Advances in Biological Research 2 (1-2): 26-33, 2008
ISSN 1992-0067

Chronic Exposure to Aluminum Chloride in Mice: Exploratory Behaviors and Spatial Learning

Ouafa Rebai and Nour Eddine Djebli

Département de Biologie, Faculté des sciences, Université de Mostaganem 27000, Algerie

Abstract: The effect of Aluminum chloride was investigated to describe the associated behavioral and brain modifications. Albino mice were administered with drinking distilled water containing AlCl(3) (50 mg/kg/day) and control group with drinking double distilled water only for 3 months. After cessation of treatment, locomotor activity, hole-board test, forced swimming, black and white Test box and Morris water maze were evaluated to assess anxiety, responses to stress and learning memory of animals. In anxiety-related behaviors, Al exposed mice were more active in the locomotor activity and holeboard tests and they did not become hyperactive in the porsolt's swimming test. They were less affected by the light conditions in the central area of black and white test box. Al-treated mice had impaired spatial working memory, with lower performance at Morris water maze. The brains of experimental animals, studied by optical microscopy, have revealed damage in the hippocampus and cortex, including neurofibrillary degeneration, which can be due to the accumulation of Aluminium in these regions. Results of this study demonstrate that Al neurotoxicity may play an important role in the development of anxiety disorders, depression and memory deficit in mice, these alterations may also play an important role in the development of Alzheimer diseases in the Al-treated animals.

Key words: Aluminum chloride · Mice · Cognition · Learning · Memory

INTRODUCTION

Aluminum (Al) is one of the most abundant metals in the earth's crust. Human exposure to Al has been increasing over the last decades. This element appears mainly in food products and in drinking water derived from both natural sources and treatment methods [1]. Al has the potential to be toxic for humans. Patients on dialysis [2] or on long-term treatment with total parenteral nutrition [3] have been shown to accumulate this metal in different organs. The human toxicological effects include encephalopathy [2], bone disease [4] and anemia [5]. Finally, Al is a possible contributing factor in Alzheimer's disease [6]. Evidence for the contribution of Al to Alzheimer's disease (AD) remains contradictory [7, 8], however, epidemiological studies have indicated a link between Al in drinking water and AD and a variety of human and animal studies have implicated learning and memory deficits after Al exposure [9-11].

In fact, it has been stated that the use of Al as an experimental neurotoxicant has recapitulated virtually every feature of the neurodegenerative spiral afflicting Alzheimer's patients [12]. Additional evidence suggests that Al accumulates in the human brain with aging [13]. Neurotoxicity from exposure to Al is known to result in impairment of learning memory and cognition function both from clinical observations and from animal experiments [14-16]. Crapper *et al*. reported that the concentrations of Al in the brains of AD patients were significantly high [17]. Long-term administration of soluble salt of Al to rats worsens their learning ability together with diminished cholinergic function and the rats become lethargic [14, 18]. Role of Al intoxication in neurodegenerative diseases has been recently emphasized [19-21].

To asses a spectrum of learning and memory functions, a battery of tests is needed [22-24]. A functional observational battery with locomotor activity, hole-board test, forced swimming and Black and White Test Box measurements was used. Theses tests were selected to assess emotionality, anxiety and/or responses to stress in animals. The Morris water maze [25-27] was selected to assess spatial discrimination learning, since no food deprivation was necessary. Two types of Morris maze tasks were used consecutively, one evaluate spatial reference memory and one to address spatial working memory.

Corresponding Auhtor: Dr. Ouafa Rebai, Département de Biologie. Faculté des sciences - Université de Mostaganem 27000, Algerie

The present studies were undertaken to assess the neurotoxicity of Aluminium by setting out to represent the possible effects of this toxin on behaviour and memory, after chronic exposition of mice. Additionally, histological study of mice's brains was designed to assess the effects of Aluminum chloride exposure on the viability of cells in the hippocampus, a structure known to contribute significantly to spatial learning.

MATERIALS AND METHODS

Animal Treatment: The study was performed on a total of forty 8-week-old male Albinos mice, weighing about 35 g. Animals were housed in stainless steel cages in a temperature (22±4°C) and photoperiod (12h light/dark cycle) controlled room. Aluminum treated animals received distilled water containing Aluminum chloride ($AlCl_3$) at a dose of 50 mg/kg/day [28], for a period of 3 months, controls were treated with double distilled water. During the time of experiments, animals had free access to standard laboratory diet and tap water. Body weight was recorded daily but no significant differences were observed between the groups.

