
	2	48.40 ± 9.48	41.22 ± 9.99	12.00 ± 9.99	19.70 ± 9.48	40.20 ± 9.48

There was a significant difference between the first and second values for rearing parameters of the animals over time ($p < 0.0001$). There was no significant difference between groups ($p = 0.3910$) and group–time interaction ($p = 0.7976$). The number of rearing in the groups decreased in the second value compared to the first value. At the end of the experiment, the groups with the lowest number of rearing were AD and ADSO, while the group with the highest number was the ADS group (Table 1, Fig. 2aE).

There was a statistically significant difference between the groups in the first and second measurements of grooming parameters of the animals ($p < 0.05$). No significant difference was found between the first and second measurements in the evaluation made according to the time effect ($p = 0.6658$) and group–time interaction ($p = 0.2862$). The control group had the most grooming time in both measures (Table 1, Fig. 2aF).

Morris water maze test

Latency to platform and distance moved parameter were analyzed of the training trials of MWM; distance moved, latency to the platform, number of passes through the target quadrant and time spent in the target quadrant parameters were analyzed of the probe test of MWM.

A significant difference was observed in group–time interaction ($p < 0.05$) at the latency to platform parameter during the training trials of MWM (4 days) (Fig. 3).

A significant difference was observed in group-time interaction ($p < 0.05$) at the distance moved parameter during the training phase of MWM (4 days). A significant difference was observed between the ADSO group and the CS group in the distance moved parameter on the 3rd day of the training phase ($p < 0.05$). On the 4th day, a significant difference was found between the AD, ADS groups and the C, CS and ADS groups ($p < 0.05$) (Fig. 4).

Latency to the platform was compared between groups, no statistically significant difference was found between the groups ($p = 0.0908$). The control group found the platform in less time compared to the other groups. The ADS group reached the platform in a shorter time than the AD and ADSO groups. Latency to the platform for the AD group and the ADSO group was close to each other (Table 2, Fig. 5A). When the distance moved during the test phase of MWM were compared, a statistically significant difference was found between the groups ($p < 0.0001$) (Table 2, Figs. 5B and 6). In the comparison between groups, a significant difference was found between the AD group and the other groups ($p < 0.05$). When the distance moved during the MWM test phase was compared, there was a visible difference between the groups. The parameter of the number of passes from the target quadrant was found statistically significant between the groups ($p < 0.05$). It was observed that the ADS group had more passages through the target quadrant than the other groups. In the comparison of the number of passes through the target quadrant between the groups, the AD group was found to be significant compared to the ADS and CS groups, the ADS group compared to the ADSO group, and the ADSO group compared to the C and CS groups ($p < 0.05$) (Table 2, Figs. 5C and 7A). In the MWM test phase, a statistically significant difference was found between the groups for the parameter of the time spent in the target quadrant ($p < 0.05$). A significant difference was observed between the CS group and the AD and ADSO groups in terms of their stay in the target quadrant ($p < 0.05$) (Table 2, Figs. 5D and 7B).

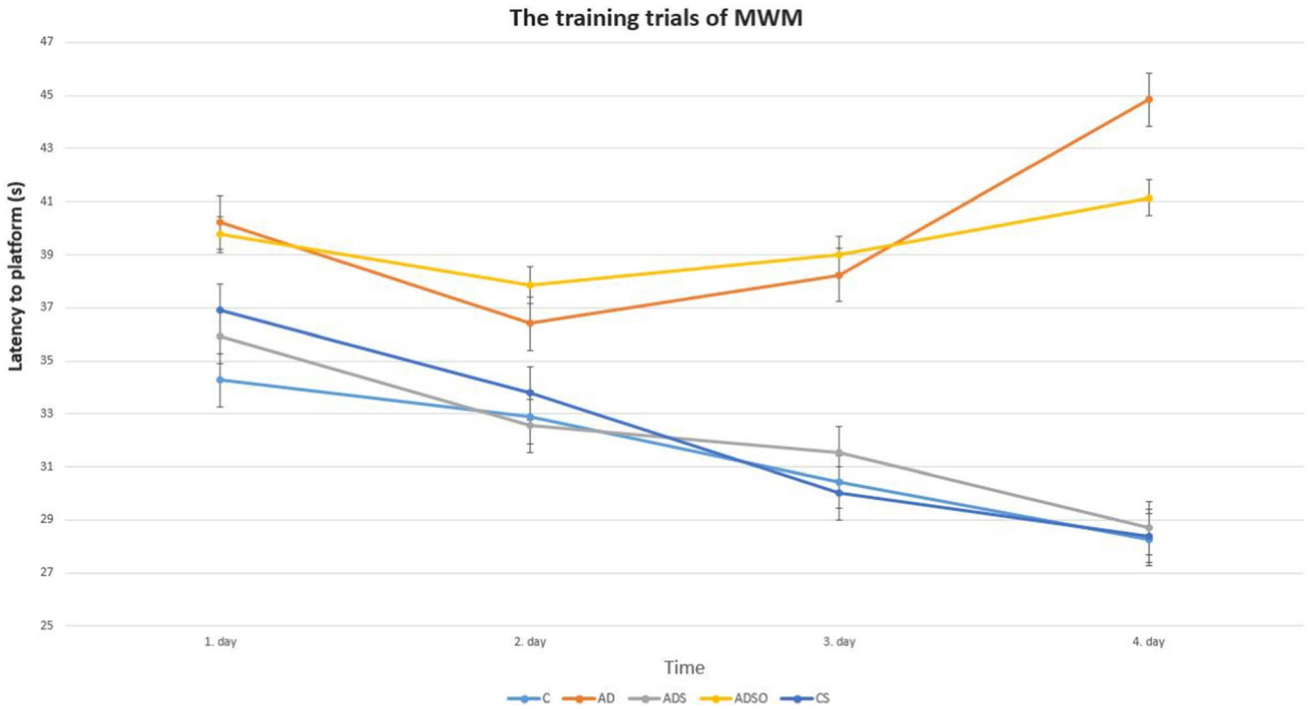

Fig. 3 Significant difference was observed in group–time interaction ($p < 0.05$) at the latency to platform parameter during the training trials of MWM (4 days). *C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group

