Neurotoxicity Research (2022) 40:2046–2060
https://doi.org/10.1007/s12640-022-00599-z

RESEARCH ARTICLE

Effects of Subchronic Aluminum Exposure on Learning, Memory, and Neurotrophic Factors in Rats

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Received: 17 June 2022 / Revised: 19 October 2022 / Accepted: 24 October 2022 / Published online: 7 November 2022

Abstract

Aluminum (Al) is a neurotoxin that gradually accumulates in the brain in human life, resulting in oxidative brain injury related to Alzheimer's disease (AD) and other diseases. In this study, the learning and memory of rats exposed to different aluminum concentrations (0.0 g/L, 2.0 g/L, 4.0 g/L, and 8.0 g/L) were studied, and the learning and memory of rats were observed by shuttle box experiment. With hematoxylin and eosin staining, Western blot, immunofluorescence, and RT-PCR, the morphology of nerve cells in the hippocampus of rat brain were observed, and the levels of activator protein-1 (AP-1) gene and protein, nerve growth factor (NGF), neurotrophin-3 (NT3), glial cell line-derived neurotrophic factor (GDNF), and brain-derived neurotrophic factor (BDNF) gene and protein level, etc. The experimental results showed that subchronic aluminum exposure damaged learning and memory in rats. The cognitive function damage in rats was more evident after increasing the aluminum intake dose. The more aluminum intake, the more pronounced the histological changes in the hippocampus will be. The expression level and protein content of neurotrophic factors in the hippocampus of rats showed a negative correlation with aluminum intake. In this experiment, we explored the mechanism of aluminum exposure in learning and memory disorders, and provided some data reference for further elucidation of the damage mechanism of aluminum on the nervous system and subsequent preventive measures.

Keywords Aluminum · Rat · Hippocampus · Learning and memory · Neurotrophic factor · Activator protein-1

Introduction

Aluminum (Al) is widely distributed worldwide and is a nonessential element found in nature (Priest et al. 1998). It can be found in all aspects of our lives, such as food, drinking water, beverages, and aluminum drugs (Kandimalla et al. 2016). In China, the consumption and proportion of small Mackey foods, milk formulas, and some wheat-based food samples (such as deep-fried dough sticks) in the dietary structure of children are relatively high, with aluminum concentrations as high as 3070 mg/kg. Among Chinese infants whose dietary aluminum exposure exceeded 8.0% of Provisional Tolerable Weekly Intake, wheat flour, deep-fried dough sticks and cakes, and steamed bread were listed as the three most contributing foods. Chinese infants 0–3 years of age did not exceed the PTWI threshold for Al, but more attention should be paid to the health risks of exposure to Al from wheat-based foods. In China, wheat is the primary source of dietary aluminum exposure (Cao et al. 2019). Legumes, candies, and cereals had the highest aluminum content in the Italian regions. Because of its small molecular weight, Al^{3+} can penetrate the blood–brain barrier and mainly gather in the striatum, brain stem, hippocampus, frontal cortex, and other brain regions (Kaur et al. 2006).

Epidemiological studies have shown that people living in areas with high levels of aluminum in drinking water are more likely to be diagnosed with Alzheimer's disease (Krewski et al. 2007). A meta-analysis involved 1208 participants and 613 AD cases. Compared with controls, AD

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cases have proved that the brain, serum, and cerebrospinal fluid levels of aluminum in individuals with AD have been significantly increased (Virk and Eslick 2015). Furthermore, many epidemiological and clinical studies have compared it with Alzheimer's disease (AD). Wang et al. (2016) reviewed 8 epidemiological studies to determine that exposure to Al is related to AD. According to the meta-analysis of the cohort study, a significant correlation was found between aluminum exposure and AD risk. A total of 10,567 individuals were included and exposed to Al from drinking water and occupational exposure. The follow-up period was 8 to 48 years. Mild cognitive impairment (MCI) is considered a transitional stage between normal cognitive aging and AD (Petersen et al. 2014). A recent meta-analysis conducted by Meng et al. investigated the prevalence of MCI among Al workers on duty. We found that higher plasma A1 levels are associated with a higher risk of cognitive impairment. Surveys on the prevalence of MCI are usually conducted among the elderly, and occupational workers may be ignored. The average age of the cases is much younger than the general MCI population. Therefore, Al in young people may impair cognitive function (Meng et al. 2019). Animal experiments also show that various animal studies have shown that the acute use of $AlCl_3$ in this study can cause obvious behavioral defects. There are apparent cognitive deficits and neuropsychiatric disorders in rats injected with $AlCl_3$. The experimental rats showed evident antioxidant enzymes, cholinergic, serotonergic, dopaminergic dysfunction, and DNA fragmentation (Liaquat et al. 2019). After the rats were exposed to aluminum for 2 months, their learning and memory abilities were reduced, and the production was high. The content of Aβ1-42 is high in the brain of mice. In addition, β-secretase increased significantly, while the different subtypes or components of α-secretase (especially ADAM metallopeptidase domain 10 and a disintegrin and metall oproteases 17) decreased significantly. Therefore, the cleavage of APP (amyloid protein precursor) by β-site-APP cleavageenzyme-1 increased, while the cleavage of APP by α-secretase decreased (Wang et al. 2014). The spatial memory ability of rats after subchronic aluminum exposure for 6 months decreased (Zhang et al. 2013). Adult rats are exposed to aluminum for 12 months. The cognitive function of aged rats is worse than that of normal aged rats (Walton 2009).

Aluminum intake leads to impairment of learning and memory ability in animals, and the structural basis of morphology is attributed to average ultrastructural damage of hippocampal neurons and synapses. Activator protein-1 (AP-1) transcription factor, which is composed of Fos family proteins (FOS, FOSB, FRA1, and FRA2) dimerized with Jun family proteins (JUN, JUNB, and JUND) (Eferl and Wagner 2003), pieces of evidence have shown that AP-1 protein is related to synaptic plasticity and regulates the survival and apoptosis of neurons and finally affects learning and memory (Alberini 2009; Herdegen and Leah 1998). AP-1 inactivation leads to the downregulation of various neurotrophic factors (NTFs), slowing neurons' functional recovery and aggravating neuronal death. Previous reports have shown that the expression of nerve growth factor (NGF) and neurotrophic factor 3 (neurotrophin-3, NT3) can be regulated by heterodimer AP-1 (Hengerer et al. 1990). Inhibit AP-1 in cells, then NGF expression will decrease (Dai et al. 2020). Glial cell-derived neurotrophic factor (GDNF) is also a direct target gene of c-Jun and plays an essential role in the survival of neurons (Fontana et al. 2012). AP-1 is also part of the positive feedback loop of brain-derived neurotrophic factor (BDNF), and the upregulation of BDNF depends on the transcription factors of the AP-1 family (Tuvikene et al. 2016). Studies have shown that the decrease of NTFs leads to decreased brain function, such as decreased memory, attention, and learning ability (Sousa Fernandes et al. 2020). To provide experimental reference data for in-depth elucidation of the neurotoxicity mechanism of aluminum, in this study, we established an animal model of cognitive impairment using drinking water containing aluminum and determined the effects of aluminum exposure on neurotrophic factors by molecular biology techniques to explore the role of neurotrophic factors in learning and memory.

Materials and Methods

Chemicals

$AlCl_3$ was obtained from China Pharmaceutical Chemical Reagent Co., Ltd., Shenyang, and dissolved in distilled water. All other chemicals used for atomic absorption spectrophotometry were guaranteed reagents. A standard Al reference material was obtained from the National Center for Standard Reference Materials (Beijing, China).

Our group looked up LD_0, LD_{50}, LD_{100} of aluminum, and calculate the pair distance $i = (\log LD_{100} - \log LD_0) / (n - 1)$ according to the preliminary test results, and calculate the dose of each group and the amount of sample added. In 1989, the World Health Organization and the Food and Agriculture Organization of the United Nations (WHO/FAO) officially defined aluminum as a food pollutant to be controlled, and put forward the provisional intake standard of aluminum for human body, that is, the allowable intake every 7 days is 7 mg/(kg·bw), which is equivalent to the daily allowable intake of 1 mg/(kg·bw). In the General Standard of Food Additives of the International Codex Alimentarius Commission (CAC), aluminum-containing food additives are also included. According to aluminum in food, the weekly tolerance intake of the exposed group was 7.0 mg/kg and the safety factor between human and animals

was 100 times, and the drinking water of rats and mice was determined. At last, we decided to set the aluminum exposure concentration at 2.0 g/L, 4.0 g/L, and 8.0 g/L.

