RESEARCH ARTICLE

Aluminum Exposure Induces Time-Dependent Cognitive Decline, Anxiety, and Brain Aluminum Accumulation in Rats

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Abstract

BACKGROUND: Aluminum, a nonessential element, can accumulate in the brain and has been implicated in neurodegenerative disorders such as Alzheimer's disease (AD). Although previous studies have examined aluminum neurotoxicity, many focus on a single time point, limiting insight into how aluminum accumulates over time. This study addresses that gap by investigating time-dependent aluminum accumulation and associated neurobehavioral changes in rats at 14, 28, and 42 days.

METHODS: Male Wistar rats were administered 200 mg/kg/day aluminum chloride ($AlCl_3$) via oral gavage for 14 (acute), 28 (subacute), and 42 (subchronic) days. Cognitive and anxiety-like behaviors were assessed using the 2-novel object recognition (2NOR) test, spontaneous alternation Y-maze, and open field test (OFT). Brain aluminum levels were quantified using inductively coupled plasma mass spectrometry (ICP-MS).

RESULTS: There were impairments in spatial and non-spatial memory and increased anxiety-like behavior across all exposure durations (p<0.05). Non-spatial memory performance decreased by 50.5%, 37.7%, and 56.2% on day-14, -28, and -42, respectively. Spatial memory significantly declined by 34.3% and 43.2% on day-14 and -42, respectively, while the 20.0% decrease at day-28 was not statistically significant. Anxiety-like behavior increased, with center zone entries reduced by 37.6%, 64.9%, and 62.9% across the same time points. Brain aluminum concentrations were significantly elevated in all aluminum-exposed groups compared to controls, with increases of 2,622.6%, 314.7%, and 969.3% on day-14, -28, and -42, respectively (p<0.05). The increase was not strictly proportional to exposure duration, suggesting possible homeostatic regulation. Weight and liver assessments confirmed the subtoxic nature of the exposure.

CONCLUSION: Exposure to aluminum for 42 days induces behavioral deficits and increases brain aluminum levels, which may support its potential relevance as a model to study aluminum-induced neurotoxicity.

KEYWORDS: aluminum exposure, cognitive function, anxiety, temporal progression, neurobehavioral changes

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Introduction

Aluminum poses risks to human health, particularly through brain accumulation, which may contribute to neurodegenerative disorders like Alzheimer's disease (AD).(1,2) Exposure occurs via inhalation and ingestion, especially through airborne particles or food contaminated during processing or packaging.(3,4) Although gastrointestinal absorption is typically below 1%, transport via plasma

proteins like transferrin allows gradual brain accumulation (1,5), raising concerns over its role in neurodegeneration. Postmortem analyses of human brain tissue have reported aluminum concentrations up to eightfold higher in elderly individuals with AD compared with age-matched controls. (1) Epidemiological studies also suggest that long-term aluminum exposure, particularly in drinking water, may increase dementia risk in older adults (2), highlighting the potential public health significance of this metal.

One key mechanism of aluminum-induced neurotoxicity is oxidative stress. Aluminum generates reactive oxygen species (ROS), which damage lipids, leading to cellular dysfunction.(6) This damage triggers neuroinflammation by activating microglia and astrocytes and contributes to neuronal injury.(7) Additionally, aluminum activates cell death pathways, including apoptosis and pyroptosis, worsening neuronal loss and cognitive decline.(8,9)

Due to its neurotoxic properties, aluminum is frequently used in AD research, with aluminum chloride ($AlCl_3$) known to induce AD-like features in experimental models.(9) AD is characterized by amyloid-β plaques and neurofibrillary tangles, which drive progressive cognitive decline.(10) Its slow onset requires a long-term approach to understand disease progression.(11) However, most studies assess aluminum's impact at a single time point, limiting insights into its temporal effects on neurobehavior.

Environmental aluminum exposure is an emerging concern, with growing evidence linking it to neurodegenerative outcomes. While previous studies have focused on immediate neurotoxic effects, the present study investigates subchronic aluminum exposure and its association with early behavioral changes, which may reflect the subtle onset of cognitive dysfunction. Therefore, this study aims to investigate aluminum-induced neurotoxicity in a rat model by examining brain aluminum accumulation and behavioral alterations at 14, 28, and 42 days of exposure.

Methods

Experimental Animal

Adult male Wistar rats (8 weeks old, 200–250 g) were obtained from the Laboratory Animal Resource Unit at Universiti Kebangsaan Malaysia, Malaysia. All animals were healthy, active, and free from signs of illness or injury at the start of the experiment. No specific exclusion criteria were applied, and no animals were removed during the study. Rats were housed in pairs with *ad libitum* access to food and water and fed a standard commercial diet. All cages were kept in the Behavioral Laboratory, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, under controlled conditions (22–24°C, a 12-hour light/dark cycle). Handling procedures were standardized for all rats to reduce stress, and the animals were allowed to acclimate for 7 days before the induction of $AlCl_3$ (Chemiz, Shah Alam, Malaysia). This experiment was conducted in accordance with Universiti Kebangsaan Malaysia's ethical guidelines and principles for the use of laboratory animals and was approved by the Universiti Kebangsaan Malaysia Animal Ethical Committee (Approval No. FSK/2023/FARAH WAHIDA/15-FEB./1303-FEB.-2023-APRIL-2025).

