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RESEARCH

Effects of miR-204-5p and Target Gene EphB2 on Cognitive Impairment Induced by Aluminum Exposure in Rats

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Abstract

Aluminum is a common environmental neurotoxin. Aluminum ions can cross the blood–brain barrier and accumulate in different brain regions, damage brain tissue, and cause cognitive impairment, but the molecular mechanism of aluminum neurotoxicity is not precise. This study investigated the effects of miR-204-5p, target gene EphB2, and downstream signaling pathway NMDAR-ERK-CREB-Arc on cognitive dysfunction induced by aluminum exposure. The results showed that the learning and memory of the rats were impaired in behavior. The accumulation of aluminum in the hippocampus resulted in the damage of nerve cell morphology in the CA1 region of the hippocampus. The expression level of miR-204-5p was increased, and the mRNA and protein expressions of EphB2, NMDAR2B, ERK1/2, CREB, and Arc were decreased. The results indicated that the mechanism of impaired learning and memory induced by aluminum exposure might promote the expression of miR-204-5P and further inhibit the expression of the target gene EphB2 and its downstream signaling pathway NMDAR-ERK-CREB-Arc.

Keywords Aluminum · Cognitive function · Epigenetics · miR-204-5P · EphB2

Introduction

Aluminum (Al) is ubiquitous in the environment, and the primary natural source is rock. At present, Al has entered the biosphere from the lithosphere. The main way Al enters the body is by mouth (from food, drink, drugs) and inhalation. It can enter the brain through capillaries or from the olfactory epithelium of the nasal cavity when inhaled and accumulate in different areas, thereby damaging brain tissue and causing cognitive impairment [1]. Epidemiological investigations have shown that occupational exposure to Al can lead to cognitive dysfunction in Al factory workers [2]. Excessive consumption of Al can alter associated proteins and impair learning and memory in rats, according to animal studies [3]. Exposure to adverse factors in early life affects the growth and development of infants and young children and plays an essential role in developing childhood diseases [4]. Infants are more likely to be exposed to dietary pollutants than adults due to their low body weight and limited diet types. Studies have found that baby food, such as infant formula food and infant complementary food, contains different levels of heavy metals which may result in the accumulation and pollution of toxic metals due to the influence of raw materials or operations affected by the processing methods [5]. The central nervous system of school age children is in a period of rapid growth and development, and the major organs and systems of the body have not yet matured and are more sensitive to toxic chemicals [6]. To protect children from exposure to environmental toxins, it is crucial

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to research environmental Al exposure during growth and development.

Small non-coding RNAs known as microRNAs (miRNAs) have a length range of 18 to 25 nucleotides and are crucial in the control of gene expression. The plasticity of hippocampus neurons and the capacity for spatial learning and memory may be impacted by abnormal miRNA expression [7]. MiR319, miR390, miR393, miR319a.2, and miR398 have been identified as having particular regulatory roles in Al toxicity signaling networks [8]. Li discovered that Al may abnormally downregulate the production of miR-29a and miR-29b1, which would then inhibit the upregulation of BACE1 and ultimately result in an increase in Aβ [9].

Erythropoietin producing hepatocellular receptor (Eph) is the most prominent family of tyrosine protein kinases. According to the kind of their binding subclass homologous ligands, EphrinAs or EphrinBs, EphA and EphB receptors are categorized. Through its association with ephrins, the transmembrane tyrosine receptor EphB2 can induce tyrosine phosphorylation in the cytoplasm, further activating the intracellular response. Its malfunction impairs learning and memory because EphB2 regulates glutamatergic synaptic transmission, neuronal morphogenesis, and gene expression [10]. Recent studies have confirmed that age-related increase in miR-204 levels can inhibit the expression of target gene EphB2, thereby leading to cognitive dysfunction [11], and it exerts anti-epileptic effects in Mg^{2+} free models in hippocampal neurons by regulating TrkB and downstream ERK1/2-CREB signaling pathways [12]. In addition, some researchers speculated that down-regulation of miR-204-5P may alleviate isoflurane induced cognitive behavioral disorders in rats by targeting EphB2 [13].

N-methyl-d-aspartate receptor (NMDAR) is an ionic glutamate receptor consisting of three subunits (NR1, NR2 (A-D), and NR3) [14]. NMDARs are highly expressed in the cerebral cortex and hippocampus, mediate excitatory synaptic transmission, and are closely related to cognitive function. Increased expression of NMDARs can improve learning and memory function, while inhibition of NMDAR can lead to impairment of spatial cognitive function [15]. Previous studies have shown that EphB2 knockout mice can decrease the number of NMDARs at synapses [16] and that Eph forward signal promotes tyrosine phosphorylation of the NMDAR subunit (NR2B) through Src family kinases to promote NMDA receptor transmission [17].