Animals

All experimental animals were provided by the Animal Center of Shenyang Medical College. Mature Wistar rats weighed 220 ± 10 g were healthy and clean. All animals were maintained at a constant temperature (24 ± 1 °C) on a 12:12 light–dark cycle. Animals were observed for 7 days before mating (female: male = 1:1). Mating success and date of conception are indicated by observing the copulatory plugs. Pregnant rats were randomly divided into one control group and three Al-treated groups, with 10 rats in each group. This experiment simulated the way of drinking water in human daily life, and supplied water resources 24 h a day for 12 weeks. From the first postnatal day (day 0), females in each dose group received distilled water solutions containing 0.0 g/L, 2.0 g/L, 4.0 g/L, and 8.0 g/L aluminum trichloride, respectively. Before weaning, pups ingested aluminum by sucking their mother's milk, and after weaning, they self-consumed distilled water solution containing $AlCl_3$ 0.0 g/L, 2.0 g/L, 4.0 g/L, and 8.0 g/L. The exposure time lasted for 12 weeks from the first day of rat birth. The rats were sacrificed, and the whole brain tissue was taken and stored at −80 °C, and then various indicators were measured. The sex of the rats used in each experiment was female: male = 1:1. Feeding and test operations shall comply with the requirements of the Animal Test Ethics Committee of Shenyang Medical College.

Animals were divided into two groups, one group was used for HE staining pathology and histochemistry, and the other group was used for biochemical analysis, measurement of aluminum content, BDNF, and other indicators after ethology. All experimenters were trained carefully, and all animal treatments were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as adopted and promulgated. All efforts were made to minimize the number and suffering of animals.

Shuttle Box Testing

All rats were tested for behavioral training and memory in the shuttle box (DSC-2, Shenyang Medical College) at 9 a.m. in a testing room. The instrument consists of two compartments (50, 16, 18 cm) separated by a 1.2 cm hurdle. The conditioned stimuli (CS) were light (7 W bulbs located on the central ceiling of each compartment) and the sound produced by a buzzer (2400 Hz, 63 dB), which were delivered simultaneously. The unconditioned stimulus (US) was an electric foot shock of a maximum of 1.2 mA delivered through stainless steel rods covering the floor. Each cycle was composed of a 10 s CS, an interval of 4 s, and then the 5 s US. The interval between the two cycles was the 20 s. One trial lasted 20 cycles. The training session consisted of two trials per day for five consecutive days. The test session was performed 7 days after the training session under the same conditions. Prior to the first session, the rats were familiar with this box for 5 min. Crossings during this period were considered a measure of locomotor activity. Crossings to the other compartment in CS and US were considered active avoidance reaction (AAR) and passive avoidance reaction (PAR), respectively. The environment was kept dim and calm during the experiment. Escape time and escape latency in AAR were recorded to evaluate each test's learning and memory performance, and the shock number and shock time in PAR were recorded.

Determination of Al^{3+} Content in the Hippocampal Neurons by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Each hippocampus in each group was accurately weighed to take 0.2 g; a total of 24 were taken, put into the polytetrafluoroethylene digestion tube, added mixed acid composed of 5 ml high-grade pure nitric acid and 1 ml high-grade pure hydrochloric acid, sealed it, and put it into the porcelain tube. Digesting the tissue in the ultra-high pressure microwave digestion instrument for 50 min, and made the blank control simultaneously. After the tissues were completely digested, the sample solution was moved to a volumetric flask with 20% nitric acid and set to 50 ml. Detect the aluminum content with an ICP-MS instrument. The content of detected aluminum value minus the background one of the blank control was the final hippocampal aluminum ion content per rat.

H&E Staining

After the rats were sacrificed, the brain tissue was quickly taken out on the ice, and the excess bloodstains were rinsed. The brain tissue was placed in 4% paraformaldehyde for fixation, and stored at 4 °C for more than 1 month. Brain tissue was trimmed, washed overnight with running water, dehydrated in gradients, embedded in paraffin, and sectioned coronally (thickness 4 μm). The samples were successively immersed in the following solutions for dewaxing: toluene (I) at 37 °C for 10 min, xylene (II) at 37 °C for 10 min, absolute ethanol (I) for 10 min, absolute ethanol (II) for 10 min, 95% ethanol for 5 min, 90% ethanol for 5 min, 80% ethanol for 5 min, 70% ethanol for 5 min, hematoxylin–eosin (HE) (KeyGen, Jiangsu, China) staining, neutral gum sealing, and then placed at average temperature for 2 h. After the neutral gum was dried, it was placed under the microscope (Ci-E, Nikon, Japan) for observation and photographing, and the

morphological conditions of hippocampal neurons in each group were recorded and preserved.

Real-Time RT-PCR

A sample of 50 mg hippocampus tissue from 6 rats per group was homogenized on ice. Total RNA was extracted with Monzol™ (Monad, Wuhan, China). The OD260/OD280 ratio and RNA concentration were measured by spectrophotometer. According to the instructions, 1 μg of RNA was reversely transcribed into cDNA using MonScript™ RTIII All-in-One Mix (Monad). The qPCR was performed with the presence of SYBR Green intercalating dye with MonAmp™ ChemoHS qPCR Mix (Monad). Rat GAPDH internal reference primer, upstream primer: 5′-GACATGC-CGCCTGGAG AAAC-3′; downstream primer: 5′-AGCCCAGGATGCCCT TTAGT-3′. Rat β-actin internal reference primer, upstream primer: 5′-TTCCAGCAGATG TGGATCAG-3’; downstream primer: 5′-AGAGAAGTGGGGTGGCTTTT-3’. Fos subunit of AP-1 mRNA primer sequence: upstream primer: 5′-TACTACCATTCCCCAGCCGA-3′; downstream primer: 5′-GCGTATCTGTC-AGCTCCCTC-3′. NGF mRNA primer sequence: upstream primer: 5′-TCGCTCTCCTTCACA-GAGTTT-3′; downstream primer: 5′-TAGAAAGCTGCG TCCTTGGC-3′. NT-3 mRNA primer sequence: upstream primer: 5′-TAAAGAAGCCAGGCCAGTCA-3′; downstream primer: 5′-AGT-CAGTGCTCGGACGTAGG-3′. GDNF mRNA primer sequence: upstream primer: 5′-GACT-TGG GTTTGGGCTACGA-3′; downstream primer: 5′-TGGTAA ACCAGGCTGTCGTC-3′. BDNF mRNA primer sequence: upstream primer: 5′-ACAGTATTAGCGAGTGGGTCAC-3′; downstream primer: 5′-GAACATACGATTGGGTAG TTCG3′. The PCR reaction conditions were as follows: pre-denaturation at 95 °C for 10 min, followed by denaturation at 95 °C for 10 s and 40 cycles at 60 °C for 60 s. The Applied Biosystems 7500 fast system was used to process the data, and the $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression level of AP-1 mRNA, NGF mRNA, NT-3 mRNA, GDNF mRNA, and BDNF mRNA. We used Primer Premier 5 design tool to design primers according to the principles of primer design, and verified the specificity of primers in Primer-BLAST, and conducted multiple preexperiments to verify the reliability of primers.

Western Blot Detection

The cytoplasmic protein and nucleoprotein in the hippocampal tissue of rats in each dose group were extracted according to the kit's instructions for extracting nucleoprotein and cytoplasmic protein (Beyotime, Shanghai, China), and the whole process was operated on ice. Finally, that protein concentration was determine according to the instruction of the BCA protein concentration determination kit (Beyotime), subpackaged, and stored at −80 °C. According to the instructions of the BCA kit (Beyotime), the concentration of each protein was determined, followed by incubation in the dark at 37 °C for 30 min, and the microplate reader was set at A562 nm for the determination of the absorbance value of the sample. With protein content (μg) as the horizon coordinate and absorbance value as the vertical coordinate, a standard curve was drawn to obtain the protein concentration of the sample, which was adjusted to 5 mg/L protein solution. In the western experiment, the loading amount was 50 μg. And the protein concentration of each sample was calculated and adjusted to 3 or 5 μg/μl. The extracted protein was mixed with the loading buffer, fully denatured in a metal bath at 100 °C for 8 min, and stored in the refrigerator at −80 °C. According to the instructions of the SDS-PAGE gel preparation kit (Beyotime), 10% separation gel and 5% concentration gel were prepared, slightly wrapped with cling film, and placed at 4 °C overnight.