Experimental Design

Thirty-six male Wistar rats were randomly divided into three groups (n=12 per group), each representing a different aluminum exposure timeline: 14 days (acute), 28 days (subacute), and 42 days (subchronic). These classifications were based on the Organization for Economic Cooperation and Development (OECD) guidelines.(12-14) Each group was further divided into two subgroups (n=6) for control and aluminum-exposed groups. All animals remained alive throughout the study period, and each group maintained the planned sample size (n=6). Based on previous study (15), six animals per group were deemed sufficient for analysis. The sample size was also supported by GPower analysis, which indicated that 24 animals would provide a power of 0.87 (effect size=0.8, α=0.05); however, 36 animals were used to improve robustness and account for potential variability. Additionally, the "E value" (E=36−6=30) slightly exceeds the ideal range of 10–20 but still reflects adequate degrees of freedom for statistical analysis.(16) This sample size is appropriate for detecting significant differences in behavioral and cognitive outcomes over time.

Control groups received distilled water (dH_2O), while aluminum-exposed groups were administered 200 mg/kg/day $AlCl_3$ via oral gavage at approximately 8:30 AM daily for 14, 28, or 42 days. A 100 mg/mL stock solution was prepared. Each morning, rats were weighed, and the volume of stock solution required to achieve the correct dose based on each rat's body weight was calculated. Distilled water was then added to adjust the total gavage volume to 2.0 mL per rat. Behavioral analyses were performed at three time points: baseline (day-0), midpoint (day-7, -14, and -21), and endpoint (day-14, -28, and -42). Results expressed relative to day-0 (baseline set as 1). These time points were selected to capture behavioral progression over the exposure period. At the end of each time point, all rats were euthanized for brain

tissue collection and aluminum content analysis. Rats were anesthetized with carbon dioxide gas and, once unconscious and immobilized, were immediately decapitated for brain tissue collection. The experimental design was summarized in Figure 1.

Mean Weight Change

Animal weights were recorded daily, and the mean weekly weight change for each animal was calculated. Monitoring body weight provides an assessment of the systemic impact of the treatment and ensures that any observed behavioral or cognitive changes are not solely due to general health deterioration. The mean body weight change (%) was calculated: [(current weight - initial weight)/initial weight]×100.

Neurobehavioral Analysis

Each rat's behavior was recorded on the day of behavioral testing. All videos were labeled with rat IDs and assessed in a blind manner. Assessors scoring the rats' behavior were unaware of group assignments (control or aluminum-exposed). The 2-novel object recognition (2NOR), Y-maze, and open field (OFT) tests were scored independently, and all results were cross-checked for consistency.

2NOR Test for Measurement of Non-Spatial Memory

The 2NOR test was used to assess non-spatial memory in rats, based on their tendency to explore novel objects more than familiar ones.(17) The apparatus consisted of a square-shaped open arena with dimensions of 40×40×40 cm. Rat behavior was monitored and recorded using a camera positioned above the arena. The test involved three phases: familiarization, training, and testing.

In the familiarization phase, rats were placed in the box for 3 minutes without any objects. During the training phase, rats were placed in the box with two identical objects and allowed to explore them for 6 minutes. In the final testing phase, the procedure was like the training phase, except that one of the objects was replaced with a novel object. The apparatus was cleaned with 70% ethanol (Systerm, Shah Alam, Malaysia) between each phase and before testing a new rat. This procedure was repeated for all the rats.

The time spent by the rats exploring each object during the test phase, including behaviors such as licking, sniffing, and touching, was recorded. The following parameters were measured: the time (in seconds) spent exploring the familiar object (TF) and the time (in seconds) spent exploring the novel object (TN). The preference percentage (%) was calculated: [TN/(TN+TF)]×100.