Under normal circumstances, the mitogen activated protein kinase phosphatase (MAPK) family member extracellular signal-regulated kinases 1 and 2 (ERK1/2) are involved in cell proliferation, differentiation, and maturation. Increases in the level of ERK phosphorylation have a protective effect on neurons, whereas decreases in the level of ERK phosphorylation promote neuronal apoptosis [18]. The plasticity and survival of NMDAR dependent neurons are mediated by the ERK signaling cascade, according to studies [19, 20]. ERK activation is the downstream of NMDA receptor activity. ERK phosphorylation is related to memory function, and the induction and maintenance of LTP [21]. Normally expressed NMDAR dependent signaling activates the ERK1/2 cascade, including CREB and BDNF activation [22]. A well-known important regulator of transcription that is influenced by brain activity is CREB. ERK activates CAMP response original binding protein by activating ribosome S6 kinase, thus initiating CREB-dependent transcription. CREB activates immediate early gene (IEG) expression in an activity- and learn-dependent manner [23, 24]. IEG is a group of proto-oncogenes, primarily c-Fos, c-Jun, and Arc, that can encode transcription factors and have the ability to couple short-term signals with long-term changes [25]. The cytoskeletal protein Arc/Arg3.1 is essential for synaptic plasticity, learning, and memory in the human brain, which are all aspects of IEG [26].

At present, the regulatory effect of epigenetic regulation in the form of miR-204-5p on cognitive impairment induced by Al remains unclear. Therefore, we studied the effects of miR-204-5p with the target gene EphB2 and downstream signaling pathway NMDAR-ERK-CREB-Arc on cognitive dysfunction caused by Al exposure.

Materials and Methods

Experimental Animals and Treatment

Forty mature Wistar rats (170–200 g) were purchased from Changsheng Biotechnology. The animals were acclimated at the Animal Experimental Center of Shenyang Medical College for seven days. Feeding conditions are 22 ± 2 ℃ at room temperature, 50 ~ 60% humidity, 12 h light and dark cycle, and free access to water and food. The water of rats was distilled water, and the food was selected as the Experimental Rat Food (Qianmin Feed Co., LTD., Shenyang, China). The Shenyang Medical College's Animal Experimental Ethics Committee gave its blessing to this work (No. SYYXY2021021403). $AlCl_3$ was dissolved in distilled water (Sinopharm Shenyang). This setting of Al^{3+} concentration is referred to the previous experimental setting of our research group [27].

Animal Mating and Grouping

A total of 24 female rats and 12 male rats were selected for 2:1 co-cage mating, and it was found that the female rats were impregnated by white vaginal plug test in the following day. Pregnant rats were divided into 3 dose groups (2.0 g/L, 4.0 g/L, 8.0 g/L $AlCl_3$) and a blank control group (normal saline 0.0 g/L $AlCl_3$) with 6 rats in each group. On

the day of delivery, the female rats in each group ingested Al by drinking different concentrations of $AlCl_3$ solution. Before weaning, the infant rats consumed Al from breast milk. After weaning, they drank different concentrations of $AlCl_3$ solution by themselves. All the rats in the four groups drank water freely during the experiment. From the first day of life (day 0), daily food and water intake were recorded, and weight was measured once a week for 90 days. By observing the ratio of average daily water intake to body weight, it was found that the average daily water intake of rats was relatively constant, indicating that the intake of Al was consistent with the experimental design and relatively reasonable. After exposure, the rats underwent a water maze experiment. After the neurobehavioral experiments ended, the rats were anesthetized for sampling, and then 6 hippocampal samples (male: female = 1:1) in different dose groups were randomly selected to determine various indexes. All animals are treated strictly with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. We did our best to reduce the number and suffering of animals.

Morris Water Maze

After 1 day of exposure, the rats in each dose group underwent water maze adaptation training for 5 days. After the adaptive training, the rats rested for 1 day and began the formal experiment. The cognitive function of rats was tested by positioning navigation and space exploration. Positioning sailing experiment: Rats were placed into the water facing the side wall of the first quadrant midpoint, and the escape latency and total swimming distance of the rats were observed. Space exploration experiment: The platform was removed, and the rats were thrown into the pool facing the wall. The free swimming time of each rat in the pool was 60 s, and the residence time of the rats in the first quadrant and the number of crossings of the target platform were recorded in the 60 s. All data are recorded automatically by Water Maze Systems (Stoeling, USA).

ICP-MS

The rats in each dose group were randomly selected to measure the Al content. ICP-MS operation procedure is as mentioned above [28].

Hippocampal Organ Coefficient

The rats in each dose group were randomly chosen, anesthetized by intraperitoneal injection of urethane, decapitated, placed on ice, and quickly extracted bilateral hippocampus with tweezers and weighed. After the above steps were completed, they were stored in liquid nitrogen at − 80 ℃. Hippocampal organ coefficient = hippocampal weight (g)/body weight (g) × 100%

Transmission Electron Microscope

In each dose group, the rat brain tissues fixed in 2.5% polyglutaraldehyde solution were rinsed with PBS buffer, and 1% osmic acid to solid sample for 2 h. The samples were dehydrated by ethanol gradient, and the tissues were embedded. The tissue was cut into slices with a thickness of 60 ~ 80 nm and stained with lead citrate and uranyl acetate. The sections were placed under the transmission electron microscope (Nikon Corporation, Japan) to observe the ultrastructure of the rat hippocampus.

Immunohistochemistry

The seahorses in each group were washed overnight with water and paraffin-embedded to make 4-μm sections. Sections were stained after dewaxing. A rabbit anti-CREB antibody (1:50) was added, incubated overnight at 4 ℃, biotin-labeled IgG polymer was added, incubated at 37 ℃, Streptomyces antibiotic protein-peroxidase was added, and incubated at room temperature. DAB color-developing solution was added, rinsed with running water, and dehydrated with alcohol. The sections were sealed and analyzed under an optical microscope, and photos were taken. Image-Pro Plus 6.0 software was used to read the average optical density of slices.