SDS-PAGE electrophoresis (041BR180487, Bio-bad, the USA) was performed, and the comb teeth were vertically pulled out. Then the solidified gel was transferred into the electrophoresis tank by Protein Electric Transfer Device (EC250-90, Bio-bad, the USA). A newly configured running buffer which was a high internal and low external state was added to inside and outside of the electrophoresis tank to form a voltage difference. Pre-dyed marker (Thermo, USA) 7.5 μl was added to the first hole of each rubber plate, and then 10–15 μl of the protein sample to be tested was added sequentially, and the record was made. The protein sample was heated in the thermostatic metal bath at 100 °C for 8 min and 4 °C for later use. Electrophoresis conditions: voltage 80 V, electrophoresis for 2.5 h. Immersing the separation gel in transbuffer, and shake at medium speed for 30 min. The PVDF membrane (Millipore, Germany), slightly larger than the separation gel, was cut according to the size of the separation gel, activated in methanol (Concord, Tianjin, China) for 10 s, and shaken at medium speed for 30 min after passing through ddH_2O. The sponge PVDF membrane, separation gel, etc. were sandwiched in sequence and placed in a transfer printing tank equipped with a transbuffer, which was placed at −20 °C for cooling 2 h before the separation gel was passed. Electrokinetic conditions: voltage 100 V, transfer membrane 45 min. The membranes were sealed in 5% skimmed milk powder (Yili, Neimenggu, China) for 2 h, and the medium was washed by phosphate buffered solution (PBST) (Solarbio, Beijing, China) for 45 min and three times with the slowest shaking speed, after which the membranes were cut into strips and marked.

The bands were washed in PBST for 45 min, and the primary antibodies were added in the diluted ratio of Fos subunit of rabbit anti-AP-1 (ABclonal, Wuhan, China) (1:1000), rabbit anti-NGF (1:1000) (ABclonal), rabbit anti-NT-3 (1:800) (ABclonal), rabbit anti-GDNF (1:1500) (ABclonal),

and rabbit β-actin (1:2000) (ABclonal), respectively. The bands were shaken for 1 h and kept at 4 °C overnight. The next day, the platform was rewarmed for 30 min, and the PVDF membrane was taken out from the primary antibody incubation solution. The membrane was washed three times with PBST, 15 min each time. Then, 1:10,000 secondary antibody hybridization solution was added, and the platform was shaken for 1 h. The bands were taken out and washed with PBST for 45 min three times. Enhanced chemiluminescence (Vazyme, Nanjing, China) was used to emit light, develop, and acquire the gel electrophoresis image analysis system (Tanon-5200Multl, Tanon, Shanghai, China) results. The gray value of each sample was analyzed with Image J software, and the results were expressed as sample gray value/internal reference gray value.

Immunofluorescence Staining

After the rats were anesthetized, the hearts were rapidly perfused and fixed with 4% paraformaldehyde solution. The heads were decapitated immediately after perfusion, and the brain tissues were quickly taken out on ice, fixed in 4% paraformaldehyde, and stored at 4 °C. Brain tissues were embedded in paraffin and coronal section (thickness: 4 μm), and the slides were collected and placed overnight at room temperature to make the slices closely connected. The slices were baked at 68 °C for 90 min, dewaxed with xylene for 20 min, dehydrated with graded alcohol, placed in 3% hydrogen peroxide solution to inactivate endogenous enzymes, and washed 3 times with 0.02 mol/L PBS (pH 7.2–7.4), 5 min each time. They were immersed in 0.01 mol/L citrate buffer (pH 6.0) and heated to repair the antigen. After cooling, wash with PBS 3 times, 5 min each time. Ten percent normal goat serum (containing 0.3% Triton X-100) was blocked for 30 min, and rabbit anti-rat BDNF antibody (1:200) was added dropwise at 4 °C overnight. TRITC goat anti-rabbit secondary antibody was added on the next day and incubated at 4 °C for 24 h in the dark. After drying and mounting, the fluorescence intensity of hippocampal CA1 and CA3 regions was observed by confocal laser scanning microscope (Ci-E, Nikon), and the mean fluorescence intensity (mean) value was used to represent the content of BDNF protein in the hippocampal CA1 and CA3 regions.

Statistical Analysis

SPSS 23.0 statistical software was used to analyze the data obtained from the experiment, and the variables were tested for homogeneity of variance. Kolmogorov–Smirnov test was used and all of the variables follow a normal distribution. When $P > 0.05$, the sample was considered to be from the population with homogeneity of variance. Repeated measurement ANOVA was carried out for weight, and other experiments were compared between groups by one-way ANOVA. The LSD method was used when the overall variance was homogeneous, and the Dunnett's *t*-test was used when the overall variance was unequal. When $P < 0.05$, the difference between groups was statistically significant. The Pearson correlation coefficient (r) was used to analyze the correlation between aluminum exposure dose and each index, and when $P < 0.05$, the correlation between the two was considered to be statistically significant.

Results

Analysis of Rats' Body Weight

According to the experimental results, the body weight of rats in each group increased during the 12 weeks, and the trend of body weight gain in rats was declining with increasing exposure (Fig. 1). The results of one-way ANOVA showed that there were significant differences in the weight data of each group at the 4th week: $F = 46.148$, df = 3, $P = 0.000$ ($P < 0.05$); at the 8th week, there were significant differences in the weight data of each group: $F = 91.766$, df = 3, $P = 0.000$ ($P < 0.05$); and at the 12th week, there were significant differences in the weight data of each group: $F = 55.654$, df = 3, $P = 0.000$ ($P < 0.05$). This shows that there are significant differences in weight data at 4, 8, and 12 weeks.

The repeated measure ANOVA of the weight data showed that $P = 0.482$ ($P > 0.05$) was obtained by the Mochili spherical test, which was consistent with the spherical hypothesis. The tests of within-subject effects (time) were

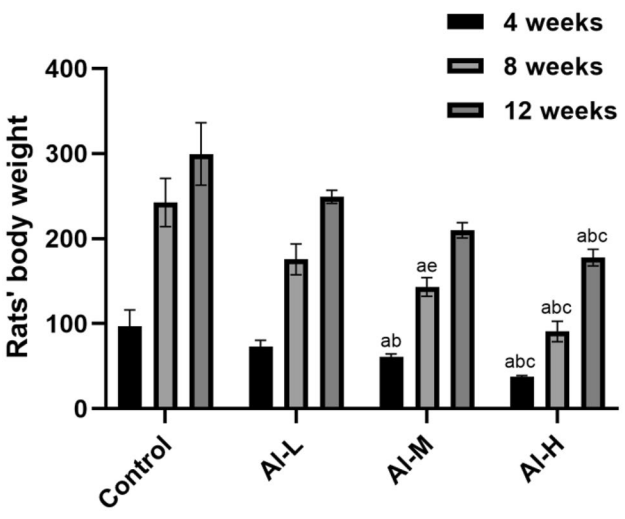

Fig. 1 Body weight change in different stages of rat in each group. [a]$P < 0.01$ vs Control, [b]$P < 0.01$, [e]$P < 0.01$ vs Al-L, [c]$P < 0.01$ vs Al-M, $n = 8$

statistically significant: $F = 1924.312$, df = 2, $P = 0.000$ ($P < 0.05$), indicating that there were significant differences in the weight data of rats from the fourth week to the twelfth week. The interaction effects (time*group) were statistically significant: $F = 27.889$, df = 6, $P = 0.000$ ($P < 0.05$), which indicated that with the prolongation of subchronic aluminum exposure time, the weight of rats in the control group was different from that in the experimental group. Tests of between-subject effects (group) were statistically significant: $F = 84.827$, df = 3, $P = 0.000$ ($P < 0.05$), which indicated that different aluminum dose exposures would affect the weight gain of rats. Using Al-L represents the low dose group, Al-M represents the medium dose group, and Al-H represents the high dose group in the following results.