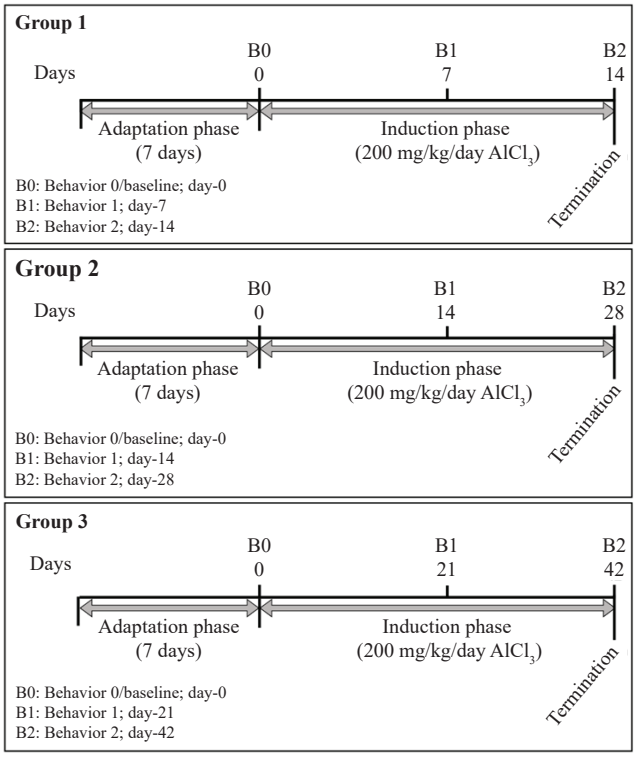

Figure 1. Timeline of behavioral assessments in rats following different durations of aluminum exposure. This figure illustrates the experimental timeline for three groups of rats subjected to different durations of aluminum exposure: 14 days (Group 1), 28 days (Group 2), and 42 days (Group 3). Each group undergoes an adaptation phase followed by an induction phase, during which rats receive daily doses of 200 mg/kg of $AlCl_3$. Behavioral assessments are conducted at three time points: baseline (B0) at the start of the induction phase (day-0), B1 at an intermediate time point (day-7, -14, and -21 for Groups 1, 2, and 3, respectively), and B2 at the end of the exposure period (day-14, -28, and -42 for Groups 1, 2, and 3, respectively).

Y-Maze Test for Measurement of Spatial Memory

The Y-maze test was conducted to assess the spatial memory of rats based on their natural tendency to avoid revisiting the same arm repeatedly.(18) The apparatus consisted of a black acrylic three-arm Y-maze, with each arm measuring 50 cm in length, 10 cm in width, and 30 cm in height. The arms were positioned at a 120º angle from each other and labeled A, B, and C accordingly. Each rat was placed in one of the arms as the starting point and allowed to explore the maze freely for 10 minutes. An entry into an arm was counted when all four paws of the rat were inside that arm. The total number of arm entries was recorded using a camera positioned above the maze.

The following parameters were measured: the number of alternations, defined as the rat visiting all three arms (A, B, and C) consecutively without revisiting the same arm, and the total number of arm entries. The percentage of

alternation (%) was calculated: [the number of alternations/(total number of arm entries-2)]×100. Between each animal test, the apparatus was cleaned thoroughly to prevent odor trails that could affect behavioral outcomes.

OFT Assessment for Measurement of Anxiety-Like Behavior

OFT was used to assess the anxiety levels of rats based on their natural exploratory behavior. Their instinct to explore may lead them to frequently enter the center of the arena. Rats with higher anxiety levels tend to remain near the edges of the arena rather than venturing into the center.(19) The test was conducted in a black square box with dimensions of 90×90×90 cm. Each rat was placed at the edge of the arena and allowed to roam freely for 10 minutes, with their movements monitored and recorded by a camera positioned above the arena. The frequency of the rats entering the center zone was measured, with an entry counted when all four paws of the rat were inside the center zone.

Aluminum Content Analysis

The protocol for aluminum analysis was adapted from a previously published article.(20) Harvested brains were stored at -80°C until the samples were processed. To minimize metal contamination, all glassware was soaked in 1% nitric acid (HNO_3) overnight and thoroughly rinsed with distilled water before use. Only metal-free equipment was utilized throughout the procedure to reduce the contamination risk further. For aluminum analysis, the entire brain was used, with freeze-dried tissue weights ranging from 0.2 to 1 gram, which were recorded for each sample. The brain processing began with the freeze-dried brains being soaked in 70% HNO_3 (Universal Solutions, Newport News, VA, USA) overnight. The following day, the samples were heated at 80°C for 20 minutes using a heating block and then cooled to room temperature. Next, 0.6 mL of hydrogen peroxide (H_2O_2) (R&M Chemicals, Semenyih, Malaysia) was added to each sample. Once the effervescence had ceased (approximately 30 minutes), the samples were heated to 70°C for 15 minutes and then cooled to room temperature. All samples were then filtered using a syringe and 0.22 µm membrane filter. Then, all samples were diluted at a ratio of 1:51 in 1% nitric acid (60 µL of sample plus 3 mL of 1% HNO_3). The samples were subsequently analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), based on aluminum-27 (Al_{27}) isotope detection. External calibration was performed using multi-point standard curve, which demonstrated excellent linearity (R^2=0.999954). Each sample was analyzed in triplicate, and relative standard deviations (RSD) were confirmed to be below 5% indicating high analytical precision. Procedural blanks were included to monitor contamination, and all blank values were below the detection limit. The aluminum values obtained (in ppb) were normalized to sample weight and reported as ppb per gram of brain tissue (ppb/g).