Western Blot

Hippocampal tissue was added to RIPA lysate for cleavage, and the protein concentration of the sample was calculated using the BCA protein detection kit. The total protein of the hippocampus was isolated by SDS-PAGE to detect EphB2, NMDAR2B, ERK1/2, and Arc. The transferred PVDF film was put into TBST solution, 5% skimmed milk powder sealing solution was added, and sealed in a shaker for 2 h. A primary antibody was added to the cut PVDF membrane: rabbit anti-rat EphB2 recombinant antibody (1:1000), rabbit anti-rat NMDAR2B antibody (1:1000), rabbit anti-rat ERK1/2 (1:1000), rabbit anti-rat Arc polyclonal antibody (1:1000), and rabbit anti-rat β-actin antibody (1: 2000), put in a shaker for 1 h, 4 ℃ overnight. On the next day, the 1:5000 secondary antibody hybrid solution was added and incubated at room temperature for 30 min. The protein strip was enhanced with ECL chemiluminescence kit, and the resulting images were analyzed by Image J software.

RT-PCR

Total RNA was extracted from the hippocampus of each group using Monzol™ reagent, the RNA concentration and purity were detected by ultramicro spectrophotometer (Thermo), and cDNA was synthesized by reverse transcription according to the specification of miRNA cDNA first chain synthesis reagent. PCR was performed using the SYBR Green fluorescent quantitative PCR kit in the 7500 HR Fast Real-time PCR system (Applied Bio-systems, USA). The primer sequences used are shown in Supplementary Table 1. Amplification was performed using the GeneAmp PCR 9700 thermal cycler (ABI, USA), including predenaturation (95 ℃, 30 s), 40 cycles: denaturation (95 ℃, 5 s) and annealing (60 ℃, 30–34 s). Using GAPDH as the internal reference, the relative expression level of each mRNA was calculated by the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Statistical results were expressed as mean ± standard deviation. SPSS 25.0 software was used to analyze and process the experimental data. In the statistical analysis, the variance homogeneity test of the dependent variables was first carried out. When the variance of the sample population was homogeneous, one-way analysis of variance (ANOVA) was used for inter-group comparison, and the LSD method was used for pairwise comparison between groups. At 95% confidence level, $P<0.05$ indicated that the difference was statistically significant. 0.0 g/L $AlCl_3$: $^{*}P<0.05$; 2.0 g/L $AlCl_3$: $^{\#}P<0.05$; 4.0 g/L $AlCl_3$: $^{+}P<0.05$.

Results

Body Weight

The weight changes of rats in each group were shown in Fig. 1, and we selected the weight of rats at 0, 4, 8, 12 weeks for statistical analysis. The spherical test Mochley W = 0.143, $P<0.05$, which did not satisfy the spherical distribution hypothesis. We observed Greenhouse Geissertime in statistical analysis. The intra time effect was significant: df = 1.796, $F=2994.453$, $P<0.05$, partial $\eta^2=0.993$. The intra subject effect of time × group was significant: df = 5.387, $F=36.373$, $P<0.05$, partial $\eta^2=0.845$. The group intersubjective effect was significant: df = 3, $F=260.963$, $P<0.05$, partial $\eta^2=0.975$.

Behavioral Changes in Rats

Positioning Navigation

The escape latency and swimming distance of rats were related to the Al dose during positioning navigation ($r=0.8$, $P<0.05$, $r=0.611$, $P<0.05$) (Fig. 2A, B). The escape latency of rats was significantly changed in the Al dose (df = 3, $F=20.627$, $P<0.05$). The escape latency of 4.0 g/L $AlCl_3$ group was higher than 0.0 g/L $AlCl_3$ group and 2.0 g/L $AlCl_3$ group. The swimming distance of rats was significantly different in the Al dose (df = 3, $F=8.575$, $P<0.05$).

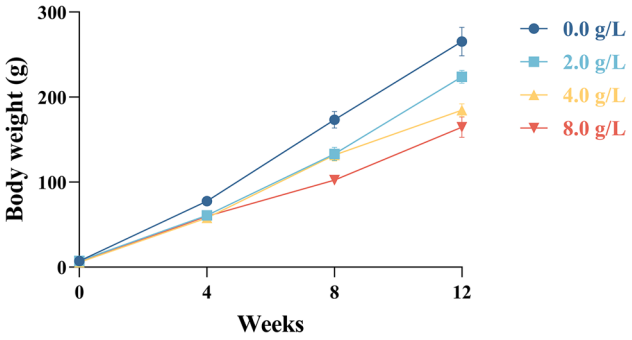

Fig. 1 Effects of Al on weight of rats ($n=6$)

Space Exploration

The increase of Al dose during space exploration was negatively correlated with the number of crossing platforms (limited time was 60 s) and residence time in the target quadrant ($r=-0.884$, $P<0.05$; $r=-0.923$, $P<0.05$) (Fig. 2C, D). The number of platform crossings in rats was reduced in the Al dose (df = 3, $F=28.011$, $P<0.05$). Compared with the 0.0 g/L $AlCl_3$ group, the number of rats crossing the platform in the other three groups was decreased. The target quadrant residence time of rats was significantly different in the Al dose (df = 3, $F=43.497$, $P<0.05$). Compared with the 0.0 g/L $AlCl_3$ group, the target quadrant residence time of rats in the other three groups was decreased.