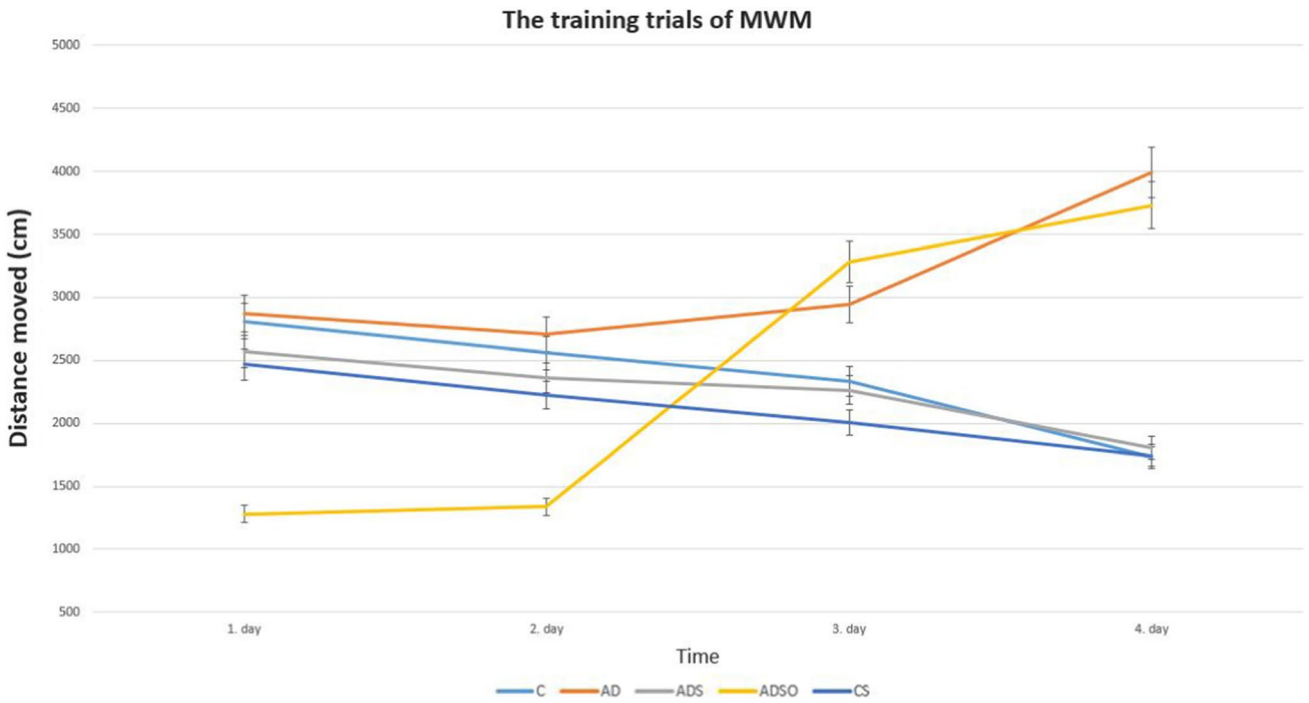

Fig. 4 Significant difference was observed in group–time interaction ($p < 0.05$) at the distance moved parameter during the training trials of MWM (4 days). *C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group

Table 2 Latency to the platform, distance moved (cm), number of passes through the target quadrant and time spent in the target quadrant parameters (s) in the MWM test (*C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group)

AM ± SE	C	AD	ADS	ADSO	CS
Latency to the platform parameter (s)	9.40 ± 6.12	34.25 ± 7.49	25.85 ± 6.12	33.05 ± 7.49	21.90 ± 5.66
Distance moved parameter (cm)	5749.62 ± 652.5	10,387 ± 687.81	6586.84 ± 729.5	8476.40 ± 652.5	4551.89 ± 652.5
Number of passes through the target quadrant parameter	20.00 ± 3.32	8.66 ± 3.50	32.75 ± 3.71	6.60 ± 3.32	28.70 ± 3.32
Time spent in the target quadrant parameter (s)	22.61 ± 1.81	15.38 ± 1.90	22.90 ± 2.02	17.10 ± 1.81	25.50 ± 1.81