Locomotor Activity: Mice were tested in acrylic cages (45 x 25 cm) divided into 16 equal squares. The number of crossed squares was recorded for each mouse per time of 5 min for 20 min investigated.

Holeboard: Exploratory behaviour of mice in a novel environment was measured as previously described using a hole-board test [29]. This method is used for measuring the response of the mice to an unfamiliar environment. The apparatus consisted of a grey wooden box (50×50×50 cm) with four equidistant holes 3 cm in diameter in the floor. Head-dipping behaviours were checked for 20 min with sample intervals of 5 min.

Black and White Test Box: As described [30], this model permits simple and quick evaluation of the anxious behaviour and its modification compared between an illuminated compartment and a dark one. The test boxes consist of two compartments, the first of these, coloured matt black and the other one, matt white. Both compartments are separated by a wall with a 70x70 mm opening in its base. Total time spent in each compartment was recorded for 20 min with sample intervals of 5 min.

Forced Swimming: Mice were placed in a Plexiglas cylinder (10 cm internal diameter, 50 cm high) filled with 25-26°C water (10 cm height). Duration of the experiment was 6 min; the behavior of the animals was evaluated between the first and sixth minute for 5 min. The immobility time was measured, a mouse was judged to be immobile when it remained floating in the water, making only those movements necessary to keep its head above the water [31].

Morris Water Maze: The maze consists of a circular pool (1.2 m in diameter and 0.47 m high) made of white plastic [27, 32]. The pool was filled to a depth of 20 cm with water (24°C-25°C) that was made opaque by the addition of non toxic white paint. An escape platform (10 cm in diameter), made of white plastic with a grooved surface for a better grip, was submerged 0.5 cm under the water level. The animal has to swim until it finds the hidden platform. The animal generally uses cues outside the maze to develop a spatial map of the environment and guide its performance. The pool was divided into four equal quadrants labeled N (north), O (east), S (south) and W (west). Their order of use was randomized daily. The time the mouse needed to find the hidden platform (latency) so that it can stop swimming was recorded. A trial was started by placing the mouse into the pool close to the rim, facing the wall of the tank into one of the four quadrants. The mice were given four days of training with four 60-seconds training trials per day. During a spatial reference memory (SRM) training, the platform was always placed in the same spatial location of the pool (NE quadrant) throughout the training period in both paradigms. During spatial working memory (SWM) training, the escape platform was placed from the edge of the pool in one of the four possible locations (designated N, S, E and W).

Preparation of Brain Tissue: At the end of exposure, animals were food deprived for 16h and killed and then brains were quickly removed. Segments were fixed in Bouin's solution and paraffin-embedded. Serial sections of 5 µm were obtained with a Leica microtome. For histologic observation, deparaffinized sections were stained according to conventional histological and histochemical stains [33, 34]. Coloration in Harry's hematoxyline 2 min (to filter before use) and for contrast with 1% eosin aqueous 40 seconds.

Statistical Analyses: The data are expressed as means with S.E.M. The statistical significance of differences between groups was assessed with an analysis of

variance (ANOVA) followed by Student Newman-Keuls method for post hoc analysis. Statistical significance was assessed at an alpha level of 0.05.

RESULTS

Behavioral Measurements: The effects of Aluminum chloride on exploratory behavior in mice are shown in Fig. 1. The locomotor activity of mice was assessed by the number of crossing squares noted as scores per time of 5 min for the 20 min investigated. Result showed that the activity of intoxicated mice was higher than controls; it was significant at the first 5 min (Fig. 1*A*). As shown in Fig. 1*B*, Aluminum did not significantly modify head-dipping behaviors.