Al^{3+} Content

We used the ICP-MS method to measure Al^{3+} content. As shown in Fig. 3, Al dose was positively correlated with Al^{3+} content in the hippocampus ($r=0.943$, $P<0.05$). There were significant differences in Al^{3+} content among all groups. The Al^{3+} content of 4.0 g/L $AlCl_3$ group was higher than that of the 0.0 g/L $AlCl_3$ group and 2.0 g/L $AlCl_3$ group.

Hippocampal Organ Coefficient

Al dose was correlated with the hippocampal organ coefficient ($r=0.469$, $P<0.05$) (Fig. 4), and the hippocampal organ coefficient increased and had significant changes in each group (df = 3, $F=3.314$, $P<0.05$). Compared with the 0.0 g/L $AlCl_3$ group, hippocampal organ coefficients in the 2.0 g/L $AlCl_3$ group and 8.0 g/L $AlCl_3$ group were increased.

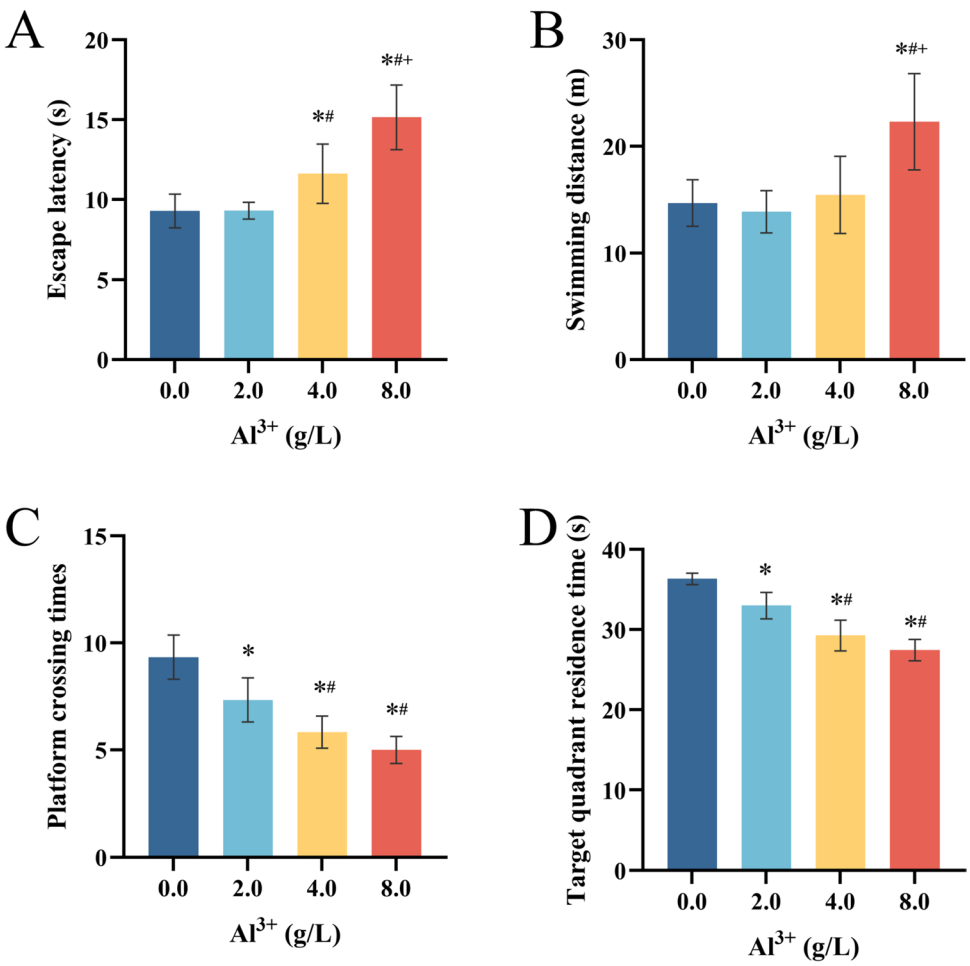

Fig. 2 Effects of Al exposure on behavior in rats ($n=6$)

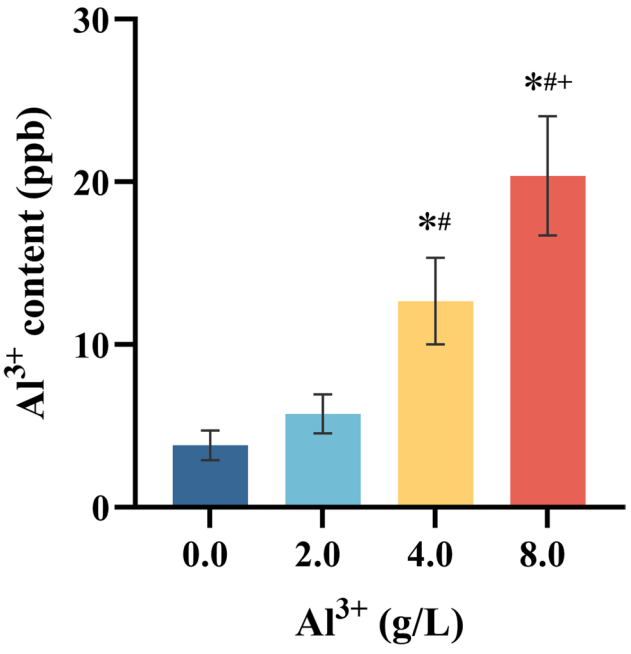

Fig. 3 Hippocampal Al^{3+} content in rats ($n=6$)