Statistical Analysis

All data were analyzed using GraphPad Prism 9.3.1 (GraphPad Software, Boston, MA, USA) and are presented as mean±standard error of the mean (SEM). Mean weight change and behavioral data were subjected to mixed-model analysis of variance (ANOVA), where both between-subject factors (groups) and within-subject factors (time points) were included to test for interactions. A two-way ANOVA was performed for aluminum content analysis, examining the effects of exposure duration and group, along with their interaction. Assumptions of normality and sphericity were checked, and any violations were addressed using Greenhouse-Geisser. Statistical significance was set at $p<0.05$, with post-hoc comparisons conducted using Tukey's test to adjust for multiple comparisons.

Results

Effect of $AlCl_3$ Induction on Body Weight in Rat Models

The body weight changes was illustrated in Figure 2. As shown in Figure 2A, there was no significant difference in mean body weight between the aluminum-exposed and control groups during the initial 14 days of aluminum exposure. However, after 14 days (2 weeks) of continued aluminum exposure, a significant difference began to emerge between the aluminum-exposed and control groups, as illustrated in Figures 2B and 2C.

Morphological Alterations in Liver Tissue Following AlCl3 Induction in Rat Model

The gross morphological analysis of liver tissues from the aluminum-exposed and control groups were presented in Figure 2. After 14 days of aluminum exposure, the livers of the control group (Figure 2D) and the aluminum-exposed group (Figure 2E) appeared uniform in reddish-brown color, with smooth surfaces free of nodules, bumps, or irregularities. Similar observations were made after 28 days of exposure, where the control group (Figure 2F) and the aluminum-exposed group (Figure 2G) displayed the same normal appearance. Even after 42 days of aluminum exposure, the livers in both the control group (Figure 2H)

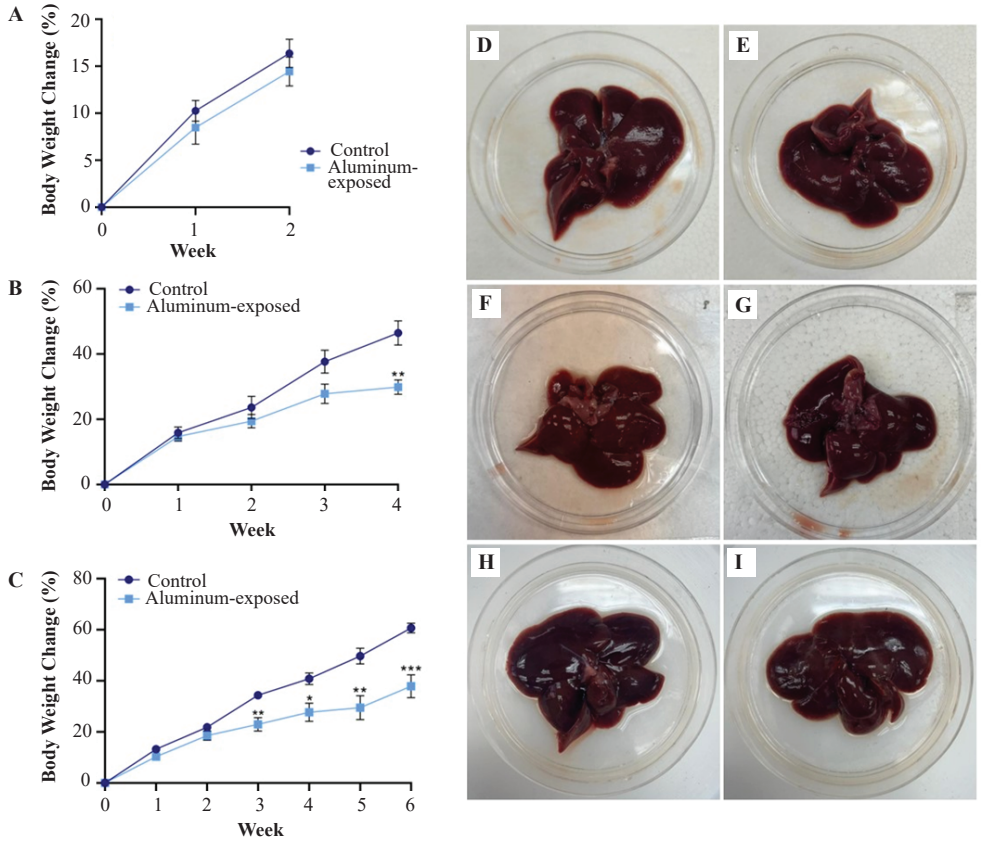

Figure 2. Body weight changes and liver morphology in rats following subchronic aluminum exposure. A-C: Mean body weight change (%) in rats exposed to 200 mg/kg/day $AlCl_3$ via oral gavage for 14 days (A), 28 days (B), and 42 days (C). Statistical analysis was performed using mixed-model ANOVA. Data are presented as mean±SEM (n = 6). Post hoc: *p<0.05, **p<0.01, ***p<0.001 versus control group. D–I: Gross liver morphology of rats after aluminum exposure. Group 1: control (D), aluminum-exposed (E); Group 2: control (F), aluminum-exposed (G); Group 3: control (H), aluminum-exposed (I). No visible signs of hepatotoxicity, such as discoloration or nodular lesions, were observed in any group.

and the aluminum-exposed group (Figure 2I) maintained a consistent, healthy morphology.