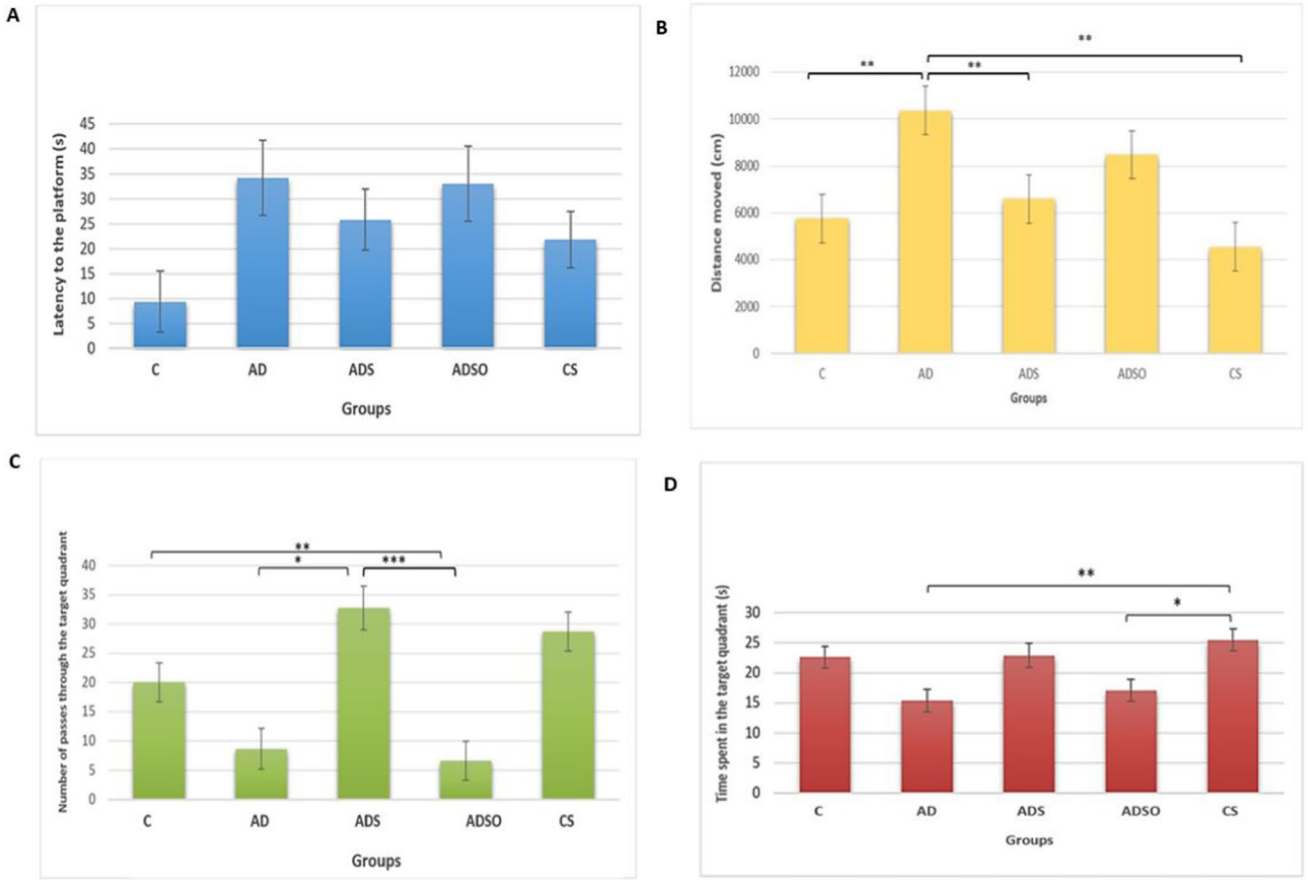

Fig. 5 Latency to the platform (s), distance moved (cm), number of passes through the target quadrant and time spent in the target quadrant parameters (s) in the probe test of the MWM. No significant difference was found for the group effect in the latency to the platform parameter ($p = 0.0908$). A significant difference was found between the groups in the distance moved parameter ($p < 0.01$). The parameter of the number of passes through the target quadrant was found significant between the groups ($p < 0.05$) ($p < 0.01$) ($p < 0.0001$). A significant difference was found between the groups in the parameter of the time spent in the target quadrant. The duration of AD and ADSO groups in the target quadrant were significantly lower than the CS group ($p < 0.05$, $p < 0.01$). *C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$)

Histopathological analysis

In the histopathological analysis, degeneration was observed in the pyramidal neurons of the hippocampus in the groups in which the AD model was created compared to the control group. Aβ1-42 reduced the thickness of the neuronal layer and the number of pyramidal neurons in some regions of CA3 (Fig. 8).

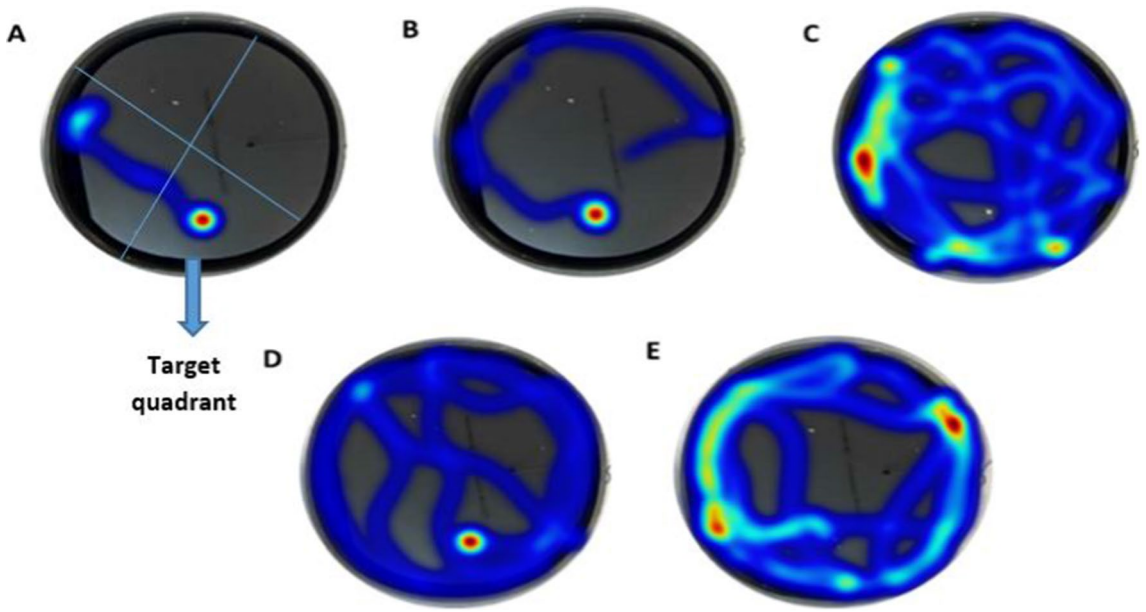

Fig. 6 Heatmap of MWM test in software program. The target quadrant is the south (marked) quadrant in the MWM. (**A**: C group, **B**: CS group, **C**: AD group, D: ADS group, **E**: ADSO group) *C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group

