Aluminum chloride impairs learning and memory by P2X7 and A2A-receptor stimulations in hippocampus of mice

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Abstract

Aluminum (Al) is considered a neurotoxic agent for biological systems and is recognized as a risk factor for neurodegenerative diseases. Al exposure occurs through the environment, water and human diet. The Al cationic form (Al$^{3+}$) has been associated with major deleterious effects on the central nervous system. The aim of this study was to investigate a possible mechanism that links Al and neurodegeneration in vivo. Here, we evaluated memory tests, purinergic signaling and inflammatory markers during long-term oral exposure to Al of mice in the total hippocampus. For this study, Swiss mice were divided into three groups: control (CT) group, AlCl$_3$ 50 mg/kg group and AlCl$_3$ 100 mg/kg group. The animals were orally treated with saline or Al$^{3+}$ at AlCl$_3$ form for 30 days. The memory parameters, body and brain weight, Al$^{3+}$ levels, DNA damage, enzyme activities, purinergic receptors and cytokine densities were assessed on the hippocampus of mice intoxicated with Al$^{3+}$. Our results reveal that Al$^{3+}$ was able to reduce brain weight and accumulate in the hippocampus of animals treated with 100 mg/kg of salt. In addition, Al$^{3+}$ causes memory deficits and DNA damage. The adenosine triphosphate (ATP) hydrolysis was also affected by Al$^{3+}$. Our results point to an increase in nucleoside triphosphate diphosphohydrolase (NTPDase), 5´-nucleotidase (5´-NT) and adenosine deaminase (ADA) activities. In addition, Al$^{3+}$ increases P2X7 and A2A receptor density, as IL-1$\beta$ proinflammatory cytokine. Taken together, we suggest that Al$^{3+}$ can cause memory loss through DNA damage and alterations to the purinergic system through the proinflammatory process.

1. Introduction

Aluminum (Al) is one of the most abundant elements in the Earth's crust, and its biological function has not been defined [1]. Compounds containing Al are ubiquitous in the environment and have been used in the manufacturing industry for centuries, including in consumer goods, kitchen utensils, food additives, water treatment, medicaments and cosmetics [2, 3]. The main route by which the metal is absorbed by the body is by oral ingestion; it can be excreted by and/or bioaccumulate in various organs, including the central nervous system (CNS) [4].

Al$^{3+}$, the bioavailable and most toxic form of Al, may cross the blood–brain barrier (BBB), resulting in a risk factor for neurodegeneration, as this metal is considered a neurotoxin [5]. Several post-mortem studies of patients with Alzheimer's disease (AD) patients have shown the high content of Al$^{3+}$ in the brain, which triggers characteristic lesions such as senile plaques and neurofibrillary tangles [6, 7]. In addition, other neurodegenerative diseases, such as Parkinson's disease (PD), dialysed encephalopathy (DE) and amyotrophic lateral sclerosis are also closely associated with chronic accumulation of Al$^{3+}$ [8–10].

Findings point to the hippocampus being a region vulnerable to Al-accumulation. Al exposure could induce cognitive impairment, affecting the memory and learning in an animal model, similarly to early
symptoms associated with AD patients [11–13]. However, the mechanisms of Al-induced memory deficits and neurodegeneration remain unclear.

One important step in memory consolidation is neurotransmission. It has been reported that adenosine triphosphate (ATP) is considered a fast excitatory neurotransmitter, being co-released with other neurotransmitters, such as acetylcholine [14]. Extracellular ATP is involved in several pathophysiological events, including neurotransmission and neuromodulation [15]. The specific role of ATP in biological systems depends on binding with specific receptors and it plays different crucial functions in the CNS [16, 17].

The levels of nucleotide are controlled by cell surface enzymes, known as ectonucleotidases [18]. The enzymes involved in this signaling cascade include the ectonucleoside triphosphate diphosphohydrolase (NTPDase), which hydrolyzes ATP to adenosine diphosphate (ADP) and/or adenosine monophosphate (AMP), followed by 5’-nucleotidase (5′-NT), which hydrolyzes AMP to adenosine, and finally, this molecule is deaminated to inosine by adenosine deaminase (ADA) [18, 19].