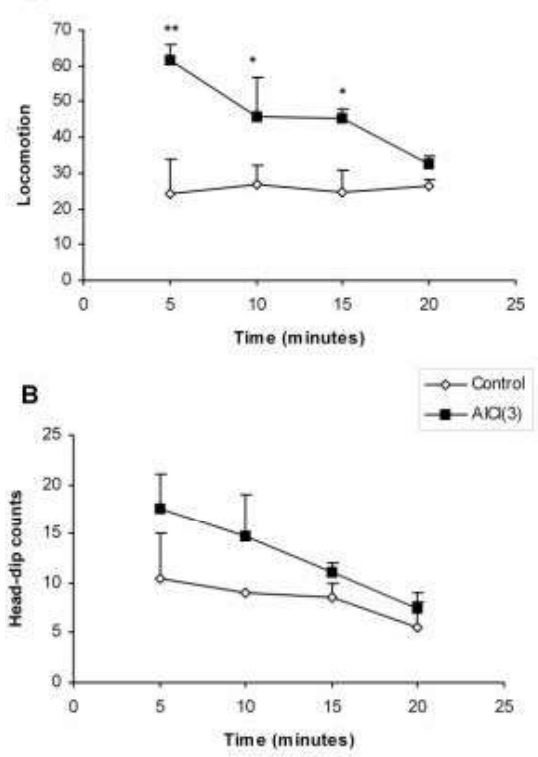

Fig. 1: Effects of exposure to AlCl(3) on locomotor activity (A) and hole-board (B) tested in mice administered 0 (control, n = 20) and 50 mg/kg/day AlCl(3) (n = 20) during 3 months. Crossing squares and head-dipping movements were recorded over a 20-min period during 5 observation sessions. Each point represents the mean ±S.E.M of 20 Al-treated and 20 control mice. Groups were compared using two-way ANOVA (Student-Newman-Keuls post hoc analysis) ($^{*}P<0.05$, $^{**}P<0.01$)

The validity of a black and white test box to measure changes in mouse exploratory behaviour relevant to assessment of anxiety was investigated by variation of the illumination within the test box. No significant difference was seen in black and white box test (Fig. 2*A*), however, time spent in light was higher than dark for intoxicated group. No significant difference for control group was noted. In the forced-swimming test, active escape periods alternated with periods in which the animals were completely inactive or made only the movements necessary to keep their head above water. As shown in Fig. 2*B*, Al treated mice spend more time immobile in this test than control animals, Aluminum chloride significantly increased immobility time ($p<0.05$).

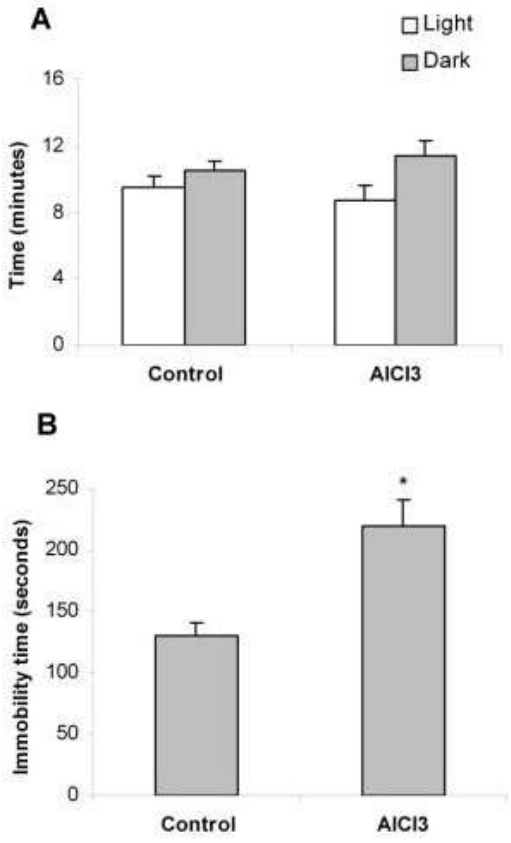

Fig. 2: Effect of AlCl(3) on the black and white shuttle box (A) and the forced swimming (B) tested in mice after 10 weeks of orally administration of AlCl(3). Each column represents the mean with the ±S.E.M of 20 Al-treated and 20 control mice. Groups were compared using one-way ANOVA (Student-Newman-Keuls post hoc analysis) ($^{*}P<0.05$)

