

Amniotic Extract as a Therapeutic Strategy in an In Vivo Model of Early-Stage Alzheimer's Disease

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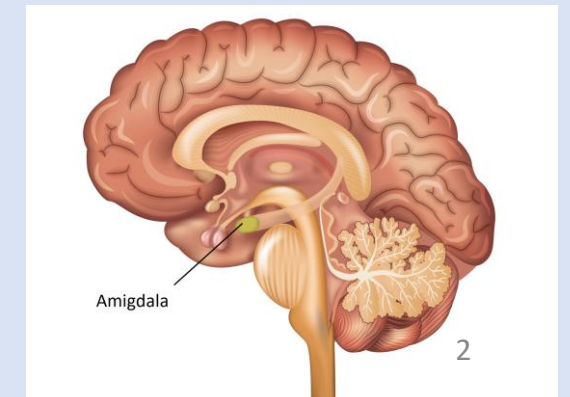
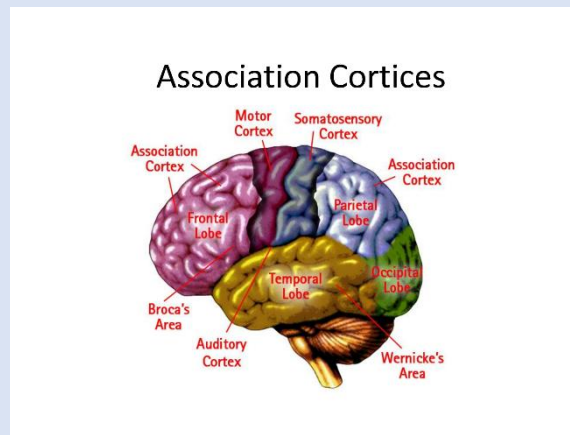
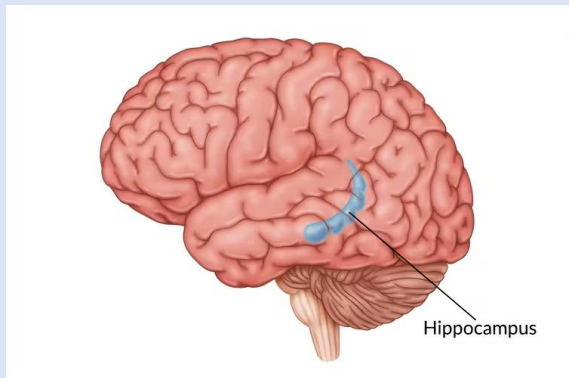
Alzheimer's Disease (AD)

- AD is an irreversible, progressive neurodegenerative disease characterized by persistent neuroinflammatory processes. Its major clinical manifestation is the loss or impairment of virtually all forms of memory, including short-term, long-term, working, episodic, emotional, spatial, and autobiographical memory.

The disease is accompanied by nervous tissue atrophy, neuronal and synaptic loss, and subcellular and functional abnormalities.

Neuropathological and Neuroinflammatory Features:

- Amyloid plaque formation – amyloid-beta peptide (1–41)
- Neurofibrillary tangle formation – tau protein
- Polarization of microglia, the CNS-resident macrophages, toward the pro-inflammatory M1 phenotype
- Predominantly affects pyramidal neurons
- The most severe neurofibrillary changes occur in the hippocampus, amygdala, and association cortex



Pathophysiology of the Disease

The disease induces severe, irreversible inflammatory processes in specific brain regions, with the following physiological manifestations:

Cognitive and memory impairment

Neuroimmune imbalance

Limited neuronal regeneration

Progressive neuronal death in the brain

Synaptic degeneration

Degeneration and reduction in brain volume as the substrate of higher nervous function

Because neuronal degeneration and irreversible inflammatory processes are major features of AD, identifying strategies for neural tissue repair and regulation of inflammation is of critical importance.