Analysis of Water Consumption and Body Weight Ratio of Rats

According to the experimental results, the water consumption to body weight ratio of rats in each group increased during the 12 weeks (Fig. 2). The results of one-way ANOVA showed at the 4th week: $F = 1.121$, df = 3, $P = 0.357$ ($P > 0.05$); at the 8th week: $F = 1.602$, df = 3, $P = 0.211$ ($P > 0.05$); and at the 12th week: $F = 1.523$, df = 3, $P = 0.230$ ($P > 0.05$). The results showed that there was no significant difference at the 4th, 8th, and 12th week, indicating that adding aluminum chloride solution to the drinking water of this experiment had no effect on the drinking water quantity of rats. Figure 2 shows the amount of water per kilogram of body weight of rats in 24 h.

Analysis of Body Weight, Brain Coefficient, Hippocampal Coefficient, and Hippocampus/Brain in Rats

At week 12, the body weight of rats in the exposure group gradually decreased (Fig. 3A), and there were significant differences in the weight data of each group: $F = 55.654$, df = 3, $P = 0.000$ ($P < 0.05$), and the differences were statistically significant. The brain coefficient of rats (Fig. 3B) in the high dose group was higher than that in the control, low, and medium dose groups, $F = 18.599$, df = 3, $P = 0.000$, and the differences were statistically significant ($P < 0.01$, $P < 0.01$, $P < 0.01$). The hippocampal coefficient of rats (Fig. 3C) in each exposure group had no significant change, $F = 2.516$, df = 3, $P = 0.079$, and the differences were not statistically significant ($P > 0.05$). The hippocampus/brain of rats (Fig. 3D) in each exposure group had no significant change, $F = 0.238$, df = 3, $P = 0.869$ ($P > 0.05$), and the differences were not statistically significant.

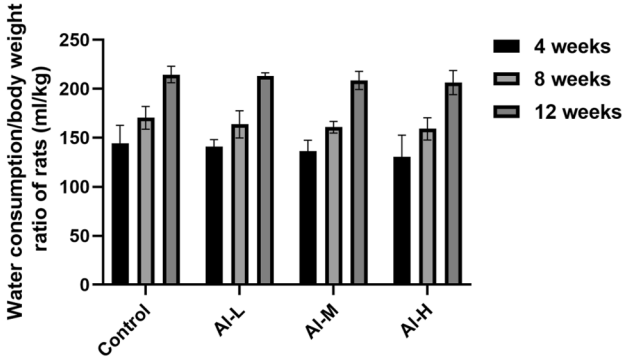

Fig. 2 Water consumption and body weight ratio of rats in each group. $n = 8$

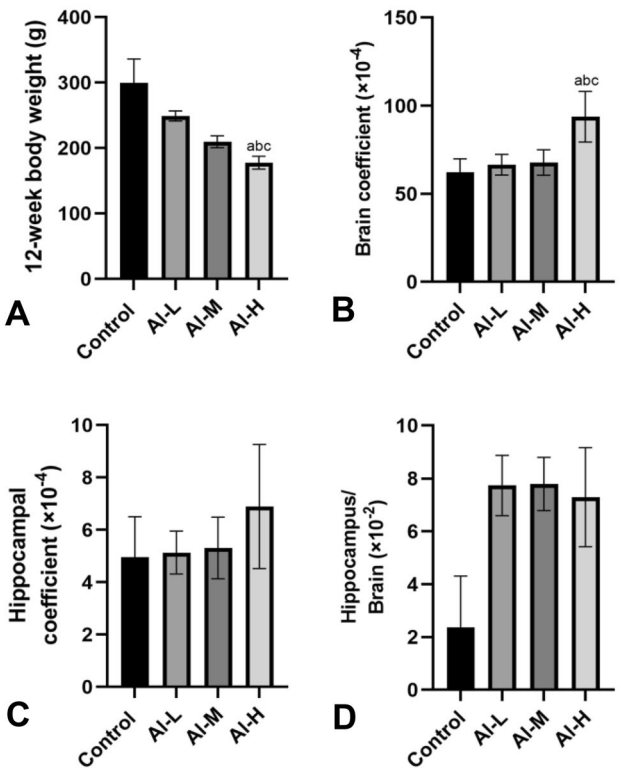

Fig. 3 Comparison of 12-week body weight, brain coefficient, hippocampal coefficient, and hippocampus weight/brain weight among rats of each group. $^{a}P < 0.01$ vs Control, $^{b}P < 0.01$ vs Al-L, $^{c}P < 0.01$ vs Al-M, $n = 8$. Brain coefficient ($\times 10^{-4}$) = brain weight/body weight. Hippocampus coefficient ($\times 10^{-4}$) = hippocampus/body weight

Effects of Aluminum Exposure on Long-Term Memory in Rats

Shuttle box experiments were performed on rats 7 days after training to evaluate the effect of aluminum on long-term memory in rats.

In the passive avoidance response index, the electric shock number of rats in each dose group was increased significantly with the increase of aluminum exposure dose.

One-way ANOVA results: $F = 40.81$, df = 3, $P = 0.000$ ($P < 0.05$). The electric shock number of rats in the high dose group was significantly higher than that in the control, low, and medium dose groups ($P < 0.01$, $P < 0.01$, $P < 0.01$). The electric shock number of rats in the medium dose group was significantly higher than that in the control and low dose groups ($P < 0.01$, $P < 0.05$). The electric shock time of rats in each dose group increased significantly with the increase of aluminum exposure dose. One-way ANOVA results: $F = 54.869$, df = 3, $P = 0.000$ ($P < 0.05$). The electric shock time of rats in the high dose group was significantly higher than that in the control, low, and medium dose groups ($P < 0.01$, $P < 0.01$, $P < 0.01$). The electric shock time of rats in the medium dose group was significantly higher than that in the control and low dose groups ($P < 0.01$, $P < 0.01$). The electric shock times of rats in the low dose group were significantly higher than those in the control group ($P < 0.05$).

Among the active avoidance indexes, the active avoidance time of rats in each dose group was significantly increased with the increase of aluminum exposure. One-way ANOVA results: $F = 52.218$, df = 3, $P = 0.000$ ($P < 0.05$). The active avoidance time of rats in the high dose group was significantly higher than that in the control, low, and medium dose groups ($P < 0.01$, $P < 0.01$, $P < 0.01$). The active avoidance time of rats in the medium dose group was significantly higher than that in the control and low dose groups ($P < 0.01$, $P < 0.01$). The active avoidance time of rats in the low dose group was significantly higher than that in the control group ($P < 0.05$). The active avoidance latency of rats in each dose group was significantly increased with the increase of aluminum exposure. One-way ANOVA results: $F = 115.218$, df = 3, $P = 0.000$ ($P < 0.05$). Among them, the active escape latency of rats in the high dose group was significantly higher than that in the control, low, and medium dose groups ($P < 0.01$, $P < 0.01$, $P < 0.01$). The active avoidance latency of rats in the medium dose group was significantly higher than that in the control and low dose groups ($P < 0.01$, $P < 0.01$).

The experimental results showed that aluminum exposure reduced the passive avoidance ability and active avoidance ability of rats, reflecting impaired learning and memory ability, and the higher the dose, the more serious the injury (Fig. 4).