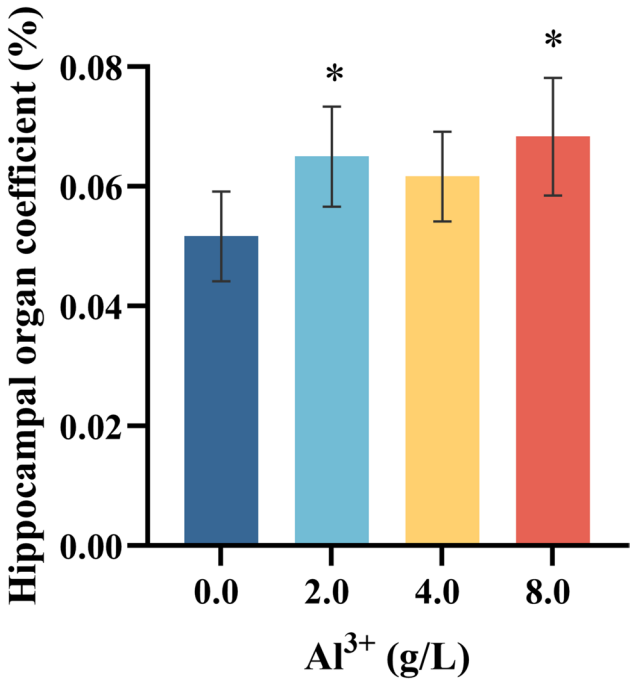

Fig. 4 Hippocampal organ coefficients in each group ($n=6$)

Morphology of Nerve Cells in CA1 Region Exposed to Al

Under transmission electron microscopy, hippocampal neuron cells in the CA1 region are shown in Fig. 5. The cell structure of the 0.0 g/L $AlCl_3$ group was regular, the membrane edges were clear, the structure was intact, the nucleus was regularly round, and the chromatin was evenly distributed. The cell morphology of the 2.0 g/L $AlCl_3$ group was relatively small. The cells in the 4.0 g/L $AlCl_3$ group and 8.0 g/L $AlCl_3$ group showed significant changes, including cell structure deformation, incomplete nuclear structure and invagination, nuclear membrane shrinkage, nuclear pyknosis, and chromatin pyknosis and edge aggregation to a certain extent, which indicated that Al exposure could cause damage to hippocampal neurons.

Changes of mRNA Expression Levels

We obtained the expression of target RNA by PCR (Fig. 6). The miR-204-5P expression was increased, and the mRNA expression of EphB2, NMDAR2B, ERK1, ERK2, CREB, and Arc was decreased with the increase in Al dose. It can be observed in Fig. 6A that the miR-204-5P expression increased with the increase of Al dose ($df = 3$, $F = 4.16$, $P < 0.05$; $r = 0.597$, $P < 0.05$). Compared with the 0.0 g/L $AlCl_3$ group, the miR-204-5P expression in the other three groups was increased to a certain extent.

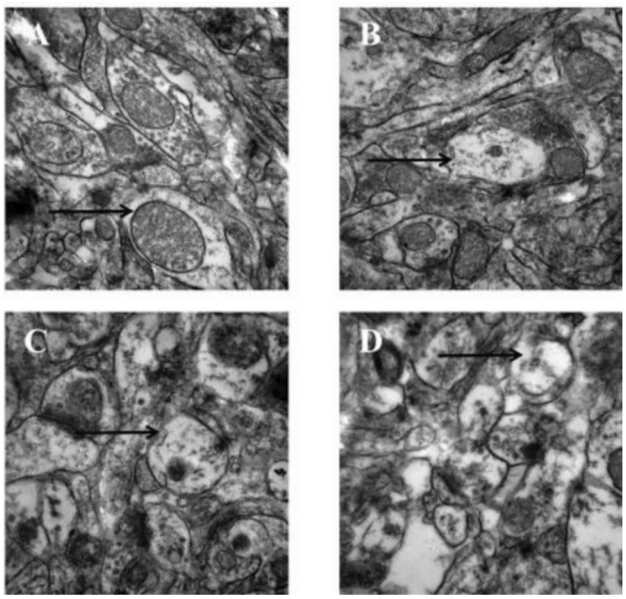

Fig. 5 Rat hippocampal CA1 nerve cells (TEM). **A**: 0.0 g/L $AlCl_3$; **B**: 2.0 g/L $AlCl_3$; **C**: 4.0 g/L $AlCl_3$; **D**: 8.0 g/L $AlCl_3$. Magnification: 50 K. Arrows point to significant neuronal cell structures in each dose group

The EphB2 mRNA expression in all groups was decreased with Al dose ($df = 3$, $F = 20.654$, $P < 0.05$; $r = -0.861$, $P < 0.05$) (Fig. 6B). Compared with 2.0 g/L $AlCl_3$ group, the EphB2 mRNA expression in the 4.0 g/L $AlCl_3$ group and 8.0 g/L $AlCl_3$ group was decreased.