Signaling through P2 and P1 receptors is finished by the hydrolysis of ATP by ectonucleotidases [19]. The role of ATP acting via P2X<sub>7</sub> receptors leads to the inflammatory condition, by pro-inflammatory cytokine release, such as IL-1β and has been associated with cell damage and/or apoptosis events [20].

Additionally, ATP nucleoside derivate, adenosine, is signaling molecules involved in the neuroprotection and neuromodulation of the CNS [21]. These effects were observed as the result of the A1 receptor binding, since this is the predominant subtype in cerebral areas [22]. However, in brain impairment conditions, studies point to the increased regulation of A2A receptors being responsible for the cognitive decline in memory performance [23].

In this regard, we evaluated the possible effects of long-term exposure of Al on an animal model in the memory parameters and purinergic signaling of animals treated with Al<sup>3+</sup> in the AlCl<sub>3</sub> form. Against this background, we propose to investigate a mechanism, to data insufficiently investigated, responsible for the effects of Al<sup>3+</sup> on the CNS.

2. Material And Methods

2.1. Animals

For this study, 30 Swiss male mice with a mean age of 60 days weighing 25 ± 5 g were kept in boxes (30 x 20 x 13 cm) containing five animals each, under a 12 h light/dark cycle with controlled temperature and humidity (25°C, 70%, respectively). The animals went through an adaptation period of 10 days and were fed with commercial feed and water ad libitum.

All animal procedures were approved by the Institutional of Animal Care and Use from the UFSM (protocol number: 2712020517/2017).
2.2. Aluminum treatment

Aluminum in the chloride form (AlCl\textsubscript{3}; molecular weight 133.34 g/mol; purity > 99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and was diluted in ultrapure water to a final concentration of 50 and 100 mg/kg of AlCl\textsubscript{3}, which is equivalent to 10 and 20 mg/kg of Al\textsuperscript{3+}, respectively. The AlCl\textsubscript{3} was administered orally for 30 consecutive days followed by 2 days of no treatment each week, and the concentration used was selected following the literature [24]. Note that the daily intake of Al\textsuperscript{3+} corresponds to 2–40 mg / kg / day, when considering an adult (60 kg); these values are equivalent to 3.4–68 mg / kg / day of Al\textsuperscript{3+} in mice [25].

2.3. Experimental design

Mice were randomly divided into three experimental groups: control (CT), AlCl\textsubscript{3} 50 mg/kg, and AlCl\textsubscript{3} 100 mg/kg. Aluminum or vehicle (0.9% NaCl) was administered via gavage between 11 and 12 a.m. once a day, at a volume not exceeding 10 mL/kg. After the treatment, the animals were anesthetized under isoflurane atmosphere before cardiac puncture and then euthanized. The brain was removed, and the hippocampus was isolated for consecutive analyses (Fig. 1).

2.4. Behavioral tests

On days 27–30, the animals performed the behavioral tests as shown in Fig. 1: day 27 – habituation to object recognition test (ORT) arena; day 28 – training for test; day 29 – mice performed training and after that they were subjected to short-term memory (STM) test, long-term memory (LTM) test, and the Y-maze test; on day 30 the animals were euthanized.

2.4.1. Open field

The open field was used to identify motor disabilities, which might influence the object recognition performance. During the habituation of the novel object recognition task, the animals were transferred to a 45 x 45 cm open field, with the floor divided into nine squares. During the 5 min open field session, the number of crossing responses was recorded, measuring the speed and average speed by ANY-maze 6.1 software.

2.4.2. Object recognition task

The object recognition test was performed according to Dao et al (2013) with some modifications. After habituation by open field test, two objects were added in the box. Chambers and objects were cleaned with 30% ethanol immediately before and at the end of each behavioral evaluation. Subsequently, the training and testing sessions were evaluated for 5 minutes. In the training session, the animals were exposed to two of the same objects (object A), and the exploration time was recorded with two stopwatches. The test session was carried out 4 h (STM) and 24 h (LTM) after training. In the test session, mice were placed back in the behavioral chamber and one of the familiar objects (object A) was replaced by a novel object (object B / C). The time spent exploring the familiar and the novel object was recorded. The percentage of the total exploration time that the animal spent investigating the novel object
was the measure of recognition memory. Recognition memory for both tests (STM and LTM) was evaluated by recognition index (%): \( \text{time in the novel object} / (\text{time in familiar object} + \text{time in the novel object}) \times 100 \) [26].