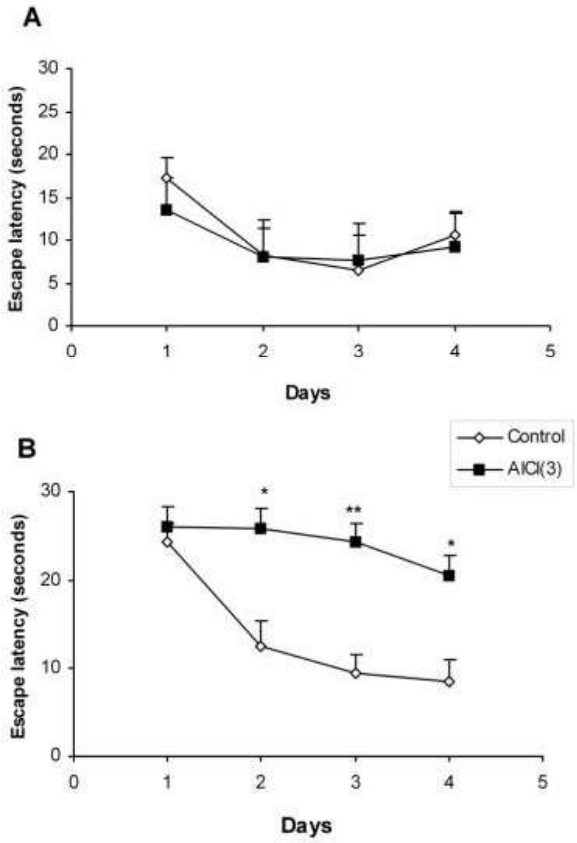

Fig. 3: Reference (*A*) and working (*B*) memory in the Morris water maze of mice administered 0 (control, n = 20) and 50 mg/kg/day AlCl(3) (n = 20) for 3 months. External cues are placed around the pool and then mice were allowed to find the platform four times daily for 4 days. In the SRM test, the platform was placed in a fixed location (NE quadrant of the pool) throughout the experiment. In the SWM test, the escape platform was placed from the edge of the pool in one of the four possible locations. A latency (in seconds) to locate the hidden platform was measured. Values are means ±S.E.M of four trials per day. Significant differences to control groups are indicated by $^{*}P<0.05$, $^{**}P<0.01$(two-way repeated measures ANOVA, Student-Newman-Keuls post hoc analysis).

Spatial Memory in the Morris Water Maze: The effects of AlCl3 administration for 3 months on reference memory and working memory are expressed as the average latencies of the mice to reach an escape platform. Global analysis revealed that in both tests all mice reduced their escape latencies over the training period (Fig. 3).

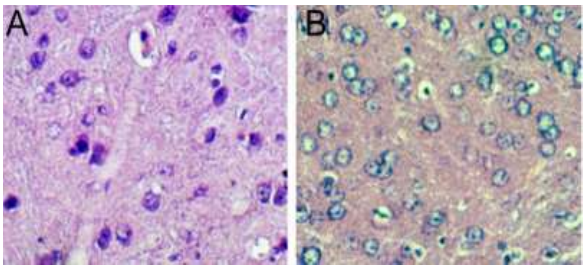

Fig. 4: Microscopic study of cerebral cortex in mouse brain. Grossly (x40). Histological sections of brain were stained with hematoxylin & eosin (H&E). Control (A). Exposed mice to 50 mg/kg/day AlCl(3) during 3 months (B).

During SRM of mice with the platform position always in the same place, the escape decreased over test days. This showed that the mice learned the location of the platform during the four test days. With the exception of day one of training, the Al-treated mice showed escape latencies comparable to their controls (Fig. 3*A*). These findings indicate no significant changes in learning between controls and Al-treated mice.

In addition to reference memory of the Morris water maze, the same mice previously tested were evaluated in the spatial working memory. SWM is indicated in this test by a reduction in escape latency over the test days (Fig. 3*B*), Al-treated mice showed significantly impairment SWM as compared with controls ($F(1, 19) = 8,23$; $p<0.01$). Control mice learned to find the platform in a time of about 10 s from day two, while Al-treated mice reached a level of escape latency in a time of about 20 s on day four.

Trend analysis performed separately for each group revealed a significant improvement in the latencies for the control mice ($F(3, 15) = 4,7$; $p<0.01$) but not for Al-exposed mice, which did not change their escape latency over the four training trials.

Nervous System: In the present study, sections of brain tissues were prepared from control and Al-exposed mice at the end of experimental time. A variety of changes were observed in the brain of Aluminum exposed mice compared with controls. Figure 4 shows a cerebral cortex of brain mice after staining with hematoxylin and eosin. Control mice demonstrating normal temporal cortex structure (Fig. 4*A*). The lesions observed in the temporal and parietal cortex of poisoned mice showed cellular hyperplasia and congestion of blood vessels (Fig. 4*B*). Additionally, the cerebral parenchyma of Al-treated