The NMDAR2B mRNA expression in all groups was decreased with Al dose ($df = 3$, $F = 16.207$, $P < 0.05$; $r = -0.804$, $P < 0.05$) (Fig. 6C). The NMDAR2B mRNA expression in 8.0 g/L $AlCl_3$ group was lower than in the 2.0 g/L $AlCl_3$ group. The ERK1 mRNA expression in all groups was decreased with Al dose ($df = 3$, $F = 21.608$, $P < 0.05$; $r = -0.714$, $P < 0.05$) (Fig. 6D, E). The ERK2 mRNA expression in all groups was decreased with Al dose ($df = 3$, $F = 9.141$, $P < 0.05$; $r = -0.687$, $P < 0.05$). The CREB mRNA expression in each group was decreased with the Al dose ($df = 3$, $F = 15.798$, $P < 0.05$; $r = -0.834$, $P < 0.05$) (Fig. 6F). The CREB mRNA expression in the 8.0 g/L $AlCl_3$ group was lower than in the 2.0 g/L $AlCl_3$ group and 4.0 g/L $AlCl_3$ group. The Arc mRNA expression in each group was decreased with the Al dose ($df = 3$, $F = 10.271$, $P < 0.05$; $r = -0.773$, $P < 0.05$) (Fig. 6G). The Arc mRNA expression in the 8.0 g/L $AlCl_3$ group was significantly lower than that in the 2.0 g/L $AlCl_3$ group and 4.0 g/L $AlCl_3$ group.

Immunohistochemistry

The CREB protein in the CA1 region was measured using immunohistochemistry (Fig. 7). The CREB protein of rats was decreased with the increase of Al dose ($df = 3$, $F = 6.516$, $P < 0.05$; $r = -0.694$, $P < 0.05$). The CREB protein in the 8.0 g/L $AlCl_3$ group was lower than that in 2.0 g/L $AlCl_3$ group.

Western Blot

The target protein was observed by the Western blot (Fig. 8). The expression of EphB2, NMDAR2B, ERK1/2, CREB, and Arc was decreased with the Al dose. Figure 8E is a bar chart of target proteins. The EphB2 protein was decreased with the increase of the Al dose ($df = 3$, $F = 21.044$, $P < 0.05$; $r = -0.905$, $P < 0.05$) (Fig. 8A). Compared with the 2.0 g/L $AlCl_3$ group, the 4.0 g/L $AlCl_3$ group and 8.0 g/L $AlCl_3$ group had a certain degree of decrease in EphB2 protein. The NMDAR2B protein was decreased with the Al dose of 4.0 g/L $AlCl_3$ group and 8.0 g/L $AlCl_3$ group ($df = 3$, $F = 55.585$, $P < 0.05$) (Fig. 8B). Compared with 2.0 g/L $AlCl_3$ group, the NMDAR2B protein in 4.0 g/L $AlCl_3$ group and 8.0 g/L $AlCl_3$ group was decreased. The ERK1/2 protein was decreased with the Al dose ($df = 3$, $F = 5.022$, $P < 0.05$, $r = -0.706$, $P < 0.05$) (Fig. 8C). Compared with the 2.0 g/L $AlCl_3$ group, the ERK1/2 protein in the 4.0 g/L $AlCl_3$ group and 8.0 g/L $AlCl_3$ group was decreased. The Arc protein was decreased gradually with the Al dose ($df = 3$, $F = 17.45$,