2.4.3. Y-maze test

The Y-maze is a well-recognized memory test; it is a three-arm maze with equal angles (120°) between all arms, each of which are 30 cm long and 5 cm wide with 12 cm-high walls. Mice were initially placed at the center of the Y-maze, and after 2 min of habituation, the sequence and number of arm entries were recorded for each mouse over 6 min period using ANY-maze 6.1 software. The percentage of trials in which the mice entered all three arms (ABC, CAB, or BCA) was recorded as an alternation to estimate short-term memory. The alternation score (%) for each mouse was defined by the equation: % alternation = \( \left[ \frac{(\text{Number of alternations})}{(\text{total arm entries} - 2)} \right] \times 100 \). The number of arm entries per trial was used as an indicator of locomotor activity [27].

2.5. \( \text{Al}^{3+} \) concentration on hippocampus and serum

The total concentration of total \( \text{Al}^{3+} \) in the serum and hippocampus of Swiss male mice were measured according to the method previously described [28]. Briefly, 100 µL of serum and 2 mL of \( \text{HNO}_3 \) (14 mol/L) were mixed in a 15 mL polypropylene tube. The mixture was heated in a water bath (70°C) for 10 min. After sample mineralization, deionized (18.2 MΩ cm) water was added to the tube in a final volume of 10 mL immediately before reading by inductively coupled plasma optical emission spectrometry (ICP OES). \( \text{Al}^{3+} \) emission line selected was at 213,856 nm and the results were expressed in µg/L.

2.6. DNA comet assay

The alkaline comet assay was performed as described by Singh et al. [29] in accordance with the general guidelines for use of the comet assay. One hundred cells (50 cells from each of the two replicate slides) were selected and analyzed. Cells were visually scored according to tail length and received scores from 0 (no migration) to 4 (maximal migration). Therefore, the damage index for cells ranged from 0 (all cells with no migration) to 400 (all cells with maximal migration). Slides were analyzed under blind conditions by at least two individuals.

2.7. Brain tissue preparation

Adult mice were anesthetized with isoflurane inside an anesthetic chamber and euthanized. The total hippocampus was weighed and allocated into test tubes. The hemisphere was homogenized 1:10 in Tris–HCl 10 mM, pH 7.2 buffer to verify enzymatic activities. All procedures described above were performed under refrigeration temperature (4 ºC).

2.8. Enzymatic assays

The E-NTPDase and 5´-NT enzymatic activities of the hippocampus were determined by the methods of Schetinger et al. (2000) and Heymann et al. (1984), respectively. The enzymatic preparation (20 µL; 8–12 µg of protein) was added to the reaction mixture and pre-incubated at 37°C for 10 min. The reaction was
initiated by the addition of a substrates (ATP, ADP, or AMP). Enzyme activities were reported as μmol Pi released/min/mg protein [30, 31].

ADA activity of the hippocampus was determined according to Guisti and Galanti (1984). Brain samples (50 μL) were incubated with 21 mM/L of adenosine pH 6.5 and incubated at 37°C for 60 min. The results were expressed as U ADO/mg protein.