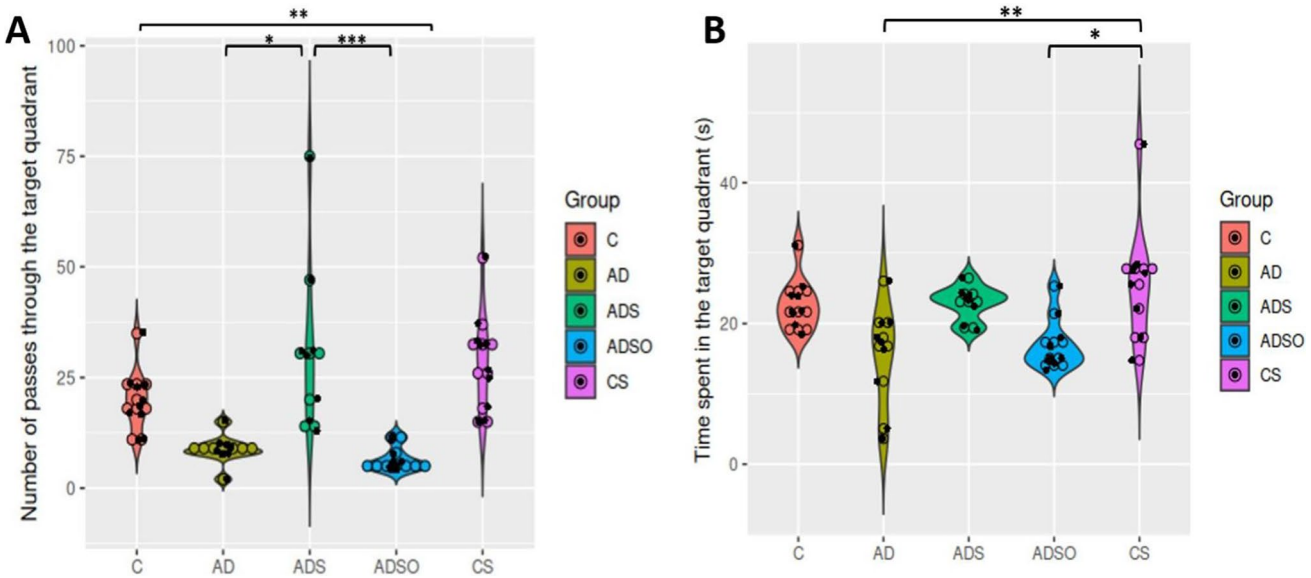

Fig. 7 The number of passes through the target quadrant and time spent in the target quadrant parameters (s) in the probe test of the MWM (Graphs with individual data points). The parameter of the number of passes through the target quadrant was found significant between the groups ($p < 0.05$) ($p < 0.01$) ($p < 0.0001$). A significant difference was found between the groups in the parameter of the time spent in the target quadrant. The duration of AD and ADSO groups in the target quadrant were significantly lower than the CS group ($p < 0.05$, $p < 0.01$). *C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$)

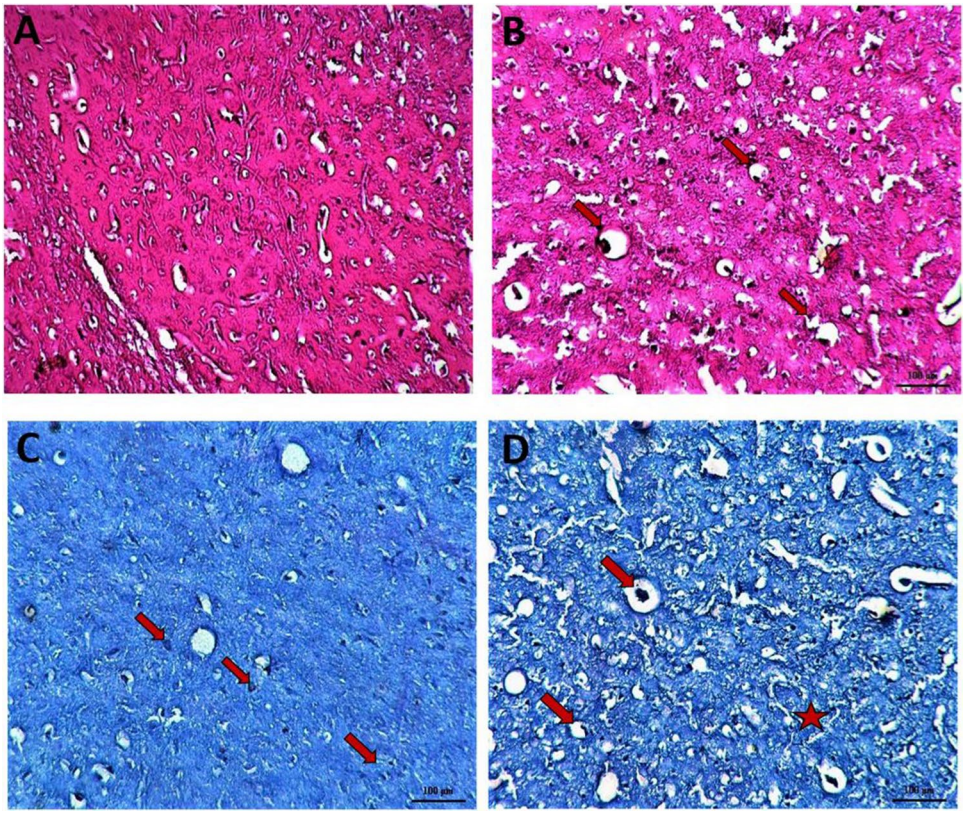

Fig. 8 Effects of Aβ1-42 on the pyramidal neuron layer of the hippocampal CA3 region. Brain sections were stained with Hematoxylin–Eosin (H&E) to evaluate histological images and with Toluidine-Blue to show degeneration in neurons. Degenerative changes were observed in the pyramidal neurons of the experimental Alzheimer model group compared to the control group. **B** The arrow shape indicates cytoplasmic vacuolation. **C** The arrow shape indicates intact neurons in the control group. **D** The star shape indicates neurons with pycnotic and irregular nuclei. The arrow shape indicates cytoplasmic vacuolation. (A- Control group B/C- AD model group, 10x, 100 µm)