Effects of $AlCl_3$ Exposure on Non-spatial Memory in Rat Models

Figure 3A illustrated the protocol for the 2NOR test, which consists of three phases: habituation, training, and testing. As shown in Figure 3B, the non-spatial memory of aluminum-exposed rats in Group 1 significantly decreased on day-14 compared to the control group. A similar trend was observed in Group 2 (Figure 3C), where the non-spatial memory of aluminum-exposed rats also significantly decreased on day-14 compared to the control group. Additionally, Figure 3C indicates that the non-spatial memory of aluminum-exposed rats in Group 2 remained significantly lower on day-28 compared to the control group. However, there was a slight improvement in memory performance from day-14 to -28. In Figure 3D, the non-spatial memory of aluminum-exposed rats in Group 3 was significantly reduced on day-42 compared to the control group.

Effects of Aluminum Exposure on Spatial Memory in Rat Models

Figure 4A illustrated the protocol for the Y-maze test, in which correct alternation between the three arms indicates intact working memory, while re-entry into a previously visited arm reflects impaired alternation behavior. As shown in Figure 4B, the spatial memory of aluminum-exposed rats in Group 1 was significantly decreased on day-14 compared to the control group. A similar trend was observed in Group 2 (Figure 4C), where spatial memory of aluminum-exposed rats was also significantly lower on day-14 compared to the control group. However, no significant difference in spatial memory was observed between the aluminum-exposed and control groups on day-28, as shown in Figure 4C. In Figure 4D, the spatial memory of aluminum-exposed rats in Group 3 showed a significant decrease on day-42 compared to the control group.

Effects of Aluminum Exposure on Anxiety-like Behavior in Rat Models

Rats that exhibited lower anxiety tend to explore the center zone of the arena, reflecting their natural exploratory behavior. In contrast, rats with higher anxiety levels tend to remain near the edges of the box rather than venturing into the center, as shown in Figure 5A. Figure 5B showed that the anxiety level in the aluminum-exposed group significantly increased on day-14 compared to the control group. A similar trend was observed in Group 2, where the anxiety level in the aluminum-exposed group was significantly higher on