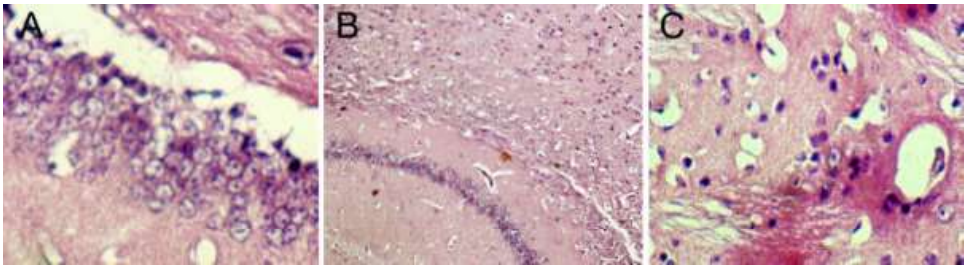

Fig. 5: Microscopic study of hippocampus in mouse brain. Grossly (x40). Histological sections of brain were stained with hematoxylin & eosin (H&E). Control (A). Exposed mice to 50 mg/kg/day AlCl(3) during 3 months (B)-(C)

mice showed several lesions. A massive cellular depletion in the hippocampal formation (Fig. 5*B*), oedema and necroses were more significant and moderate neurofibrillary degeneration is quite illustrated (Fig. 5*C*).

DISCUSSION

In the present study, the effects of Al exposure were investigated to describe the associated behavioral and brain modifications. Mice were poisoned with Aluminium chloride (AlCl3) orally at 50 mg/kg/day in drinking water for 3 months. The animals of the control group received drinking double distilled water only during this period. Results showed that Al increased the activity scores of locomotor and head-dipping. Previous reports have shown that Subchronic exposure to AlCl(3) in mice may diminish motor activity and grip strength, but motor coordination was not impaired [28]. The hyperactivity observed in our data could be the result of stress conditions. Head dipping on a hole-board is frequently used as an indicator of exploratory tendencies in rodent studies. Drugs with diverse pharmacological properties alter head dipping suggesting that many neurotransmitter systems are involved in the expression of exploratory behaviour [35]. It has been shown that changes in head-dipping behavior may reflect the anxiogenic and/ or anxiolytic state of animals [36]. Based on these results, it seems likely that enhanced in exploratory behavior in Al mice during the holeboard test and locomotor activity may reflect the anxiety response of an animal to an unfamiliar environment. Additionally, Black and White Test Box was performed as described [30]. The test was based on the adversive properties of the open field and in which anxiolytic drug-induced ease of exploratory activity is compared between an illuminated compartment and a dark one. This model permits simple and quick evaluation of the anxious behaviour and its modification by pharmacological agents. Results showed that Al-exposed mice spent less time in dark than light, this suggest the anxiety of intoxicated animals. In our present study forced swimming test showed a significantly increased immobility time of Al-treated mice. This test is a common behavioural test for assessing depression in which animals have given up the hope of escape and depression remains controversial [37], drugs with antidepressant activity reduce the time during which the animals remain immobile [31, 38].

These observations were important for the evaluation of the cognitive tests in since impaired physiological functions or changed motor performance may conflict with learning and memory tests. Most learning paradigms that require configural associations require a fully functioning hippocampus; however learning paradigms that can be solved using only elemental associations can be solved without input from this structure [39]. The Morris water escape task can test of the hippocampus in learning and memory. Results of the Morris water test documented that Al-treated mice showed no impairment in spatial learning and memory in reference version of the test. In the SRM test, the chloride Al-treated mice showed latencies comparable to controls littermates of reaching the platform when swimming to a stationary hidden platform marked by a visible cue. However, because the SRM version of the Morris water test dose not require the development of spatial mapping strategy for its solution [40, 39, 25]. The spatial working memory training paradigm used in the present study required that animal is started in a different position for each trial (different random quadrants); it can no longer rely on finding the platform in relation to one cue. The animal now must build a cohesive spatial representation of the room to find the platform. Results demonstrated a significant impairment in spatial learning and memory indicated by a decrease in escape latency of Al-treated mice compared with controls. These findings are consistent with previous reports [41-43] that have demonstrated a spatial memory

deficit in mice and rat after Aluminum exposure. The memory impairment in AD patients is often difficult to specify precisely because of the heterogeneity of psychopathology, confounding impairments in other faculties, or difficulties in determining the duration of illness, but the general consensus is that the working memory system is compromised first at early stages of the disease development [39, 44, 45, 46, 47]. In this respect, the impairment in working memory observed in the Al-exposed mice may likely correspond to early clinical stages of the disease.