### 2.9. Western blot

Samples of the total hippocampus were homogenized in ice-cold radioimmunoprecipitation assay buffer (RIPA buffer) with 1mM protease and phosphatase inhibitors and centrifuged at 12,000 rpm at 4°C for 10 min. The protein concentration was determined using the BCA Protein Assay Kit (Sigma-Aldrich, EUA). The diluted samples were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Amersham Biosciences, UK). After blocking, the membrane samples were incubated overnight at 4°C with the primary antibodies: P2X<sub>7</sub> (dilution 1:500, Santa Cruz Biotechnology, CA, USA); A1 (dilution 1:500, Santa Cruz Biotechnology, CA, USA); A<sub>2A</sub> (1:800, Santa Cruz Biotechnology, CA, USA); IL-1β (dilution 1:1000; cell signaling, MA, USA); and IL-10 (dilution 1:1000, Santa Cruz Biotechnology, CA, USA). Membranes were incubated with anti-rabbit or anti-mouse secondary antibodies (dilution 1:10.000, Santa Cruz Biotechnology, CA, USA) for 90 min at room temperature. The membranes were incubated with an enhanced chemiuorescent substrate (Amersham Biosciences) and analyzed with an Amersham Imager 600 (GE Healthcare Life Sciences, EUA). The membranes were re-probed and tested for β-actin immunoreactivity as a control for protein concentration [32].

### 2.10. Protein determination

Protein levels were measured by Coomassie blue method as previously described by Bradford, using bovine albumin serum as standard [33].

### 2.11. Statistical analysis

Results were expressed as mean values ± standard error of the mean (SEM).

Statistical analysis was assessed by one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test using the GraphPad Prism (Version 5.0) software. Differences between mean values were considered statistically significant at *p < 0.05.

### 3. Results

#### 3.1. Effects of Al on brain and body weight

To verify the effects of Al-treated mice, we measured body and brain weight at the end of the experiment. The results are presented in Fig. 2A. With regard to the body weight, there were no significant differences among the groups (p < 0.05). For the brain weight (Fig. 2B), we observed a reduction in turn of 0.230 g on brain of mice treated with AlCl<sub>3</sub> at 50 and 100 mg/kg when compared to control group (p < 0.05).
3.2. Al\(^{3+}\) levels on serum and hippocampus

One crucial question regarding the experimental model of Al exposure, used in this study, is whether the ion was able to cross the gastrointestinal tract and access the brain. Thus, the serum and hippocampal Al\(^{3+}\) levels were quantified. As can be observed in Fig. 3, animals treated with 50 and 100 mg/kg of AlCl\(_3\) increased the Al\(^{3+}\) concentration in the serum to values of 0.98 µg/g and 0.56 µg/g, respectively, when compared to the control group. In addition, for hippocampal Al\(^{3+}\) levels, only animals in the group treated with 100 mg/kg were levels higher (0.27 µg/g) (p < 0.05).

3.3. Al\(^{3+}\) induced memory impairment in mice

Before the analyses of memory tests, it is necessary to evaluate the locomotor activity of mice to verify whether the treatment affected this parameter or not. Figure 4 demonstrates that AlCl\(_3\) did not alter the number of crossings, speed and average speed after the treatment when compared to the control group. In memory tests, Fig. 5 shows that AlCl\(_3\) induced impairment on short- and long-term memory in the novel object recognition test only at 100 mg/kg. In addition, AlCl\(_3\) groups treated with 50 and 100 mg/kg was able to cause short-term memory loss in the Y-maze test, as observed in Fig. 6 (p < 0.05).

3.4. Al\(^{3+}\) causes genotoxicity

Genotoxicity of Al\(^{3+}\) during chronic metal exposure was measured by DNA damage using the comet assay. Figure 7A shows that Al\(^{3+}\) elongated the DNA tail. In this analysis, the cells with damaged DNA had the appearance of a comet, while undamaged cells had an intact nucleus without a tail. Different damage classes are quantified in Fig. 7B. As can be observed, the group treated with 100 mg/kg of AlCl\(_3\) had a reduced number of undamaged DNA cells and increased the class of damage until the apoptosis stage (maximally damaged) (p < 0.05).

3.5. Al\(^{3+}\) alters NTPDase, 5’-NT and ADA enzymes activities in the hippocampus of mice

The results obtained for E-NTPDase, E-5’-NT, and ADA activities are shown in Fig. 8. ATP (Fig. 8A) hydrolysis by NTPDase and AMP (Fig. 8C) hydrolysis by 5’-NT was significantly increased (35% and 22.3%, respectively) only in the AlCl\(_3\) group treated with 100 mg/kg compared with the control group. For ADP hydrolysis there were no observed differences among the groups (p < 0.05). Following the ectonucleotidase cascade, only at 100 mg/kg concentration was AlCl\(_3\) able to increase the ADA activity (55.8%) when compared to the control group (Fig. 8D).