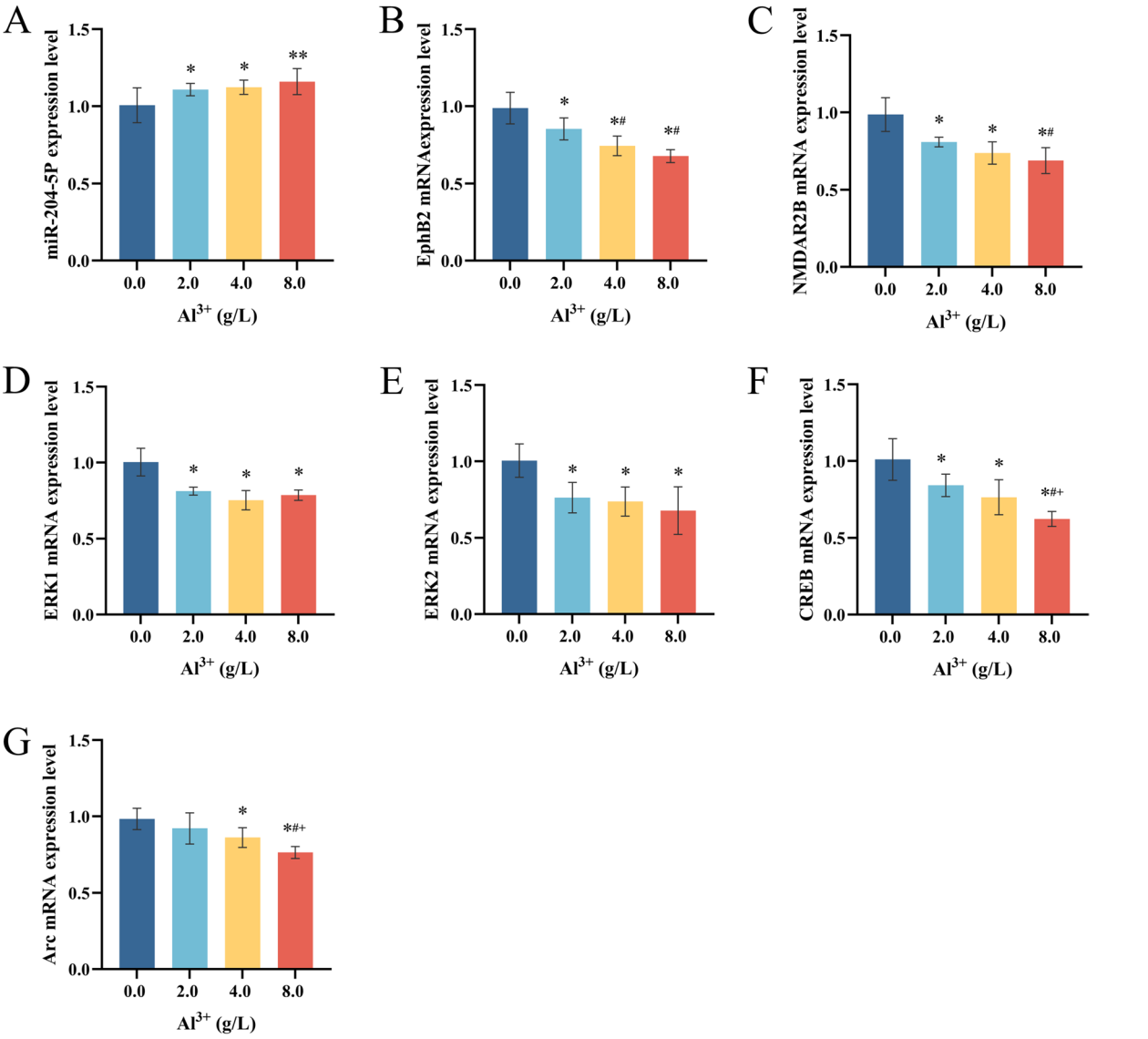

Fig. 6 Changes in target mRNA expression ($n=6$)

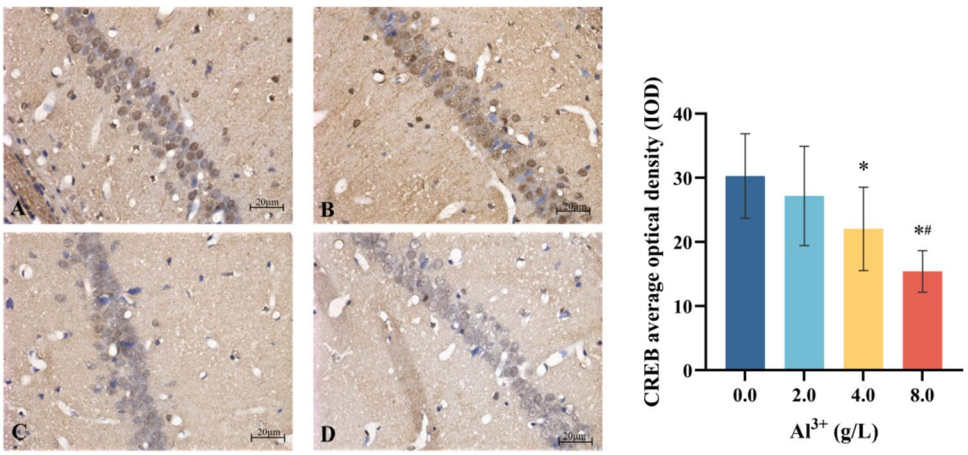

Fig. 7 Expression of CREB in the hippocampus (Immunohistochemistry). **A**: 0.0 g/L $AlCl_3$; **B**: 2.0 g/L $AlCl_3$; **C**: 4.0 g/L $AlCl_3$; **D**: 8.0 g/L $AlCl_3$. Magnification: 400.