The hippocampus and the cerebral cortex are the key structures of memory formation. Because the hippocampus is especially indispensable in the integration of spatial information, a decline in learning ability may be induced by the deterioration of hippocampal function [48, 49]. However, the frontal cortex plays a critical role in both spatial and non spatial working memory. It has been suggested that there may be domain-specific subdivisions within dorsal and ventral regions of the lateral prefrontal cortex which subserve working memory for spatial and non spatial information [50, 51]. In this study the brains of experimental animals, studied by optical microscopy, displayed a massive cellular depletion in the hippocampal formation with neurofibrillary degeneration. We observed numerous ghost-like neurons with cytoplasmic and nuclear vacuolations, which can be due to the accumulation of Aluminium in these regions [52]. Other experimental protocols have provided evidence that Al can accumulate in hippocampus and cortex [53]. Additionally, general histological alterations and glia activation after Al ingestion were probed. Evidence for stronger glia activation was observed in Al-exposed animals, indicative of an acceleration of pathological and inflammatory events by Al [54, 55]. Inflammatory responses are known to play an important role in neurodegenerative disease such as AD [56]. Recently, it has also been suggested that there may be an important link between Al, oxidative stress, inflammation and AD [57]. This is supported by our data and by other studies indicating that Al facilitates iron-induced oxidative stress *in vitro* [58], this may be the cause for Al-induced learning and memory deficits observed before severe neurodegeneration can be identified. This action may also be the basis for Al as a putatively contributing factor in AD.

In conclusion, the present study provides further evidence for the neurotoxic action of Al in the mouse brain. Administration of Al chloride in the drinking water of mice resulted in distinct morphological alterations in the brain and behavioral results indicate cognitive impairment and enhanced anxiety of mice in an unfamiliar environment. The mouse behavior in the water maze demonstrates that the impairment in spatial working memory of Al-exposed mice was caused by inefficient use of a spatial mapping strategy, however, mice showed intact spatial reference memory. This may be induced by the deterioration of hippocampal function after Al exposure.

REFERENCES

1. Yokel, R.A. and P.J. McNamara, 2001. Aluminum toxicokinetics: an updated minireview. Pharmacol. Toxicol., 88: 159-167.
2. Alfrey, A.C., G.R. LeGendre and W.D. Kaehny, 1976. The dialysis encephalopathy syndrome. Possible aluminum intoxication. N. Engl. J. Med., 294: 184-188.
3. Klein, G.L., 1993. Aluminum and hepatobiliary complications of total parenteral nutrition. Gastroenterology, 10: 1583-1584.
4. Ward, M.K., T.G. Feest, H.A. Ellis, I.S. Parkinson and D.N. Kerr, 1978. Osteomalacic dialysis osteodystrophy: Evidence for a water-borne aetiological agent, probably aluminium. Lancet, 22: 841-845.
5. Short, A.I.K., R.J. Winney and J.S. 1980. Robson, Reversible microcytic hypochromic anaemia in dialysis patients due to aluminium intoxication. Proc. Eur. Dial. Transplant. Assoc., 17: 226-233.
6. Campbell, A., 2002. The potential role of aluminium in Alzheimer's disease. Nephrol. Dial. Transplant., 17: 17-20.
7. Flaten, T., 2001. Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water. Brain Res. Bull., 55: 187-196.
8. Gupta, V.B., G. Anitha, M.L. Hegda, L. Zecca, R.M. Garruto, R. Ravid, S.K. Shankar, R. Stein, P. Hanmugavelu and K.S. Jagannatha Rao, 2005. Aluminium in Alzheimer's disease: are we still at a crossroad? Cell. Mol. Life Sc., 62: 143-158.
9. Yokel, R.A., 2000. The toxicology of aluminum in the brain. Neurotoxicology, 21: 813-828.
10. Schmidt, M.L., V. Zhukavera, D. Perl, S. Sheridan, T. Schuck, V. Lee and Q. Trojanowskij, 2001. Spinal cord neurofibrillary pathology in Alzheimer disease and Guam Parkinsonismdementia complex. J. Neuropathol. Exp. Neurol., 60: 1075-1086.