3.6. Al\(^{3+}\) modulates the purinoreceptors density on hippocampus of mice

Since we found that AlCl\(_3\) alters the ATP metabolism by augmented ectonucleotidase activities, we evaluate the purinoreceptors density in the hippocampus of mice. Western blot analysis of P2X7, A1 and A2A receptors is shown in Fig. 9. AlCl\(_3\) augmented the P2X7 receptor density only at 100 mg/kg (Fig. 9A) compared to the control group (p < 0.05). In relation to the adenosine receptors, the A1 receptor density showed no significant differences among the groups (Fig. 9B). However, animals exposed to 100 mg / kg of AlCl\(_3\) increased the A2A receptor density (Fig. 9C) when compared to the control group (p < 0.05).
3.7. Al\textsuperscript{3+} affects the cytokines density on hippocampus of mice

P2X7 receptor plays a crucial role in mediating immune response by regulating the expression of the proinflammatory cytokines. In view of that, we evaluated pro- and anti-inflammatory markers, to identify the possible neuroinflammation induced by Al\textsuperscript{3+}. The Western blot cytokine density is presented in Fig. 10, and indicates an augmented IL-1\textbeta, a proinflammatory cytokine, in animals treated with 100 mg /kg of AlCl\textsubscript{3} when compared to the control group. On the other hand, anti-inflammatory IL-10 was also determined and is decreased in Al\textsuperscript{3+}-exposed animals when compared to the control group (p < 0.05).

4. Discussion

Al is a nonessential metal prevalent in the environment and its daily exposure to biological systems is uncontrollable [3]. Epidemiological studies about Al’s clinical neurotoxicity point out that chronic exposure to metal ion can induce cognitive deficits similar to those that occur in the dementia condition [11, 34–36]. The brain is the most vulnerable organ to Al accumulation and toxicity [13]. Thus, we evaluate mechanisms linked to Al toxicity and memory parameters using an animal model of chronic exposure to the metal.

The Al\textsuperscript{3+} form, accesses the CNS by crossing the BBB, and is deposited in the brain regions [6]. The hippocampus is the important structure responsible for memory formation and consolidation, as well as neurogenesis and neural plasticity, and represents one target of Al\textsuperscript{3+} accumulation [4]. Our results shown that AlCl\textsubscript{3} at 100 mg/kg concentration was able to decrease the brain weight of mice, as well as increase the Al\textsuperscript{3+} concentration in the serum and hippocampus.

Several studies have demonstrated a possible relation with Al accumulation in the brain and the development of neurodegenerative disorders [4]. Once we had shown quantitatively that our model of Al exposure by oral administration was sufficient to reach the CNS, we evaluated short- and long-memory tests. Our findings showed that mice treated with 100 mg/kg of AlCl\textsubscript{3} did not alter their locomotor activity but their short- and long-term memory was diminished, as observed by the novel object recognition and Y-maze tests. We suggest that Al toxicity can induce irreversible cognitive deficits, possibly due to changes in the hippocampal functions.

In addition, the comet assay indicates that AlCl\textsubscript{3} treatment at 100 mg/kg caused DNA damage in blood cells detected in our experimental approaches. We suggest that genotoxicity conferred by Al\textsuperscript{3+} could cause mutations and apoptosis, contributing to cellular dysfunction, since blood cells represent a periphery marker of cellular damage. Moreover, Al\textsuperscript{3+} can interact with the plasma membrane, affecting the structure and function of DNA and important proteins [37].