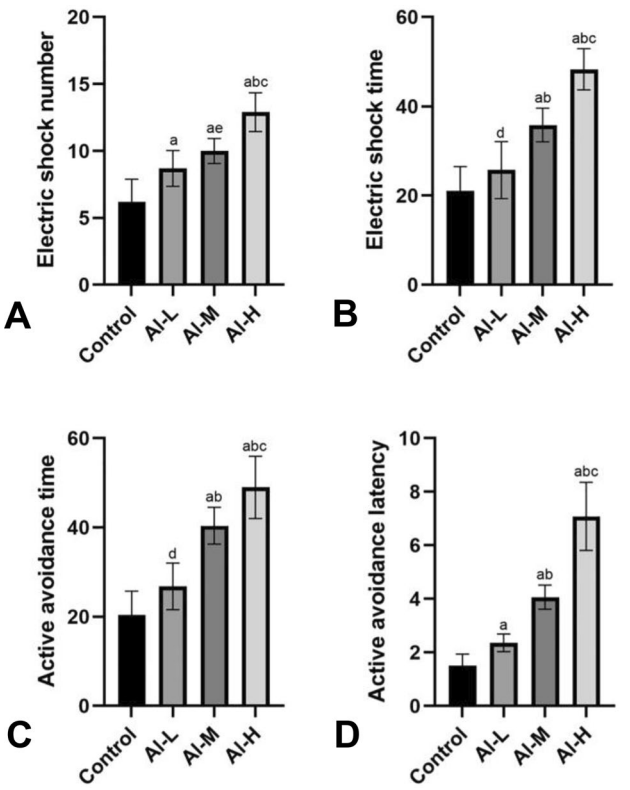

Fig. 4 Analysis of shuttle box results in rats of each group. [a]$P < 0.01$, [d]$P < 0.05$ vs Control, [b]$P < 0.01$, [e]$P < 0.05$ vs Al-L, [c]$P < 0.01$ vs Al-M, $n = 10$

Aluminum Content

Hippocampal aluminum content was measured by ICP-MS in each dose group. The results showed that aluminum content accumulated in the hippocampus increased with the increase of aluminum intake dose, and there was a positive correlation ($r = 0.987$, $P < 0.01$). One-way ANOVA results: $F = 421.259$, df = 3, $P = 0.000$ ($P < 0.05$), and the differences were statistically significant. Among them, the aluminum content in the hippocampus of the low dose group was higher than that in the control group ($P < 0.01$). Aluminum content in the hippocampus of the medium dose group was higher than that in the control and low dose groups ($P < 0.01$, $P < 0.01$). Aluminum content in the hippocampus of the high dose group was higher than that in the other three groups ($P < 0.01$, $P < 0.01$, $P < 0.01$) (Fig. 5).

Effects of Al on the Hematoxylin and Eosin Staining in the Hippocampus

In the control group, the nerve fibers were compact and tidy, with many layers, and there were many nerve cells with clear staining. After the increased intake of aluminum, the neurons in the hippocampus showed damage changes. Nerve fibers were sparse, and the gap was large. The cells were sparsely arranged, and the number of nerve cells decreased. With the increase of aluminum exposure, the staining became gradually lighter in color, and even the nuclei shrank and condensed into dark blue (Figs. 6 and 7). In this experiment, there was no variance analysis on the number of neurons.

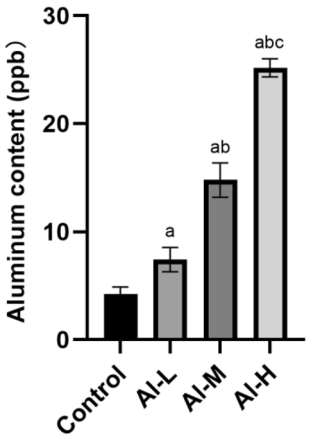

Fig. 5 Aluminum content in the hippocampus of rats in each group. [a]$P < 0.01$ vs Control, [b]$P < 0.01$ vs Al-L, [c]$P < 0.01$ vs Al-M, $n = 6$

Effects of Al on the Expression Levels of AP-1 mRNA, NGF mRNA, NT-3 mRNA, GDNF mRNA, and BDNF mRNA in the Hippocampus

RT-PCR results are shown in Fig. 8, the expression level of AP-1 mRNA in the hippocampus of rat in each exposure group was lower than that of the control group ($P < 0.01$, $P < 0.01$, $P < 0.01$). One-way ANOVA results: $F = 18.489$, df = 3, $P = 0.000$ ($P < 0.05$), and the differences were statistically significant. The expression level of AP-1 mRNA decreased with the increase of exposure dose, and there was a negative correlation ($r = -0.732$, $P < 0.01$).

The expression level of NGF mRNA in the hippocampus of rats in each exposure group was lower than those in the control group ($P < 0.01$, $P < 0.01$, $P < 0.01$). One-way ANOVA results: $F = 130.92$, df = 3, $P = 0.000$ ($P < 0.05$), and the differences were statistically significant. The expression levels of NGF mRNA in the hippocampus of the medium and high dose groups were lower than those in the low dose group ($P < 0.01$, $P < 0.01$). NGF mRNA expression level in the high dose group was lower than that in the medium dose group ($P < 0.01$). NGF mRNA expression decreased with the increase of exposure dose, and there was a negative correlation ($r = -0.936$, $P < 0.01$).

NT-3 mRNA expression level in the hippocampus of rats in each exposure group was lower than that in the control group ($P < 0.01$), and NT-3 mRNA expression levels in the hippocampus of the medium and high dose groups were lower than those in the low dose group ($P < 0.01$, $P < 0.01$). The expression level of NT-3 mRNA in the hippocampus of the high dose group was lower than that in the medium dose group ($P < 0.01$). One-way ANOVA results: $F = 28.407$, df = 3, $P = 0.000$ ($P < 0.05$), and the differences were statistically significant. NT-3 mRNA expression decreased with the increase of exposure dose, and there was a negative correlation ($r = -0.784$, $P < 0.01$).

The expression level of GDNF mRNA in the hippocampus of rats each exposure group was lower than that in the control group ($P < 0.01$, $P < 0.01$, $P < 0.01$). The expression level of GDNF mRNA in the hippocampus of the medium dose group was higher than that in the low dose group ($P < 0.01$). The expression level of GDNF mRNA in the hippocampus of the high dose group was higher than that in the low dose group, and lower than that in the medium dose group. One-way ANOVA results: $F = 179.764$, df = 3, $P = 0.000$ ($P < 0.05$), and the differences were statistically significant.

BDNF mRNA expression level in the hippocampus of rats in each exposure group was lower than that in the control group ($P < 0.01$, $P < 0.01$, $P < 0.01$), and the BDNF mRNA expression levels in the hippocampus of medium and high dose rats were lower than those in the control group ($P < 0.01$, $P < 0.01$). BDNF mRNA expression levels in the hippocampus of the medium and high dose groups were lower than those in the low dose group ($P < 0.01$, $P < 0.01$). The expression level of BDNF mRNA in the hippocampus of

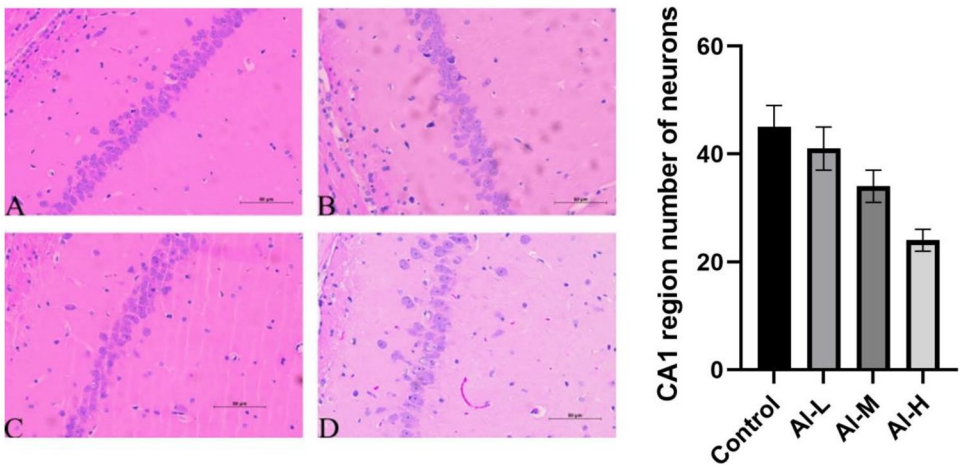

Fig. 6 Neurons in the hippocampal CA1 region of rats in each group. **A** Control; **B** Al-L; **C** Al-M; **D** Al-H (bar = 50 μm). That left panel contains representative microphotographs, right panel contains results of the densitometric analysis

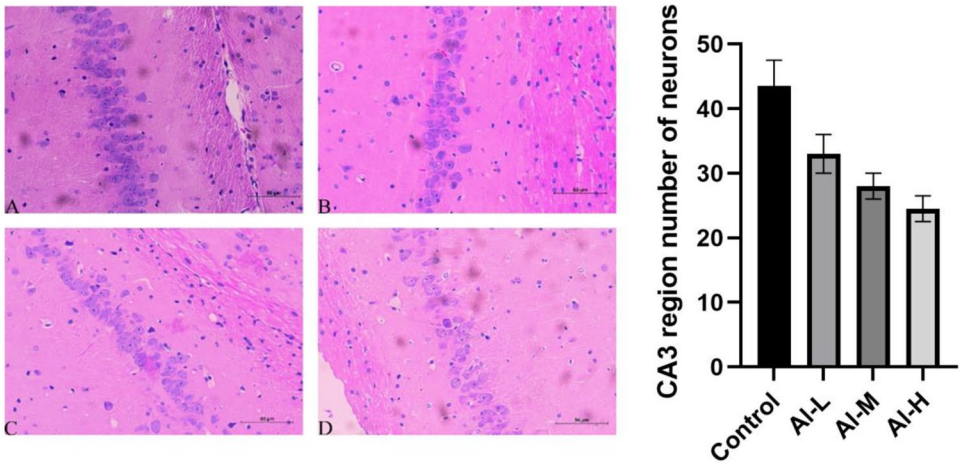

Fig. 7 Neurons in the hippocampal CA3 region of rats in each group. **A** Control; **B** Al-L; **C** Al-M; **D**: Al-H (bar = 50 μm). That left panel contains representative microphotographs, right panel contains results of the densitometric analysis

the high dose group was lower than that in the medium dose group, and the difference was not significant ($P > 0.05$).