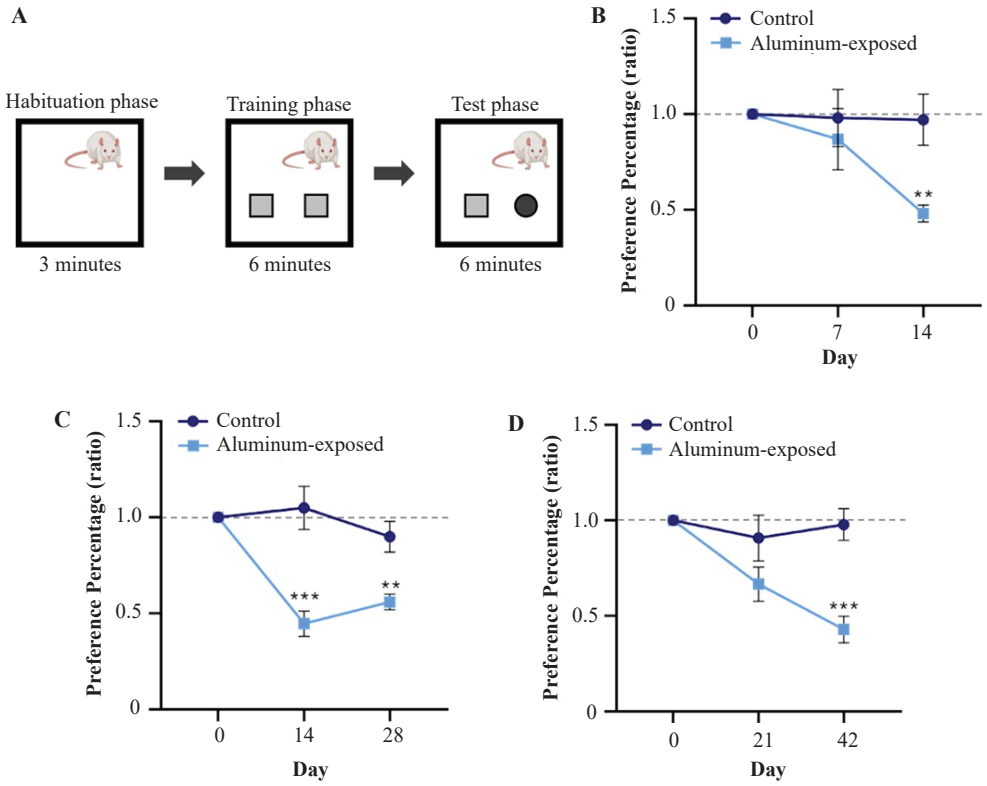

Figure 3. Effects of aluminum exposure on non-spatial memory performance in rats assessed using 2NOR test. A: Schematic representation of the 2NOR test protocol, consisting of three phases: habituation (3 minutes), training (6 minutes), and test (6 minutes). B-D: Preference percentage ratios in the 2NOR test following oral administration of 200 mg/kg/day $AlCl_3$ for 14 days (B), 28 days (C), and 42 days (D). Statistical analysis was conducted using mixed-model ANOVA, with data presented as preference ratios relative to day-0 (set as 1). Results are shown as mean±SEM (n=6 per group). Post hoc analysis: $^{**}p<0.01$, $^{***}p<0.001$ versus control group.

day-14 than in the control group, as shown in Figure 5C. Additionally, Figure 5C indicated that anxiety levels in the aluminum-exposed group continued to worsen by day-28. In Figure 5D, Group 3 showed a significant decrease in anxiety levels on day-42 compared to the control group, and no significant differences were observed between the control and aluminum-exposed groups on day-21, as shown in Figure 5D.

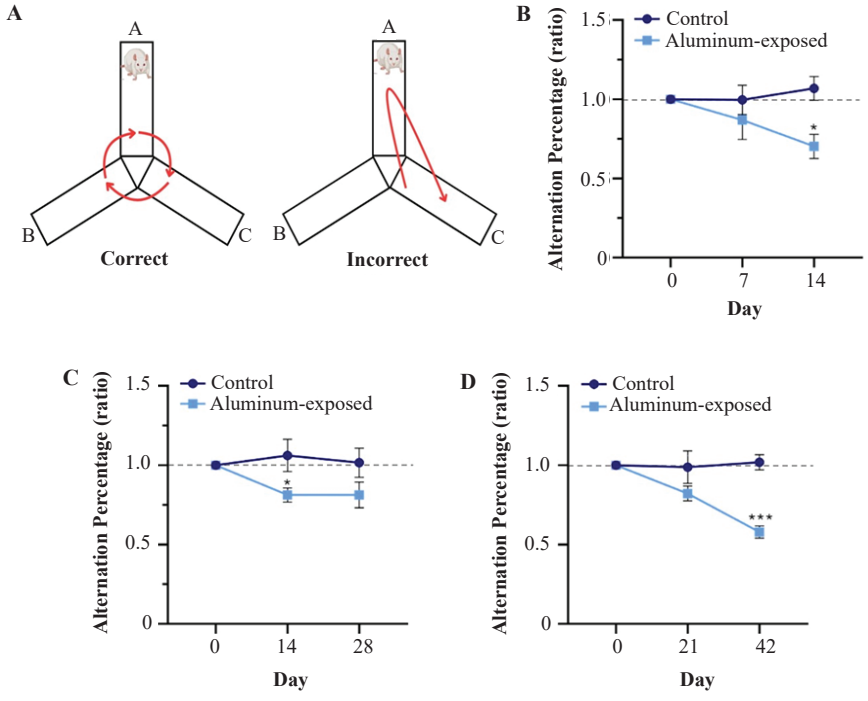

Figure 4. Effects of aluminum exposure on spatial memory performance in rats assessed using the Y-Maze test. A: Schematic representation of the Y-Maze test. In a correct sequence, the rat alternates between all three arms (A → B → C), indicating intact working memory. In an incorrect sequence, the rat re-enters a previously visited arm, reflecting impaired alternation behavior. B-D: Alternation percentage ratios after oral administration of 200 mg/kg/day $AlCl_3$ for 14 days (B), 28 days (C), and 42 days (D). Statistical analysis was performed using mixed-model ANOVA. Data are expressed as ratios relative to day-0 (set as 1) and shown as mean±SEM (n=6 per group). Post hoc analysis: $^{*}p<0.05$, $^{***}p<0.001$ versus control group.