11. Exley, C.A., 2005. The aluminium-amyloid cascade hypothesis and Alzheimer disease, Subcell. Biochem., 38: 225-234.
12. Grant, W.B., A. Campbell, R.F. Itzhaki and J. Savory, 2002. The significance of environmental factors in the etiology of Alzheimer's disease. J. Alzheimers Dis., 4: 179-189.
13. Markesbery, W.R., W.D. Ehmann, T.I. Hossain, M. Alauddin and D.T. Goodin, 1981. Instrumental neutron activation analysis of brain aluminum in Alzheimer disease and aging. Ann. Neurol., 10: 511-516.
14. Berlyne, G.M., R. Yagil, J.B. Ari, G. Weinberger, E. Knopf and G.M. Danovitch, 1972. Aluminium toxicity in rats. Lancet, 7750: 564-568.
15. McDermott, J.R., A.I. Smith, K. Iqbal and H.M. Wisniewski, 1979. Brain aluminum in aging and Alzheimer disease. Neurology, 29: 809-814.
16. Ashall, F. and A.M. Goate, 1994. Role of the beta-amyloid precursor protein in Alzheimer's disease. Trends Biochem. Sci., 19: 42-46.
17. Crapper, D.R., S.S. Krishnan and S. Quittkat, 1976. Aluminum, neurofibrillary degeneration and Alzheimer's disease. Brain, 99: 67-80.
18. Deloncle, R. and O. Guillard, 1990. Mechanism of Alzheimer's disease: arguments for a neurotransmitter – aluminum complex implication. Neurochem. Res., 15: 1239-1245.
19. Somova, L.I., A. Missankov and M.S. Khan, 1997. Chronic aluminum intoxication in rats: dose-dependent morphological changes. Methods Find Exp. Clin. Pharmacol., 19: 599-604.
20. Wu, Y.H., Z.M. Zhou, Y.L. Xiong, Y.L. Wang and J.H. Sun, 1998. Effects of aluminium potassium sulfate on learning, memory and cholinergic system in mice. Acta. Pharmacol. Sin, 19: 509-512.
21. Exley, C.A., 1999. Molecular mechanism of aluminum induced Alzheimer's disease. J. Inorg. Biochem., 76: 133-140.
22. Green, R.J. and M.E. Stanton, 1989. Differential ontogeny of working memory and reference memory in the rat. Behav. Neurosci., 103: 98-105.
23. Peele, D.B., 1989. Learning and memory: Considerations for toxicology. Am. Coll. Toxicol., pp: 213-223.
24. Schenk, F. and R.G. Morris, 1985. Between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. Exp. Brain Res., 58: 11-28.
25. Brandeis, R., Y. Brandys and S. Yehuda, 1989. The use of the Morris water maze in the study of memory and learning. Int. J. Neurosci., 48: 29-69.
26. Lindner, M.D., A.H. Balch and C.P. VanderMaelen, 1992. Short forms of the "Reference-" and "Working-Memory" Morris water maze for assessing age-related deficits. Behav. Neural. Biol., 58: 94-102.
27. Morris, R.G., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods, 11: 47-60.
28. Hu, H., Y.J. Yang, X.P. Li and G.H. Chen, 2005. Effect of aluminium chloride on motor activity and species-typical behaviors in mice, Zhonghua Lao Dong Wei Sheng Ye Bing Za Zhi., 23: 132-135.
29. Boissier, J.R. and P. Simon, 1962. The exploration reaction in the mouse. Preliminary note. Therapie., 17: 1225-1232.
30. Costall, B., M.E. Kelly, R.J. Naylor and E.S. Onaivi, 1989. The effects of ondansetron (GR38032F) in rats and mice treated subchronically with diazepam. Pharmacol. Biochem. Behav., 32: 777-785.
31. Porsolt, R.D., A. Bertin and M. Jalfre, 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther., 229: 327-336.
32. Van der Staay, F.J. and M. de Jonge, 1993. Effects of age on water escape behavior and on related acquisition in rats. Behav. Neural. Biol., 60: 33-41.
33. Bancroft, J.D. and A. Stevens, 1986. Connective tissues and stains. In Theory and practice of histological techniques. London, Churchill-Livingstone, pp: 122-124.
34. Kiernan, J.H., 1989. Methods for connective tissue. In: Histological and Histochemical methods. Theory and practice.2nd edition. Oxford: pergamon Press, 115-131.
35. Kliethermes, C.L. and J.C. Crabbe, 2006. Pharmacological and genetic influences on hole-board behaviors in mice. Pharmacology Biochemistry and Behavior, 85: 57-65.
36. Takeda, H., M. Tsuji and T. Matsumiya, 1998. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur. J. Pharmacol., 350: 21-29.
37. Gardier, A.M. and M. Bourin, 2001. Appropriate use of "knockout" mice as models of depression or models of testing the efficacy of antidepressants. Psychopharmacology, 153: 393-4.