Effects of Aluminum on Protein Levels of AP-1, NGF, NT-3, GDNF, and BDNF in the Hippocampus

The effect of Al on AP-1, NGF, NT-3, and GDNF levels in the hippocampus of rats was measured (Figs. 9 and 10). The AP-1 levels in the medium and high dose groups were significantly lower than those in the control group. One-way ANOVA results: $F = 9.466$, df = 3, $P = 0.000$ ($P < 0.05$), and the differences were statistically significant. AP-1 protein content decreased with the increase of exposure dose, showing a negative correlation ($r = -0.716$, $P < 0.01$).

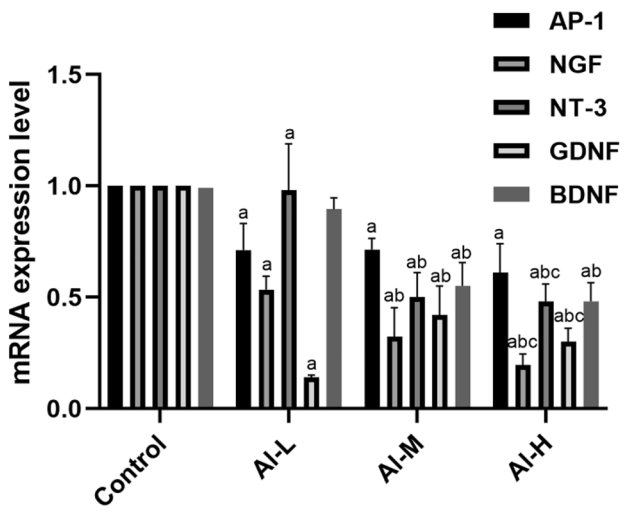

Fig. 8 Expression level of AP-1, NGF, NT-3, GDNF, and BDNF mRNA in the hippocampus of rats in each group. AP-1: $^{a}P < 0.01$ vs Control. NGF: $^{a}P < 0.01$ vs Control, $^{b}P < 0.01$ vs Al-L, $^{c}P < 0.01$ vs Al-M. NT-3: $^{a}P < 0.01$ vs Control, $^{b}P < 0.01$ vs Al-L, $^{c}P < 0.01$ vs Al-M. GDNF: $^{a}P < 0.01$ vs Control, $^{b}P < 0.01 < 0.05$ vs Al-L, $^{c}P < 0.01$ vs Al-M. BDNF: $^{a}P < 0.01$ vs Control, $^{b}P < 0.01$ vs Al-L. $n = 6$

The protein content of NGF in the hippocampus of the low dose group was lower than that in the control group ($P < 0.05$). The protein content of NGF in the hippocampus of the medium dose group was lower than that in the control and low dose groups ($P < 0.01$, $P < 0.05$). The protein content of NGF in the hippocampus of the high dose group was lower than that in the other three groups ($P < 0.01$, $P < 0.01$, $P < 0.05$). One-way ANOVA results: $F = 17.445$, df = 3, $P = 0.000$ ($P < 0.05$), and the differences were statistically significant. The protein content of NGF decreased with the increase of the exposure dose, showing a negative correlation ($r = -0.840$, $P < 0.01$).

The NT-3 protein content in the hippocampus of the medium dose group was lower than that in the control group ($P < 0.05$). The NT-3 protein content in the hippocampus of the high dose group was lower than that in the low dose group ($P < 0.05$). One-way ANOVA results: $F = 7.039$, df = 3, $P = 0.001$ ($P < 0.05$), and the differences were statistically significant. The NT-3 protein content decreased with the increase of the exposure dose, showing a negative correlation ($r = -0.687$, $P < 0.01$).

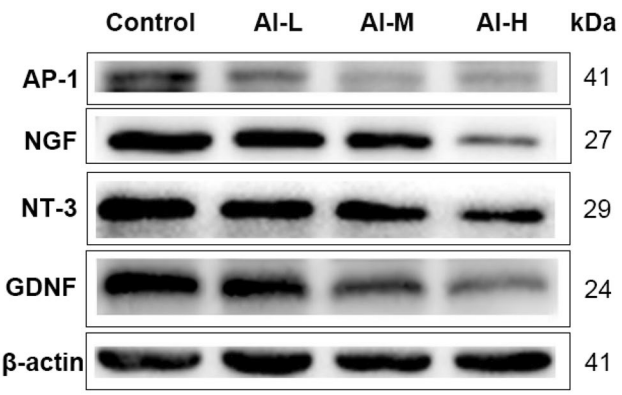

Fig. 9 Protein content in the hippocampus of rats in each group

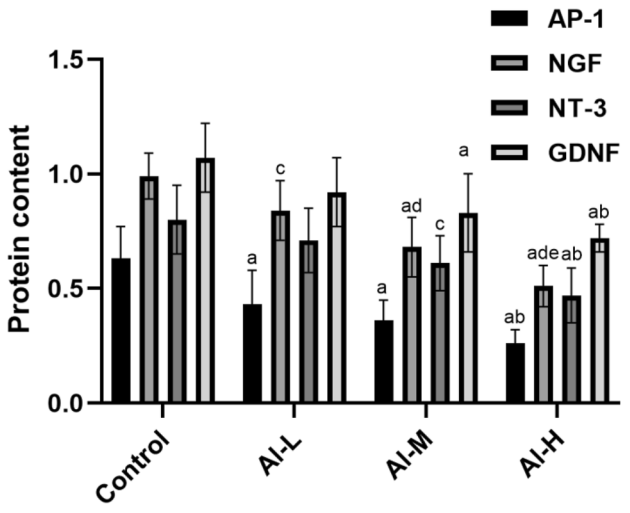

Fig. 10 Protein content in the hippocampus of rats in each group. AP-1: $^{a}P < 0.01$ vs Control, $^{b}P < 0.01$ vs Al-L. NGF: $^{a}P < 0.01$, $^{d}P < 0.05$ vs Control, $^{b}P < 0.01$, $^{e}P < 0.05$ vs Al-L, $^{c}P < 0.01$ vs Al-M. NT-3: $^{a}P < 0.01$ vs Control, $^{b}P < 0.01$ vs Al-L, $^{c}P < 0.01$ vs Al-M. GDNF: $^{a}P < 0.01$ vs Control, $^{b}P < 0.05$ vs Al-L, $n = 6$

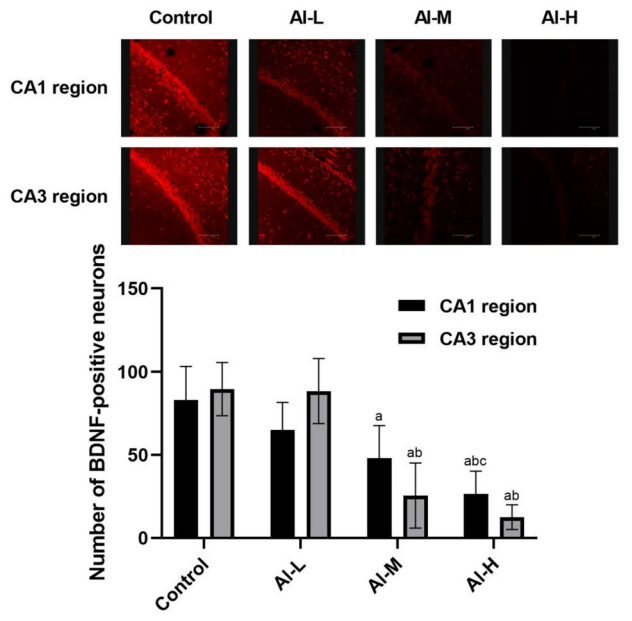

Fig. 11 BDNF protein content in the hippocampal CA1 and CA3 regions of rats in each group. $^{a}P < 0.01$ vs Control, $^{b}P < 0.01$ vs Al-L, $^{c}P < 0.01$ vs Al-M, $n = 8$. That top panel contains representative IHC microphotographs, bottom panel contains results of the respective densitometric analysis