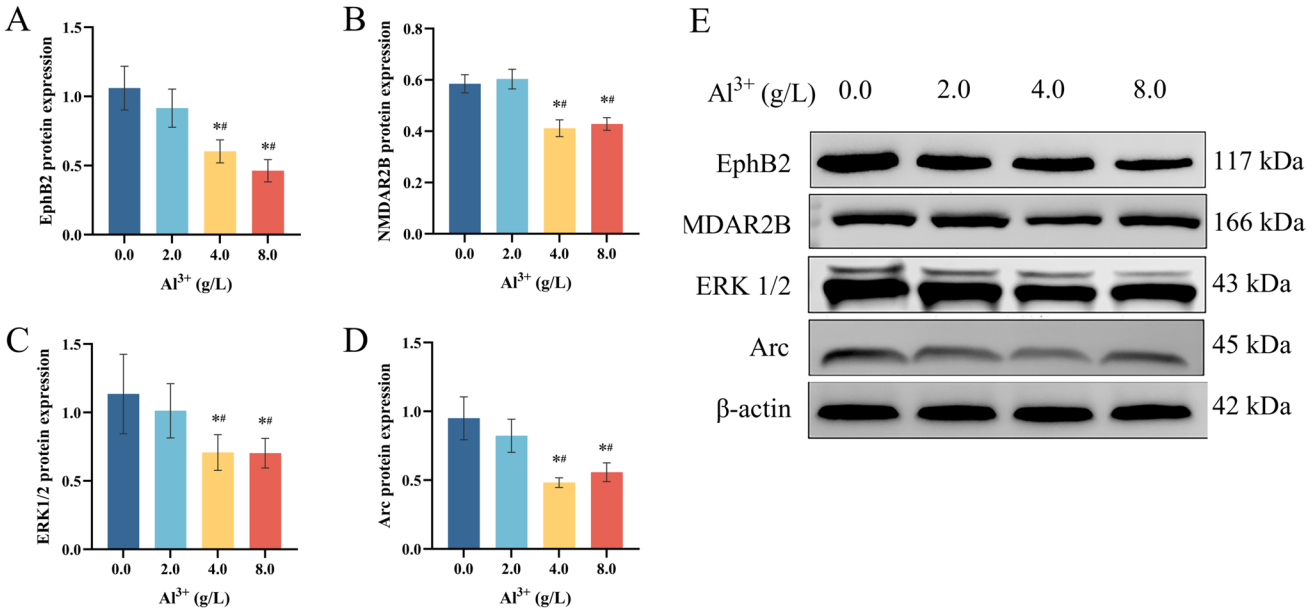

Fig. 8 Changes in protein expression ($n=6$)

$P<0.05$, $r=-0.804$, $P<0.05$) (Fig. 8D). Compared with 0.0 g/L $AlCl_3$ group and 2.0 g/L $AlCl_3$ group, Arc protein in the 4.0 g/L $AlCl_3$ group and 8.0 g/L $AlCl_3$ group was decreased.

Discussion

Al is a hazardous metal contaminant that accumulates in the environment. A number of disorders, including those that increase the chance of developing neurodegenerative diseases, can be brought on by excessive or prolonged exposure to Al [29]. However, there is a lack of comprehensive and systematic researches on the molecular mechanisms of Al-induced learning memory deficits. We found that the Al^{3+} content in the hippocampus of the Al dose groups was higher than the control group, suggesting that Al accumulation occurred in the rats, indicating that the rat model of Al exposure was built. Experimental data showed that Al exposure had an inhibitory influence on the weight growth of rats and affected the growth and development of rats, which was consistent with other studies [30]. The water maze test is considered the standard for judging learning and memory, so it was used to test the cognitive function of rats. Al exposure can reduce the spatial learning and memory ability of rats, which was kept up with the earlier results of our research team. The hippocampus is a critical brain region controlling behavior and cognition, and the CA1 region, in particular, plays a vital role [31]. An ordinary indicator of the hippocampus's growth and development is its organ coefficient. We found that the hippocampus organ coefficient was increased, suggesting that Al exposure was detrimental to rat brain development. Some studies also indicated that the hippocampal coefficient in the Al exposure groups was decreased [32]. Electron microscopy results revealed that Al may harm neurons and impair learning and memory, which is consistent with the findings of other investigations [33].

We found that miRNAs play a key role in salience plasticity. We looked at the expression of miR-204-5P gene in the beginning to further investigate the molecular mechanism of neurotoxicity brought on by Al exposure. The results indicated that the miR-204-5P gene expression gradually increased with the Al dose, indicating that the neurotoxicity of Al exposure may be caused by the up-regulation of miR-204-5P expression. Liu found that isoflurane could induce cognitive impairment, and down-regulation of miR-204-5P could inhibit neuroinflammation and played a protective role[13], which supported our findings.

EphB2 is involved in synaptic development and maturation [34]. According to the results of this experiment, we hypothesized that upregulation of miR-204-5P induced cognitive impairment caused by Al exposure through targeted inhibition of EphB2 expression. EphB2 directly interacts with NMDAR and mediates tyrosine phosphorylation of NMDAR receptor subunits. Loss of EphB2 results in a reduction in long-term potentiation and functioning synaptically localized NMDAR, which in turn causes neuronal dysfunction [35]. We found that the NMDAR2B gene and protein were decreased gradually with the Al dose, suggesting that Al exposure has an inhibitory effect on the

expression of NMDAR2B in rats. In order to further verify the role of NMDAR downstream pathway genes in learning and memory impairment induced by Al exposure, we also explored the gene and protein expression of NMDAR downstream indicators. In this study, Al exposure reduced ERK1/2, CREB, and Arc protein and mRNA expression in the hippocampus of rats, which may be related to the direct effect of Al exposure. This finding raises the possibility that NMDAR2B and its numerous downstream signaling pathways play a role in the regulation of cognitive impairment brought on by Al exposure.

In summary, Al exposure induces the abnormal miR-204-5P expression, which may inhibit the transcription and translation of its target gene EphB2 by down-regulating the miR-204-5P mRNA, and then lead to the aberrant of the downstream signaling pathway NMDAR-ERK-CREB-Arc, which ultimately lead to the impaired cognitive function in rats; thus, it is hypothesized that miR-204-5P is involved in the neurotoxicity mechanism of Al-induced neurotoxicity. However, more research is required to clarify the precise process.

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Author contributions NH and YY designed the study. WL and JG wrote the manuscript. XY and JL analyzed the data. JP, SY and DZ reviewed the references. PM and LZ reviewed and revised the manuscript. All authors had read the manuscript and agree to the publication of this study.

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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.

Competing Interests The authors declare no competing interests.

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