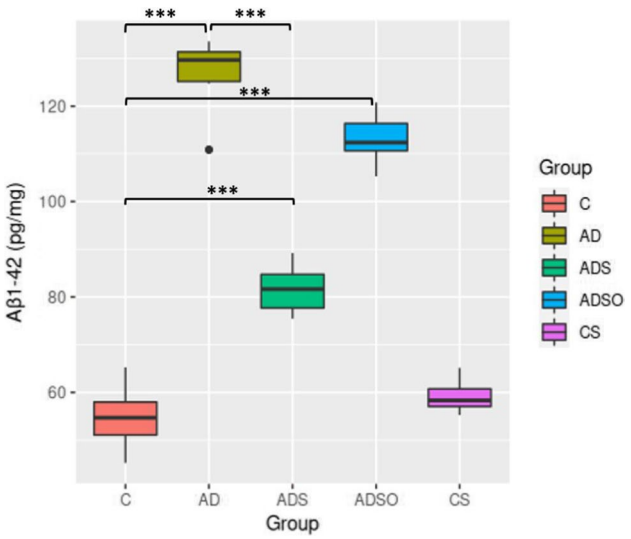

Fig. 9 The levels of Aβ1-42 in the hippocampal tissue were measured by ELISA. The levels of Aβ1-42 in the hippocampus were determined as pg/mg of tissue protein. The levels of Aβ1-42 in the AD, ADS and ADSO groups were significantly increased compared with the control group ($p < 0.0001$). In the ADS group after senktide administration, the levels of Aβ1-42 were significantly reduced in the hippocampus compared with the AD group ($p < 0.0001$). (***$p < 0.0001$). *C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group

ELISA analysis

The levels of Aβ1-42 in the hippocampal tissue were measured by ELISA. The levels of Aβ1-42 in the hippocampus were examined among different groups. The levels of Aβ1-42 in the AD, ADS and ADSO groups were significantly increased compared with the control group ($p < 0.001$). In the ADS group after senktide administration, the levels of Aβ1-42 were significantly reduced in the hippocampus compared with the AD group ($p < 0.001$). These findings indicate that senktide administration affected Aβ accumulation in the rat hippocampus (Fig. 9).

The amount of AChE was determined in the hippocampus and cortex tissues by ELISA method (Table 3). A statistically significant difference was found between groups and in group–tissue interaction ($p < 0.0001$). There was no significant difference between tissues ($p = 0.7005$). The amount of hippocampus tissue AChE was found to be statistically significant between the AD group and the CS group and between the ADSO group and the CS group ($p < 0.01$) (Fig. 10A). In the cortex tissue, a statistically significant difference was observed between the C group and all groups ($p < 0.0001$) (Fig. 10B).

ChAT amount was determined in hippocampus and cortex tissues by ELISA method (Table 3). A statistically significant difference was found between groups ($p < 0.05$), between tissues and group–tissue interaction ($p < 0.0001$). A

Table 3 AChE (pg/mg protein) and ChAT (ng/mg protein) amount in hippocampus and cortex tissues (C: control group, AD: Alzheimer's disease-like group, ADS: Alzheimer's disease-like + NK3R agonist group, CS: control + NK3R agonist group, ADSO: Alzheimer's disease-like + NK3R agonist + NK3R antagonist group)

AM ± SE	C	AD	ADS	ADSO	CS
AChEcrx	56.62 ± 2.06	41.42 ± 2.17	37.93 ± 2.30	35.23 ± 2.06	35.82 ± 2.06
AChEhpc	41.16 ± 2.06	45.93 ± 2.17	42.38 ± 2.30	46.3 ± 2.06	33.61 ± 2.06
CHATcrx	0.23 ± 0.008	0.32 ± 0.008	0.26 ± 0.009	0.33 ± 0.008	0.29 ± 0.008
CHAThpc	0.33 ± 0.008	0.27 ± 0.008	0.35 ± 0.009	0.30 ± 0.008	0.32 ± 0.008