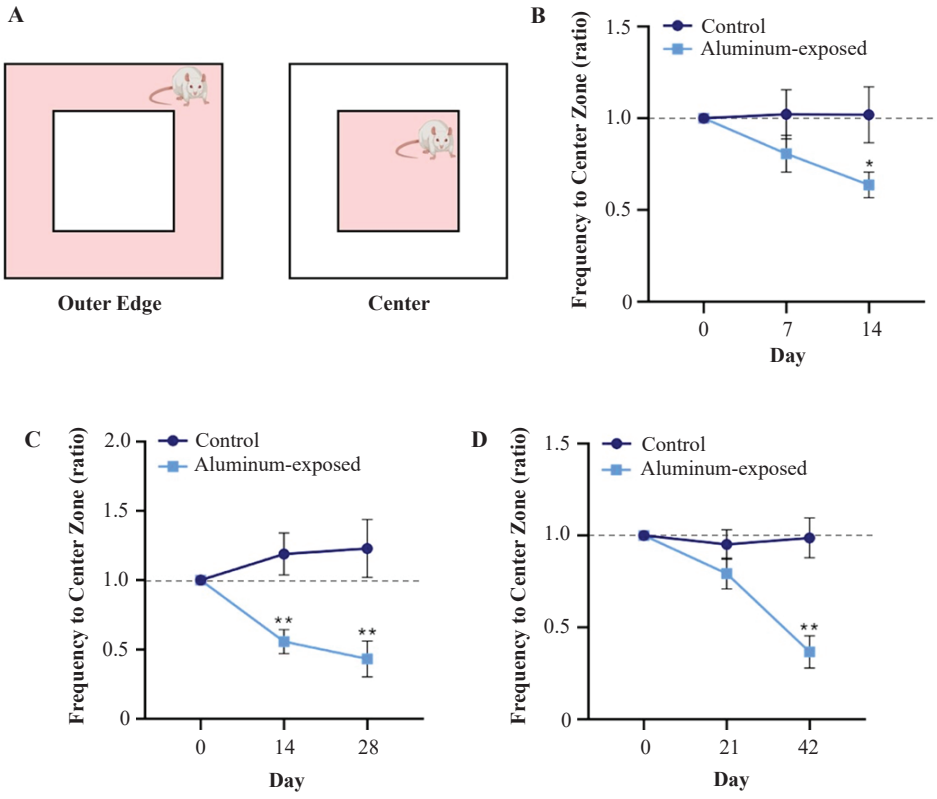

Figure 5. Effects of aluminum exposure on anxiety-like behavior in rats assessed using the Open Field Test (OFT). A: Diagram of the OFT setup. Time spent in the outer edge of the arena indicates anxiety-like behavior, while exploration of the center zone reflects lower anxiety levels. B-D: Frequency ratios of entries into the center zone following oral administration of 200 mg/kg/day $AlCl_3$ for 14 days (B), 28 days (C), and 42 days (D). Statistical analysis was conducted using mixed-model ANOVA. Data are presented as ratios relative to day-0 (set as 1) and expressed as mean±SEM (n=6 per group). Post hoc analysis: *p<0.05, **p<0.01 versus control group.

Aluminum Accumulation in The Brain Rat Models

Figure 6 showed that aluminum content in the aluminum-exposed rat brain was significantly higher on day-14, -28, and -42 compared to the control groups. Interestingly, the aluminum content in the aluminum-exposed group on day-28 showed a slight decrease compared to day-14. Overall, after 42 days of aluminum exposure, there was a marked accumulation of aluminum in the rat brain. As shown in Figure 6, the aluminum content in the aluminum-exposed group on day-42 was significantly higher compared to the aluminum-exposed groups on day-14 and -28.

Discussion

This study investigated the temporal effects of aluminum exposure on behavioral outcomes in rats. The results revealed significant impairments in both spatial and non-spatial memory, elevated anxiety-like behaviors, and increased brain aluminum accumulation across different exposure durations.

Non-spatial memory involves recalling non-locational information, while spatial memory relates to the recognition of places or directions. Both are regulated by the hippocampus.(21-23) Following 14 days of aluminum exposure, both memory domains were significantly impaired. Interestingly, by day-28, spatial memory performance showed no significant difference compared to controls, while non-spatial memory impairment persisted. This transient stabilization may be due to early compensatory mechanisms in the brain. Previous studies

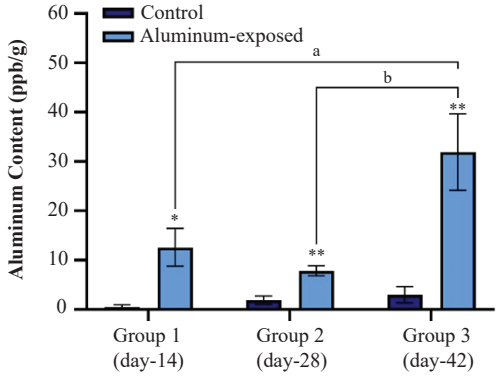

Figure 6. Progressive aluminum accumulation in the brain. Aluminum content (ppb/g) significantly increased in the brain across time points (day-14, -28, -42) in the aluminum-exposed group compared to controls. Statistical analysis was performed using two-way ANOVA. Data are presented as average±SEM (n=6). Post hoc: *p<0.05, **p<0.01 versus control group, $^a p$<0.05 *vs.* aluminum-exposed Group 1, $^b p$<0.05 *vs.* aluminum-exposed Group 2.

reported increased acetylcholine levels following aluminum exposure, which may counteract memory decline.(24) According to a previous study, slight reductions in brain aluminum levels within the initial 1–35 days of exposure support the possibility of homeostatic responses.(25) However, after 42 days, both types of memory were markedly impaired, indicating that these protective mechanisms may have been overwhelmed. These findings are consistent with previous reports of aluminum-induced cognitive decline.(26) Aluminum exposure also led to heightened anxiety-like behavior, suggesting a potential impact on the amygdala, the brain region involved in emotional processing and fear memory.(27)