The hippocampal GDNF protein content of the medium and high dose groups was lower than that in the control group ($P < 0.01$, $P < 0.01$). The hippocampal GDNF protein content of the high dose group was lower than that in the low dose group ($P < 0.05$). One-way ANOVA results: $F = 6.028$, df = 3, $P = 0.004$ ($P < 0.05$), and the differences were statistically significant. The GDNF protein content decreased with the increase of the exposure dose, showing a negative correlation ($r = -0.669$, $P < 0.01$).

With the increase of the exposure dose, the average fluorescence intensity of BDNF in CA1 and CA3 regions of the hippocampus decreased (Fig. 11). In the CA1 area, the average fluorescence intensity of the medium and high dose groups was lower than that in the control group ($P < 0.01$, $P < 0.01$). The average fluorescence intensity of the high dose group was lower than that in the low dose group ($P < 0.01$). The average fluorescence intensity of the high dose group was lower than that in the medium dose group ($P < 0.05$). One-way ANOVA results: $F = 14.866$, df = 3, $P = 0.000$ ($P < 0.05$), and the differences were statistically significant. In the CA3 area, the average fluorescence intensity of the medium and high dose groups was lower than that in the control group ($P < 0.01$, $P < 0.01$), and was lower than that in the low dose group, the difference was statistically significant ($P < 0.01$, $P < 0.01$). One-way ANOVA results: $F = 69.833$, df = 3, $P = 0.000$ ($P < 0.05$), and the differences were statistically significant.

Discussion

Food remains the most common source of human exposure to aluminum. In the case of food, aluminum is absorbed through the gastrointestinal (GI) system with an average daily human range of 3–10 mg (Yokel and McNamara 2001). Generally, only 0.1–0.4% of Al is absorbed from the GI tract (Shaw 2018). However, there is a concern that the Al in drinking water may be more easily absorbed than that at mealtime because an empty stomach promotes absorption (Flaten 2001). A smaller amount enters through the skin, such as in antiperspirants. These routes would put aluminum into the circulatory system relatively quickly, and most of this aluminum is typically rapidly removed by the kidneys (Tomljenovic 2011). This study used rats exposed to drinking water containing aluminum to study the neurotoxicity of aluminum since oral exposure is closer to the way that the body experiences aluminum exposure. In 2011, *World Health Organization* and *Food and Agriculture Organization* of the United Nations revised the weekly permissible intake of aluminum to 2 mg/kg body weight, according to the human and rat doses (mg/kg) were converted (conversion factor 6.17) (Wang et al. 2019). The entrance of Al into the brain of fetuses through the placenta and maternal milk has been confirmed by a radioisotope of Al during gestation and

lactation, which are sensitive periods for the development of the brain (Yumoto et al. 2001). In this study, neonatal rats were exposed to Al by parental lactation for 3 weeks and then fed with distilled water containing 0.0 g/L, 2.0 g/L, 4.0 g/L, and 8.0 g/L Al chloride ($AlCl_3$) for 12 weeks. The available literature clearly shows that the neurotoxicity of aluminum in the CNS manifests itself in symptoms such as deficits in learning, memory, concentration, speech, and psychomotor control, as well as increased seizure activity and altered behavior (i.e., confusion, anxiety, repetitive behaviors, and sleep disturbances) (Tomljenovic 2011). Al sequesters different transport mechanisms to traverse brain barriers actively, and incremental acquisition of small amounts of Al over a lifetime favors its selective accumulation in brain tissues. Hippocampus is one of the brain regions where most of the Al administered accumulates in it. The learning, memory, and neurogenesis centers are primarily located in the hippocampus. Many reports strongly indicated that Al harmed the central nervous system (CNS), particularly cognitive ability. Therefore, we chose the stage from parturition to postnatal 12 weeks, a necessary period of CNS development, exploring the mechanism underlying Al impairment of cognitive ability, especially long-term memory.

We observed a trend of decreased body weight in Al-treated rats, especially in 8.0 g/L $AlCl_3$-treated rats (no decrease in food intake), which was consistent with previous reports (Zhang et al. 2014). Additionally, the accumulation of Al in brain might result from the increased permeability of the blood–brain barrier (BBB) (Mooradian 1988). Dialysis-associated encephalopathy/dementia (DAE) is always associated with elevated serum aluminum levels (Rob et al. 2001). In order to more accurately reflect the exposure level of aluminum in the body, we not only detected the aluminum content in the blood but also detected the Al level in the hippocampus. The $AlCl_3$ overload was confirmed by the significant increase of $AlCl_3$ levels in plasma and brain of $AlCl_3$ exposed rats compared to the control rats with a dose-dependent manner at 2.0 g/L, 4.0 g/L, and 8.0 g/L $AlCl_3$ treated and which has been confirmed in our previous study (Zhang et al. 2014). These results indicate that increased Al retention and impaired hippocampus development negatively influence cognitive ability in animals. So, we chose the hippocampus to explore the probable mechanism of Al impairing LTM.

Another thing is that the relatively high doses of $AlCl_3$ were set in this study mainly considering as following three factors: the broad application of Al, the human being is the most sensitive species to be extrapolated, meanwhile, referring to our and other previous studies (Zhang et al. 2013, 2014; Wang et al. 2010a). Our data (Fig. 1) showed that the hippocampus or blood Al contents in $AlCl_3$-treated rats gradually increased with the increasing $AlCl_3$ dose. This implies that the model was sufficiently ready for studying the toxic effects of Al on hippocampus.

Shuttle box testing is a two-way active avoidance (TWAA) test, and it is an experimental method for judging the ability of learning and memory. It mainly observes the changes in the subject's ability to reflect the active and passive escape conditions. When the experimental animals are tested in the shuttle box test, the more electric shocks the animal receives, the longer the electric shock time, and the longer the animal's active escape latency, the worse the learning and memory ability (Izquierdo et al. 2006). Studies have shown that prenatal Al exposure impaired rat pups' performance in the passive avoidance task and postnatal Al exposure also induced neurobehavioral deficits. Separately, it was shown that transient suppression of hippocampus (especially hippocampus ventral) activity interferes with active avoidance learning (Wang et al. 2015). In the present study, to assess long-term memory, we allowed the rats to rest for 1 week after the training session and then subjected them to the same testing session of AAR and PAR. In correlation with an increasing dose of Al, the shock number and shock time in PAR increased gradually, which indicated that the learning and response abilities of Al-treated rats were defective. The escape time and escape latency in AAR prolonged gradually with an increasing Al dose, which suggested that Al-treated rats could not maintain the obtained behavioral performance and that long-term memory ability was impaired. These findings suggested that Al may impair the long-term memory of animals via its accumulation in the hippocampus.

There was evidence that AP-1 protein plays a role in synaptic plasticity and memory (Alberini 2009). AP-1 protein was involved in memory formation (Grimm et al. 1997) and regulated neuronal survival and apoptosis. Its role depended on the same cellular environment and stimulation (Herdegen and Leah 1998). The regulation of AP-1 activity depended on two main factors: the concentration and activation level of AP-1 protein after extracellular stimulation. Under physiological conditions, the protein concentration and activity of AP-1 were deficient; when the cells were stimulated, the protein level of AP-1 would increase temporarily and rapidly. The activation of AP-1 in the hippocampus may protect neurons from damage by regulating its target genes, such as NGF and BDNF (Zhang et al. 2003). AP-1 was found at the receiving end of multiple signaling pathways and regulates gene expression in response to various stimuli, including cytokines, growth factors, stress, and bacterial and viral infections. AP-1 controlled a broad range of biological processes, including proliferation, transformation, cell differentiation, cell migration, and apoptosis (Leppä and Bohmann 1999; Johnson and Lapadat 2002; Shaulian and Karin 2002; Ameyar et al. 2003). Early experiments in this research group showed that the c-fos gene and protein content in the brain of rats exposed to aluminum decreased (Zhang

et al. 2016a, b). This result was consistent with that of our experiments.