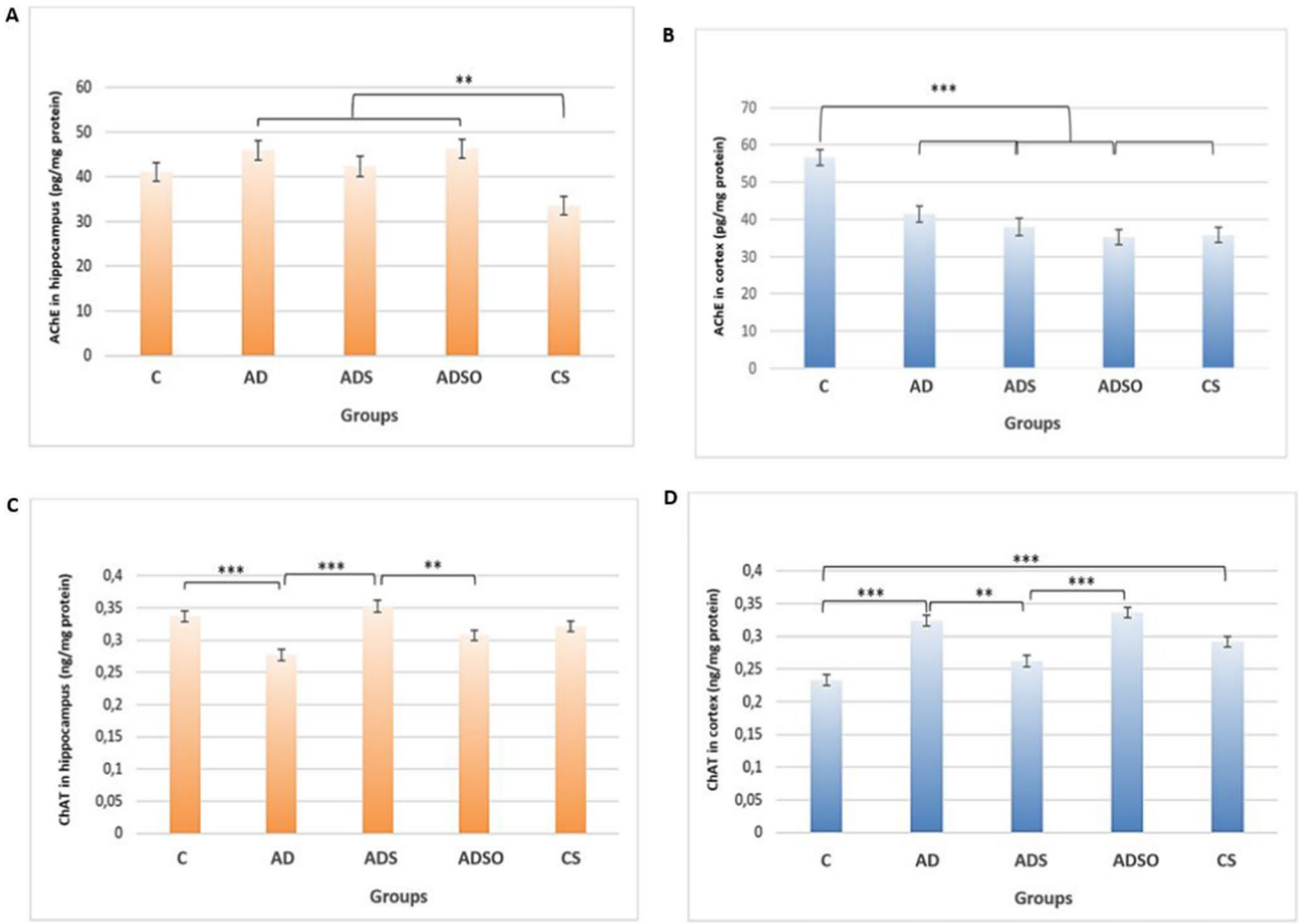

Fig. 10 AChE (pg/mg protein) and ChAT (ng/mg protein) amount in hippocampus and cortex tissues. The amount of AChE in hippocampus and cortex tissue was found to be significantly different between groups and in group–tissue interaction ($p < 0.0001$). A statistically significant difference was found between the CS group and the AD and ADSO groups in the hippocampus ($p < 0.01$). A statistically significant difference was observed between the C group and all groups in cortex ($p < 0.0001$). The amount of ChAT in hippocampus and cortex tissue was found to be significantly different between groups, between tissues and in group–tissue interaction. A statistically significant difference was found between the AD group and the ADS and C groups ($p < 0.0001$), and between the ADS group and the ADSO group in the hippocampus ($p < 0.01$). A statistically significant difference was observed between the AD group and the ADS group and the C group, between the ADS group and the ADSO group, between the ADSO group and the C group, and between the C group and the CS group in the cortex ($p < 0.01$, $p < 0.0001$). *C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$)

Table 4 Catecholamines in brainstem (pg amine/mg tissue) (*C* control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group)

AM ± SE	C	AD	ADS	ADSO	CS	P
NA	143.66 ± 20.8	110.93 ± 22.1	120.14 ± 23.6	141.16 ± 20.8	159.80 ± 20.8	0.5293
DA	43.11 ± 5.81	13.69 ± 6.16	25.60 ± 6.59	59.26 ± 5.81	28.10 ± 5.81	< 0.0001

statistically significant difference was found in the amount of ChAT in hippocampus tissue between the AD group and the ADS and C groups ($p < 0.0001$), and between the ADS group and the ADSO group ($p < 0.01$) (Fig. 10C). A statistically significant difference was observed in the amount of ChAT in cortex tissue between the AD group and the ADS group ($p < 0.01$) and the C group, between the ADS group and the ADSO group, between the ADSO group and the C group, and between the C group and CS group ($p < 0.0001$) (Fig. 10D),

Catecholamine analysis

NA and DA concentrations were evaluated in the brainstem samples in rats (Table 4). There was no significant difference between the groups in NA concentration ($p > 0.05$) (Fig. 11A). Although statistically significant was not seen, the NA concentration of the AD group was lower than all groups. DA concentration was found to be significantly different between the groups (Fig. 11B). In the comparison of DA concentrations between groups, the AD group was found to be significant compared to the C group ($p < 0.05$), and the ADS group compared to the ADSO group ($p < 0.01$).

Discussion

Although activation of NK3R has positive effects on learning processes, there is still little evidence for neurodegenerative diseases (Schable et al. 2011; De Souza Silva et al. 2013; Zlomuzica et al. 2008). In this study, we investigated the effects of NK3R agonist (senktide) administration on cognitive functions and neurobehavioral mechanisms in an experimental AD-like rat model. Locomotor activity, behavioral and memory changes were evaluated with OF and MWM tests in the experimental AD-like model. Neuronal damage was determined histopathologically. The levels of Aβ1-42 in the hippocampal tissue were measured by ELISA. Cholinergic system activation and catecholaminergic neurotransmission levels were determined by ELISA and HPLC methods. Although the peripheral effects of systemic senktide administration cannot be ignored, it has been reported that the results of the present study can be interpreted as the direct central effects of the drug, since many studies are showing the brain penetration of systemically injected senktide (Zlomuzica et al. 2008).