One important class of cell-surface proteins are the ectonucleotidases [19]. We evaluated the NTPDase, 5'-NT and ADA in total hippocampus. Our data showed an augmented activity of these enzymes in mice treated with 100 mg/kg of AlCl\textsubscript{3}. We suggest that, in this condition, ATP acts as a proinflammatory agent,
with elevated hydrolysis of nucleotides in response to possible high levels of ATP [38]. These results are in accordance with Kaizer et al. 2007, who indicated an increase in ectonucleotidase activities in the synaptosome fraction obtained from the cerebral cortex and hippocampus of rats [39]. In contrast, Schetinger et al. 1995 demonstrated that the metal ion significantly inhibited ATPase and ADPase activities in vitro of rat cortex synaptosomes and, more recently, Reichert et al. 2020 showed a reduced NTPDase in vitro in neural progenitor cells. This fact may occur due to the different time and manner of exposure to Al [40, 41].

High levels of ATP are sufficient to activate specific receptors, such as P2X7 [42]. Here, we demonstrate that AlCl₃ at 100 mg/kg was able to increase this receptor expression, developing an inflammatory process induced by Al³⁺. Similarly, Assmann et al. 2021 found that the AlCl₃ triggered an increase in gene expression and protein density of P2X7R in BV-2 brain microglial cells [43]. From the data revealed by the P2X7 receptor in our study, we analyzed the pro- and anti-inflammatory cytokines. Our results indicate that AlCl₃ at 100 mg/kg was able to elevate IL-1β proinflammatory cytokine and reduced IL-10, an anti-inflammatory cytokine. Taken together, this data configures a neuroinflammatory process. Our results are in concordance with Cao et al. 2016, who showed that AlCl₃ can induce neuroinflammation, affecting neuronal survival, and thereby leads to learning and memory deficits [11].

In addition, adenosine is a molecule related to neuromodulatory effects by binding to specific receptors [22]. The increase in adenosine deaminase activity may represent a reduced level of adenosine in the hippocampus. Thus, we evaluated the adenosine receptors A1 and A2A densities to clarify the effects of this molecule on the CNS. Our data showed no significant differences on A1 receptor density among the groups. However, we observed an increase in A2A receptor in animals treated with 100 mg/kg of AlCl₃. We suggest that adenosine acts by binding to A2A receptor, which explains the results observed on behavior tests, since this receptor is associated with cognitive decline and enhanced neurotoxicity [44–46].

In summary, our study points to the importance of the purinergic system during Al long-term exposure. ATP and adenosine represent two important molecules susceptible to metal ion metabolism, contributing to memory impairments due to the neuroinflammation process, via P2X7 and A2A receptors. Moreover, this study contributes to elucidating possible mechanisms of aluminum on CNS, inducing neurodegeneration associated with environmental factors.

5. Conclusion

In conclusion, this study reveals some mechanisms involved in the neurotoxic effects of Al³⁺-induced cognitive impairments after long-term exposure of AlCl₃ in Swiss mice. We also showed that Al³⁺ alters the purinergic cascade in the hippocampus of mice, stimulating the proinflammatory process. Thus, the explanation of the role played by purinergic signaling against external stimuli could be a powerful tool for the development of new strategies of neuroprotection.
Declarations

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Availability of data and material: All data and material generated during this study are included in this published article.

Authors’ Contributions: Vera Maria Melchiors Morsch, Maria Rosa Chitolina Schetinger and Karine Paula Reichert: experimental design and supervised the study. Karine Paula Reichert, Nathieli Biachin Bottari and Anaílen Dutra da Silva: Aluminum treatment, euthanized and molecular assays. Karine Paula Reichert, Charles Elias Assmann, Pauline da Costa, Thauan Faccin Lopes and Nathieli Bianchin Bottari: Behavioral tests, euthanized and molecular assays. Graciela Heidrich and Valderi Dressler: Aluminum concentration on serum and hippocampus. Karine Paula Reichert: collected and analyzed the data. Karine Paula Reichert, Vera Maria Melchiors Morsch and Nathieli Bianchin Bottari: wrote the manuscript.

Ethics Approval: All animal procedures were approved by the Institutional of Animal Care and Use from the UFSM (protocol number: 2712020517/2017).

Consent for Publication: Not applicable.