Experimentally, chronic exposure to aluminum can cause neuropathological changes and cognitive impairments, which were similar to those of Alzheimer's disease, including the deficiency of the neurotransmitter acetylcholine, extracellular amyloid β (Aβ) deposits, neurofibrillary tangles, and the loss of neurons (Stephen and Whitehouse 1992; Pierce and Kawas 2017). Cholinergic neurons were situated mainly in different areas of the basal forebrain (BF), the primary source of cholinergic innervation to the cerebral cortex, hippocampus, amygdala, and remaining portions of the cortical mantle (Niewiadomska et al. 2009). Neurotrophins control plasticity, differentiation, pruning, and survival of the BDNF, and the signaling of these peptides was highly altered in the course of the disorder (Skaper 2018).

NTFs are growth factors that support the development, differentiation, and plasticity of brain function throughout life. Moreover, NTFs could also maintain neuronal survival cell morphology and play critical roles in cognition and memory formation (Connor and Dragunow 1998). NGF, NT3, GDNF, and BDNF are the most widely studied neurotrophic factors.

NGF was discovered at the earliest time and played an essential role in the growth and development of peripheral and central neurons, maintaining neuron survival, etc. Mature NGF has neuroprotective effects (Keefe et al. 2017). In autopsy reports of patients diagnosed with MCI, mild AD, and severe AD, the level of NGF in the hippocampus was highest in cadavers with mild AD, followed by severe AD, and lowest in cadavers with MCI (Mufson et al. 2003). This suggests that NGF levels may be affected by differences in disease age or disease course. Experiments showed that aluminum-induced NGF expression decreased concentration-dependent, and NGF expression could not be detected when the aluminum concentration was too high (Johnson and Sharma 2003). Pregnant mice exposed to lead from gestation until weaning (postpartum day 21) decreased NGF expression in the hippocampus of their offspring, which may lead to the accumulation of brain Aβ, neurological dysfunction, and impairment of signaling pathways (Li et al. 2017). The Western blot and RT-PCR results in the present study showed that subchronic aluminum exposure reduced hippocampal nerve growth factor levels in rats. There was a negative correlation with the dose, suggesting that the learning and memory disorders in rats might be related to the decrease in NGF levels in the rat brain, especially in the hippocampus.

Neurotrophin-3 (NT-3) was a member of the nerve growth factor family and mediates trophic effects on neurons, including neuronal differentiation, neurite outgrowth, synapse formation, and plasticity (Barbacid 1994; Huang and Reichardt 2003; Ramos-Languren and Escobar 2013). NT-3 binded with the highest affinity to TrkC, a neurotrophin receptor tyrosine kinase (RTK), and activated its tyrosine kinase to drive intracellular signaling cascades (Barbacid 1994; Lamballe et al. 1991). In the rodent brain, both NT-3 and TrkC were expressed from embryonic to adult stages and continued to be highly expressed in the adult hippocampus (Maisonpierre et al. 1990; Tessarollo et al. 1993; Lamballe et al. 1994). Our antibody uptake assays revealed that NT-3 had trophic effects on native functional presynaptic terminals. NT-3 binded to TrkC and TrkB and p^{75} with a lower affinity (Barbacid 1994). NT-3 and BDNF were also secreted in a neuronal activity-dependent manner (Lessmann et al. 2003). Recent studies had found polymorphisms of NTF-3 associated with AD (Kobayashi et al. 2012; Nagata et al. 2013). The experiment found that the expression level of NT-3 mRNA and protein in mouse brain tissue induced by Dgalactose combined with $AlCl_3$ was significantly reduced, and its protein expression level was also significantly reduced in the mouse cortex and hippocampus CA1 area (Gao et al. 2014). This study showed that NT-3 level in rat hippocampus decreased after subchronic aluminum exposure, and there was a negative correlation with the dose, suggesting that the learning and memory impairment in rats might be related to the decrease of NT-3 level in rat hippocampus.

GDNF was widely distributed in the cortex, hippocampus, striatum, and spinal cord of developing and adult animals and played an essential role in the growth, proliferation, differentiation, apoptosis of neurons, and the formation of axons (Zhang et al. 2006). The expression of hippocampal GDNF was decreased after lead exposure, suggesting that GDNF was involved in lead intake, impairing learning and memory (Wang et al. 2010b). The present study results showed that subchronic aluminum exposure decreased the levels of GDNF in the hippocampus of rats and was negatively correlated with the dose of the toxicant, suggesting that the impairment of learning and memory in rats may be related to the reduced levels of GDNF in the hippocampus.

BDNF is mainly distributed in the cerebral cortex and hippocampus and is an essential regulator of synaptic degeneration and synaptic plasticity mechanisms related to learning and memory in the central nervous system. Studies had confirmed that BDNF was involved in regulating LTP (Leal et al. 2014). Our previous research revealed that Al could impair long-term memory via the impairment of late-phase long-term potentiation (L-LTP) in vivo (Zhang et al. 2013). At the same time, BDNF binds to its receptor TrkB. TrkB gains catalytic activity, activates its tyrosine kinase activity, and causes self-phosphorylation. The activated receptor can stimulate signal transduction and trigger intracellular signal cascades, including its downstream signals molecules ERK and CREB, thereby increasing synaptic connections and improving learning and memory (Zhao et al. 2018). Within the hippocampus, GDNF was co-expressed and shares similar signaling pathways with

BDNF (Pertusa et al. 2008), and BDNF was closely related to synaptic plasticity, and memory formation GDNF may also affect learning and memory processes by interacting with BDNF. Wang's study showed that in AD patients complicated with cognitive dysfunction, there was a clear correlation between the severity of the inflammatory and oxidative stress responses and the abnormal changes of BDNF (Wang et al. 2021). Increase of BDNF had been found in obstructive sleep apnea (OSA) studies in PD patients, possibly associated with increased lethargy, leading to cognitive impairment, but were subject to further study (Kaminska et al. 2022). Aluminum intake increased, and the mean fluorescence intensity of BDNF decreased in CA1 and CA3 areas of the rat hippocampus. In the CA1 area, the mean fluorescence intensity of the medium and high dose groups was lower than that in the control group, and the mean fluorescence intensity of the high dose group was lower than that in the low and medium dose groups; in the CA3 area, the mean fluorescence intensity was decreased in the medium and high dose groups compared with the control group, and in the medium and high dose groups compared with the low dose group. These results suggested that the impairment of learning and memory in rats may be associated with decreased BDNF levels in the hippocampus region of rats.

Conclusion

In summary, in this study, we found that aluminum exposure may affect the decreased expression level of neurotrophic factors in the hippocampus of rats by inhibiting the expression level of AP-1 and finally lead to learning and memory impairment in rats. The specific possible mechanism and preventive measures need to be further discussed.

Author Contribution WL and JL designed the study, wrote the manuscript, and analyzed the data. JG reviewed the references. XD and LZ reviewed and revised the manuscript. All authors had read the manuscript and agree to the publication of this study.

Funding This work was supported by the National Natural Science Foundation of China (81673226); by the Initiated Research Foundation for the Doctoral Program of Science and Technology Department of Liaoning Province, China (201601226); by the Natural Science Foundation of Education Department of Liaoning Province, China (L2015544, LJKZ1146); by the Natural Science Foundation for Innovation and Entrepreneurship Training Program of Education Department of Liaoning Province, China (201710164000038); by the Natural Science Foundation of Science and Technology Department of Shenyang City, China (17–231-1–44); by the Natural Science Foundation of Shenyang Medical College, China (20153043); by the Natural Science Foundation for Graduate Students of Shenyang Medical College, China (Y20180512); and by the Natural Science Foundation for Undergraduate Students of Shenyang Medical College, China (20179028).

Data Availability The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval The research was carried out following the National Institutes of Health Guide for the Care and Use of Laboratory Animals Standard.

Consent to Participate Not applicable.

Competing Interests The authors declare no competing interests.

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