OF test was performed at the beginning and the end of the experiment to test the locomotor activities of the animals

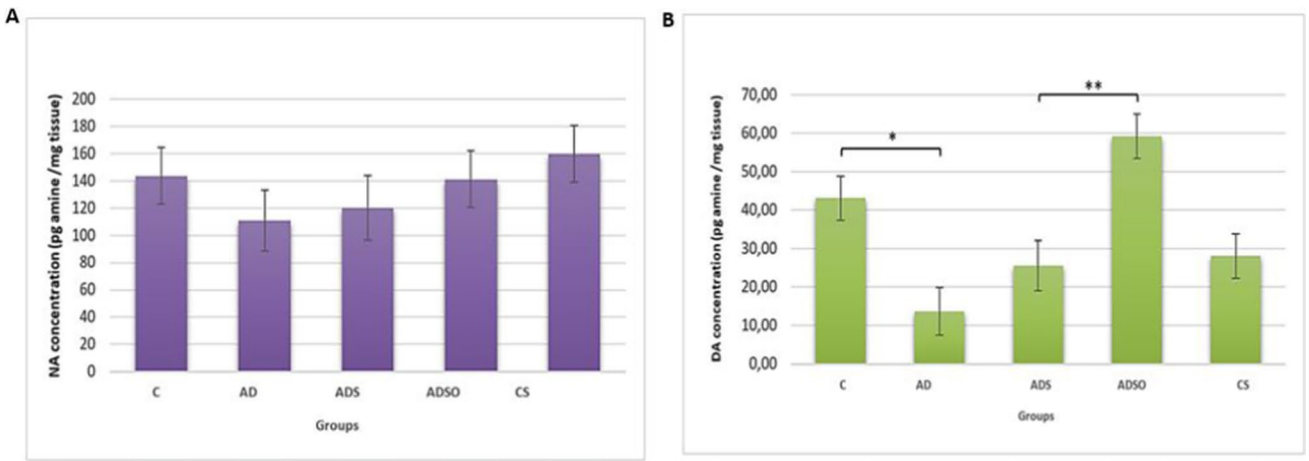

Fig. 11 Catecholamines in brainstem (pg amine/mg tissue). There was no significant difference between groups for NA concentration ($p > 0.05$). DA concentration was significant between AD and C group ($p < 0.05$) and between ADS and ADSO group ($p < 0.01$). The AD group was found to be significantly lower compared to the C group, and the ADS group compared to the ADSO group (*$p < 0.05$, **$p < 0.01$). *C* Control group, *AD* Alzheimer's disease-like group, *ADS* Alzheimer's disease-like + NK3R agonist group, *CS* control + NK3R agonist group, *ADSO* Alzheimer's disease-like + NK3R agonist + NK3R antagonist group

and to examine their anxiety levels. For locomotor activity analysis, distance moved, velocity and movement parameters were evaluated in OF test. Senktide had no effect on locomotor activity at doses of 0.1–0.4 mg/kg alone (De Souza Silva et al. 2006). In our study, it was thought that the decrease in locomotor activity may be sourced by the level of anxiety caused by the applications performed during the experiment. Anxiolytic, anxiogenic and antidepressant-like effects were observed of NK3R agonist and antagonist in OF and force swim tests. In the open field test, an anxiolytic-like effect was observed with both low dose (0.2 mg/kg) and high dose (0.4 mg/kg) of senktide (Schable et al. 2011). In another study, while NK2R agonism has memory loss and anxiogenic effects, NK3R agonists have been reported to show memory-healing and anxiolytic effects (Schable et al. 2012). In our project the distance moved, velocity, movement, the number of transitions from the edge to the center, rearing and grooming parameters were found reduced in the second measurement values. While the group–time interaction was significant in the OF test findings, there was no significant difference between the groups. The absence of a significant effect on anxiety may be due to the use of low-dose senktide. Different doses of senktide may be tried in future AD rat model studies.

Researchers found deficiencies in conditional avoidance and MWM tests in NK3R-damaged mice (Siuciak et al. 2007). In contrast, there was an improvement in an operant task and spatial learning performance. This difference in the direction of the effect suggested that it may be due to the use of different genetic backgrounds used in the production of these animals (Nordquist et al. 2008). Senktide has been found to alleviate scopolamine-induced dysfunction in its spatial working memory task (Kameyama et al. 1998). An improvement was observed with senktide injection in MWM. This effect is completely blocked by pretreatment with an NK3R antagonist. NK3R antagonist itself did not affect learning and memory alone (De Souza Silva et al. 2013). In the current study, while the shortest time to reach the platform at the testing stage in MWM is the C group, the longest is the AD group. Although it was not statistically significant, it was observed that the time to reach the platform decreased in the ADS group with the effect of senktide in probe test of MWM. In the ADSO group, this effect returned with osanetant and the time was prolonged. The fact that the distance moved parameter of the ADS group was lower than the AD group suggested that senktide had a curative effect on the learning deficits in AD. The number of passes through the target quadrant is a parameter that indicates the animals have learned the location of the platform and searched in that area. In the testing phase of the MWM, the number of passes through the target quadrant parameter of the ADS group was higher than the other groups. The number of passing through the target quadrant of the AD and ADSO groups was quite less than the other groups. This situation shows that the animals in the AD group cannot learn the target quadrant and that in the ADS group, senktide has an improving effect on learning deficits. The presence of similar findings in the ADSO group to the AD group suggested that the effects of senktide were dependent on NK3R. The fact that there was no difference in the velocity parameter in the MWM test suggested that there was no change in the locomotor activities of the animals between the groups. No difference in animal speeds means that velocity does not affect other results (latency to the platform, distance moved, number of passes through the target quadrant). Time spent in the target quadrant in the MWM indicates whether the animal has learned where the platform is. AD and ADSO groups spent the least time in the target quadrant. Groups C and ADS spent similar time in the target quadrant. With this parameter, it was observed that the ADS group showed a learning level close to the control group, and the animals in the AD and ADSO groups could not learn the target quadrant. The reversal of the positive effects of senktide with osanetant in the ADSO group suggested that senktide was dependent on NK3R. It is thought that the injection procedure may cause impairment in memory performance in the solvent-administered group. In this case, it has been stated that senktide supports memory by reducing the stress caused by the injection procedure (Kart-Teke et al. 2007). Different results in the C and CS groups may be related to this matter. If all the results of the MWM are considered, it can be interpreted that senktide has a positive effect on learning in the AD model.