38. Borsini, F. and A. Meli, 1988. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology, 94: 147-160.
39. Morris, R.G. and A.D. Baddeley, 1988. Primary and working memory functioning in Alzheimer-type dementia. J. Clin. Exp. Neuropsychol., 10: 279-296.
40. Caprioli, A., O. Ghiradi, A. Giuliani, M. Ramacci and L. Angelucci, 1991. Spatial learning and memory in the radial maze: A longitudinal study in rats from 4 to 25 months of age. Neurobiol. Aging, 12: 605-607.
41. Miu, A.C., C.E. Andreescu, R. Vasiu and A.I. Olteanu, 2003. A behavioural and histological study of the effects of long-term exposure of adult rats to aluminium. Intern. J. Neurosci., 113: 1197-1211.
42. Kaneko, N., J. Takada, H. Yasui and H., Sakurai, 2006. Memory deficit in mice administered aluminum–maltolate complex. BioMetals, 19: 83-89.
43. Roig, J.L., S. Fuentes, M. Teresa Colomina and P. Vicens, 2006. Aluminum, restraint stress and aging: behavioral effects in rats after 1 and 2 years of aluminum exposure. Toxicology, 218: 112-124.
44. Hulme, C., G. Lee and G.D. Brown, 1993. Short-term memory impairments in Alzheimer-type dementia: Evidence for separable impairments of articulatory rehearsal and long-term memory. Neuropsychologia, 31: 161-172.
45. Hodges, J.R. and K. Patterson, 1995. Is semantic memory consistently impaired early in the course of Alzheimer's disease? Neuroanatomical and diagnostic implications. Neuropsychologia, 33: 441-459.
46. Desgranges, B., F. Eustache, P. Rioux, V. de La Sayette and B. Lechevalier, 1996. Memory disorders in Alzheimer's disease and the organization of human memory. Cortex, 32: 387-412.
47. Backman, L., B.J. Small and L. Fratiglioni, 2001. Stability of the preclinical episodic memory deficit in Alzheimer's disease. Brain, 124: 96-102.
48. O'Keefe, J. and L. Nadel, 1978. The hippocampus as a cognitive map. Oxford University Press, Oxford.
49. Munoz, D.G. and H. Feldman, 2000. Causes of Alzheimer's disease. Canadian Medical Association or its licensors, 162: 65-72.
50. Wan, R.Q., K. Pang and D.S. Olton, 1997. Nonhippocampal muscarinic receptors are required for nonspatial working memory. Pharmacol. Biochem. Behav., 58: 361-367.
51. Bimonte-Nelson, H.A., C.L. Hunter, M.E. Nelson and A.C. Granholm, 2003. Frontal cortex BDNF levels correlate with working memory in an animal model of Down syndrome. Behav. Brain Res., 139: 47-57.
52. Varner, J., K.F. Jensen, W. Horvath and R. Isaacson, 1998. Chronic administration of aluminum–fluoride or sodium–fluoride to rats in drinking water: alterations in neuronal and cerebrovascular integrity. Brain Res., 784: 284-298.
53. Struys-Ponsar, C., A. Kerkhofs, A., Gauthier, M. Soffie and P. Van den Bosch de Aguilar, 1997. Effects of aluminum exposure on behavioural parameters in the rat. Pharmacol. Biochem. Behav., 56: 643- 648.
54. Yokel, R.A. and J.P. O'Callaghan, 1998 An aluminum-induced increase in GFAP is attenuated by some chelators. Neurotoxicol. Teratol., 20: 55-60.
55. Platt, B., G. Fiddler, G., Riedel and Z. Henderson, 2001. Aluminium toxicity in the rat brain: histochemical and immunocytochemical evidence. Brain Res. Bull., 55: 257-267.
56. Rogers, J.P., S. Webster, L.F. Lue, L. Brachova, W.H. Civin, M. Emmerling, B. Shivers, D. Walker and P. Mcgeer, 1996. Inflammation and Alzheimer's-disease pathogenesis. Neurobiol. Aging, 17: 681-686.
57. Campbell, A. and S.C. Bondy, 2000. Aluminum induced oxidative events and its relation to inflammation: a role for the metal in Alzheimer's disease. Cell. Mol. Biol., 46: 721-730.
58. Xie, X.X., M.P. Mattson, M.A. Lovell and R.A. Yokel, 1996. Intraneuronal aluminium potentiates iron-induced oxidative stress in cultured rat hippocampal neurons. Brain Res., 743: 271-277.