The behavioral changes observed in this study are likely linked to the gradual accumulation of aluminum in the brain, as reported in previous animal and human studies.(28,29) Notably, donors with AD have shown significantly higher aluminum levels in brain tissues compared to healthy controls, reinforcing a possible association between aluminum accumulation and neurodegeneration.(2)

A time-dependent increase in brain aluminum content was observed across all exposure durations. Interestingly, a slight reduction was detected at day-28, which may reflect early-stage clearance reported in previous studies.(25,30) This suggests that brain aluminum levels do not strictly rise with cumulative exposure but may be influenced by time-dependent physiological mechanisms that affect its retention, clearance, or redistribution. Although direct evidence for compensatory clearance is limited, these changes are hypothesized to involve detoxification pathways or the activity of astrocytes and microglia, which help maintain brain homeostasis and remove harmful substances.(31,32) However, after 42 days, aluminum levels rose sharply, suggesting that detoxification capacity may have become saturated or impaired. These findings highlight the value of longitudinal assessment in tracking aluminum-induced neurotoxicity.

Aluminum accumulation in the brain can trigger several pathogenic mechanisms, including oxidative stress, mitochondrial dysfunction, and protein aggregation.(6,9) It may also facilitate the deposition of amyloid-β plaques and neurofibrillary tangles, which is the hallmark features of AD pathology.(6,33) Although this study did not directly assess amyloid or tau pathology, the observed behavioral alterations resemble AD-like changes. Further investigation is required to determine whether subchronic aluminum exposure directly contributes to these pathological features.

The current study also evaluated general toxicity by tracking body weight and liver morphology. No significant weight differences were observed between aluminum-exposed and control rats during the early phase. However, weight changes became apparent after two weeks, which mirrors trends in other AD animal models and clinical populations.(34,35) These changes may reflect alterations in metabolism, appetite, and gastrointestinal function commonly associated with neurodegeneration.(36,37)

The gross liver morphology remained normal across all exposure groups, showing no visible signs of hepatotoxicity, such as nodules or discoloration.(38,39) These findings suggest that the 200 mg/kg/day dose of $AlCl_3$ is subtoxic at the systemic level. However, the presence of marked neurobehavioral changes in the absence of gross liver damage indicates selective neurotoxicity. Subclinical hepatic effects cannot be excluded and require further biochemical investigation.

Overall, this study shows that subchronic aluminum exposure leads to behavioral impairments that progress over time, aligning with the accumulation of aluminum in the brain. These findings support previous reports suggesting that aluminum-induced neurotoxicity involves redox imbalance, mitochondrial disruption, cell death pathways, protein aggregation, and neuroinflammation.(6,8,9,40)

By establishing a model of aluminum-induced cognitive decline across these time points, this study emphasizes the value of examining temporal dynamics in future research and provides a foundation for studies targeting molecular mechanisms and therapeutic interventions. Further exploration of molecular pathways, particularly those related to neuroinflammation and neuronal survival, may identify therapeutic targets for mitigating aluminum-induced brain damage. However, this study has some limitations. Parameters of AD, including amyloid-β plaques and neurofibrillary tangles, were not examined. Only limited time points (day-14, -28, and -42) were assessed, without intermediate days between subacute (day-28) and subchronic (day-42), or time points beyond day-42. Therefore, the peak of aluminum-induced neurotoxicity could not be determined. Future studies should include additional time points and assess classical AD pathology markers to provide a more comprehensive understanding of aluminum-induced neurotoxicity and identify potential therapeutic targets. Given rising concerns over environmental aluminum exposure, these findings also highlight its potential public health implications and the importance of preventive strategies to mitigate long-term cognitive risk.

Conclusion

This study demonstrates that aluminum exposure over 14, 28, and 42 days induces cognitive and behavioral impairments in rats, with deficits becoming most pronounced at day-42. The observed memory deficits and heightened anxiety, together with increased brain aluminum accumulation, highlight aluminum's neurotoxic potential and its possible relevance to neurodegenerative conditions such as AD. Importantly, aluminum accumulation in the brain was not strictly linear, suggesting the involvement of time-dependent physiological mechanisms such as compensatory clearance or redistribution.

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Authors Contribution

AHAA was the main writer and contributed to the manuscript from start to finish, including conceptualizing, writing, and revising the manuscript. FWI, as the main supervisor, guided the conceptualization of the study, assisted with manuscript editing, and contributed to manuscript corrections. HHF, KM, and MHZF were responsible for data collection and also assisted in reviewing the flow of the methodology section in the manuscript. HA and NAR assisted in interpreting the data and refining the flow of the results section. NFJ, SFM, and NFR contributed to reviewing, correcting, and ensuring the manuscript flow before submission.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

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