Aβ accumulation is considered a fundamental problem of AD pathophysiology. The neuritic plaque in the brain of AD patients consists of amyloid composed primarily of Aβ peptide. Methods to quantitate the Aβ peptide are, therefore, invaluable to most studies aimed at a better understanding of the molecular etiology of the disease and in assessing potential therapeutics. The ELISA is one of the most commonly used, reliable, and sensitive methods for quantitating the Aβ peptide (Schmidt et al. 2005). In the present study, we found that senktide affected the level of Aβ in the hippocampus. These findings indicate that the effect of senktide in improving learning-memory processes in AD rats might be through reducing Aβ aggregation.

It has been determined that the activity of cholinergic system can be stimulated by NK3R agonism in adult animals. Microdialysis results after systemic senktide administration showed that ACh levels increased in the frontal cortex, amygdala and hippocampus regions of aged rats. A low dose (0.2 mg/kg) was effective in increasing ACh levels in the frontal cortex and amygdala, and a high dose (0.4 mg/kg) was effective in increasing ACh levels in the hippocampus (Schable et al. 2011). The data indicate that the effects of senktide can be caused by changes in cholinergic

neurotransmission. NKB, the NK3R ligand, has been found to increase ACh levels in the hippocampus. No significant effect was seen in the frontal cortex. In the amygdala, there was an insignificant increase in ACh (Schable et al. 2012). The pharmacological stimulation of NK3R has been shown to significantly improve learning and memory in older animals. These effects were most likely mediated by increased ACh levels in the major cholinergic projection target regions of the basal forebrain and the regulation of NK3R in the hippocampus. NK3R agonism also increased ACh activity in brain regions related to learning and memory (De Souza Silva et al. 2013). Pharmacological inhibition of the metabolizing enzyme AChE has been shown to increase ACh levels and improve learning and memory in the aged organism. However, AChE inhibitors can have serious side effects on the peripheral and autonomic nervous systems. NK3R agonists have a low effect on ACh-mediated peripheral mechanisms. Therefore, it is thought to be a way with a lower risk of side effects on brain ACh activity (Pinz et al. 2018). In aged rats, subcutaneously administered senktide increased ACh in the hippocampus, amygdala and frontal cortex, and showed anxiolytic, promnestic effects (Schable et al. 2010). In this study, the amount of AChE and ChAT was determined in the hippocampus and cortex tissues by ELISA method. While the amount of AChE in the hippocampus was increased in the AD and ADSO groups, it was found to be lower in the ADS group than in these groups. On the contrary, the amount of hippocampal ChAT was lower in the AD and ADSO groups, while it was increased in the ADS group. In other words, results consistent with our hypothesis were obtained in the amount of AChE and ChAT in the hippocampus tissue. On the contrary, the results of the cortex tissue were found inconsistent. Based on the knowledge that the systemic administration of NK3R agonists has a low effect on ACh-mediated peripheral mechanisms (De Souza Silva et al. 2013), it is thought that the extent of side effects may be safer.

In AD, there is a progressive degeneration in the noradrenergic nuclei of locus coeruleus (Heneka et al. 2010; Trillo et al. 2013). In AD, it has been reported that noradrenergic innervation loss occurs in the cortex while the nigrostriatal DA system is not particularly affected (Hirao et al. 2015). Memory-enhancing effects of senktide can affect different neurotransmitter systems (Chao et al. 2014). Low doses of NK3R agonist senktide have positive effects on temporal object recognition memory in rats with midbrain DA activity damage. This effect may be due to ACh release in the medial prefrontal cortex and hippocampus. Hemiparkinsonian model mice which were treated with 0.2 mg/kg senktide exhibited intact temporal object recognition memory via the DA mechanism (Chao et al. 2015). In the present study, the brain stem NA and DA concentrations were lower in the AD group than in the other groups, while it was increased in the ADS group compared to the AD group. But there was no statistically significant difference. It was thought that the decreased catecholamine values in the AD group might be related to the deterioration of cognitive functions. However, it cannot be said that NK3R agonism improves cognitive functions through catecholaminergic mechanisms. To reach this conclusion, more detailed studies on the underlying mechanisms are required.

Conclusion

AD becomes a very important health problem with the increasing aged population. Therefore, research for the diagnosis and treatment of AD have become compulsory. Animal models are very helpful in clarifying AD pathophysiology and discovering new pharmacotherapies. In experimental AD-like models, various learning and memory tests have been conducted for a long time. Several therapeutic agents can be tried to treat cognitive impairment in AD through these models. In this study, it was observed that the learning and memory processes could be improved with NK3R agonist in the MWM test. The hippocampal AChE and ChAT levels supported the positive effects of senktide on learning via the cholinergic mechanisms. Considering that dementia is characteristic of aging and Alzheimer's disease, NK3R needs further investigation for possible pharmacological administrations in this type of cognitive decline. More effective treatment strategies can be developed for AD by making more scientific research in human and animal models.

Author contributions ROK and ZISG conceived and designed the experiments; ROK, ZISG, HS and AK performed the research; ROK, ZISG, SK, IK and MSI analyzed the data; ROK wrote the first draft of the paper; ROK and ZISG revised and approved the final manuscript ZISG supervised the study.

Funding The study was supported by the Scientific Investigations Projects Coordinatorship of Necmettin Erbakan University (SIPC Project Number: 161418005).

Data availability All materials and data are available from the corresponding author upon a reasonable request.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval The protocols of animal experiments were approved by the Local Ethics Committee of Application and Research Center of Experimental Medicine, Necmettin Erbakan University, no. 2016–050, on 30.09.2016.

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