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**Figures**

**Figure 1**

Experimental design. Schematic representation of the experimental design of this study. 1- CT - control group, 2 - group treated with 50 and, 3- 100 mg / kg of AlCl₃, STM - short-term memory and LTM - long-term memory.
| Groups       | Body weight (g) | Brain weight (g) |
|--------------|-----------------|-----------------|
|              | Onset           | End             |                  |
| Control/saline | 44.6 ± 3.6     | 42.4 ± 2.1      | 0.501 ± 2.5      |
| AlCl₃ 50 mg/kg  | 42.5 ± 2.1     | 43.0 ± 4.0      | 0.482 ± 1.8      |
| AlCl₃ 100 mg/kg | 41.7 ± 1.7     | 41.3 ± 3.1      | 0.475 ± 3.0*     |

**Figure 2**

Effects of AlCl₃ on body and brain weight of mice at the onset and after 30 days of experiment. A) Body weight, and B) Brain weight. Data are expressed as the mean + S.E.M. (p<0.05; n = 10). ANOVA and Tukey’s post-hoc test.

**Figure 3**

Al₃+ concentration (µg / g) in the serum and hippocampus of mice by ICP-OES. Each column represent the + S.E.M., n = 4 for each group (*p < 0.05).
Figure 4

AlCl₃ did not alter locomotor activity measured by open field test. (A) Number of crossing, (B) speed, (C) average speed and (D) Track plots of animal way on apparatus exploration. The following symbols have been used in these plots.

CT  AlCl₃ 50 mg/kg  AlCl₃ 100 mg/kg

S.E.M. (p<0.05; n = 10). ANOVA and Tukey’s post-hoc test.

Figure 5

AlCl₃ did not alter locomotor activity measured by open field test. (A) Number of crossing, (B) speed, (C) average speed and (D) Track plots of animal way on apparatus exploration. The following symbols have been used in these plots.
Effects of AlCl₃ on the recognition index in novel object recognition task. Oral administration of AlCl₃ at 50 and 100 mg/kg decreased the short-term memory – 4hs (A) and long-term memory -24 hs (B) as measures of recognition index in the object recognition task. Data are expressed as the mean ± S.E.M. (p<0.05; n = 10). ANOVA and Tukey’s post-hoc test.

Figure 6

Effects of AlCl₃ on spontaneous alternation in the Y-maze test. (A) Percentage of alternation, (B) Total number of arm entries. (C) Pooled heatmap representing the normalized mean time spent within the maze during the test. Data are expressed as the mean ± S.E.M. (p<0.05; n = 10). ANOVA and Tukey’s post-hoc test.
Figure 7

Effects of AlCl3 on DNA damage assessed using the comet assay. Mean comet score and comet classes in control and exposed groups. The cells were assessed visually and received class 0 (undamaged) to apoptosis (maximally damaged), according to the size and shape of the tail. Data are expressed as the mean + S.E.M. (p<0.05; n = 3). ANOVA and Tukey’s post-hoc test.
Figure 8

Effects of AlCl₃ on nucleotide hydrolysis and adenosine deaminase (ADA) activity in hippocampus from mice treated with saline or AlCl₃ at 50 and 100 mg/kg. A) ATP hydrolysis by NTPDase, B) ADP hydrolysis by NTPDase, C) AMP hydrolysis by 5’-NT, and D) adenosine deamination by ADA. Data are expressed as the mean + S.E.M. (p<0.05; n = 10). ANOVA and Tukey’s post-hoc test.

Figure 9

AlCl₃ modulates purinoreceptors density after chronic exposure of mice. A) Western blot quantification of P2X7 receptors in total membranes of the hippocampus of mice treated with saline or AlCl₃ at 50 and 100 mg/kg. β-actin was used as a loading control to normalize protein levels. Data are expressed as the mean + S.E.M. (p<0.05; n = 5). ANOVA and Tukey’s post-hoc test.
AlCl₃ alters inflammatory cytokine markers density after chronic exposure of mice. A) Western blot quantification of IL-1β proinflammatory cytokine, and B) IL-10 anti-inflammatory cytokine densities in total membranes of the hippocampus of mice treated with saline or AlCl₃ at 50 and 100 mg/kg. β-actin was used as a loading control to normalize protein levels. Data are expressed as the mean + S.E.M. (p<0.05; n = 5). ANOVA and Tukey’s post-hoc test.