Clinical Trial Outcomes and Promising Therapeutic Strategies

Due to the rising prevalence of AD (Alzheimer's disease) and limited options for effective treatment, it represents one of the most critical scientific and clinical challenges today.

Analysis of studies conducted between 2004 and 2022 showed that only 2% of interventional Phase II and III trials were successful (Luan et al., 2022).

Aducanumab (Aduhelm) is a human monoclonal antibody.

Proposed basis of efficacy: aducanumab can bind to and facilitate the clearance of β -amyloid peptides that accumulate pathologically in the brains of patients with AD (Kachungunu et al., 2025).



Sodium oligomannate (GV-971) is an oligosaccharide derived from marine products.

Proposed basis of efficacy: it modulates the gut microbiome, thereby reducing pathological infiltration of immune cells into the brain, suppressing neuroinflammation, indirectly limiting amyloid and tau accumulation, and promoting modulation of microglial polarization (Bosh et al., 2024).



Biological Characteristics of Amniotic Extract

Amniotic extract is a biological preparation obtained by processing the amniotic membrane, the inner layer of the human placenta, and contains numerous bioactive substances.

Amniotic extract contains:

Six major growth factors:

EGF – epidermal growth factor

HGF – hepatocyte growth factor

TGF- β – transforming growth factor-beta

bFGF – basic fibroblast growth factor

KGF – keratinocyte growth factor

NGF – nerve growth factor

These growth factors are associated with neuroprotection and neuroregeneration (Santa-Olalla & Covarrubias, 1999; Ding et al., 2024; ScienceDirect, 2026).

It also contains anti-inflammatory cytokines, chemokines, and other regulatory proteins.

In this context, our study focuses on amniotic extract as a potentially neuroprotective, neuroregenerative, inflammatory-modulating, yet still insufficiently studied, biological agent.

Biological Characteristics of Amniotic Extract

In an APP/PS1 transgenic mouse model, transplantation of human amniotic mesenchymal stem cells reduced A β deposition, improved spatial memory, and promoted modulation of neuroinflammation (Wang et al., 2017). In animal models, the use of amniotic-derived tissues or cells has been associated with reduced inflammatory infiltration, regression of tissue damage, and activation of regenerative processes (Heiligenhaus et al., 2001).

Amniotic fluid and its derived extract reduced inflammatory effects in RAW264.7 monocyte/macrophage cells. The levels of pro-inflammatory cytokines, including TNF- α and IL-6, decreased, whereas the anti-inflammatory cytokine IL-10 increased. Reduced actin filament staining indicated decreased M1-type polarization (He et al., 2008).



These effects suggest that amniotic-derived biological material may act both as an anti-inflammatory agent and as a facilitator of tissue remodeling.

Study Aim

To evaluate the therapeutic potential of amniotic extract in an animal model of early-stage AD symptoms.

Study Objectives

1. To establish an animal model of early-stage AD symptoms using repeated intraperitoneal AlCl_3 injections in adult male rats.
2. To perform surgical microinjection of amniotic extract into the lateral ventricles of animals in the early-stage AD model.
3. To investigate the therapeutic potential of amniotic extract by assessing:
 - Hippocampus-dependent declarative memory using behavioral tests of spatial, short-term, and long-term memory;
 - Emotional behavior by assessing anxiety-related behavior and risk-assessment indices;
 - Hippocampal neuronal morphology by evaluating dendritic arborization and synaptic number.

Experimental Groups:

**Fifteen adult male rats were used for experimental group allocation.
Each group consisted of 5 rats.**

First group (FIZ-PBS)

- Sham surgery with intraperitoneal injection of physiological saline
- Sham surgery with injection of BPS in lateral ventricles

Second group (AL-PBS)

- Induction of Alzheimer's disease model
- Sham surgery with injection of BPS in lateral ventricles

Third group (AL-AMN)

- Induction of Alzheimer's disease model
- Injection of amniotic extract

Methodology

In Vivo Modeling of Alzheimer's Disease Using Intraperitoneal AlCl_3 Injections (Thenmozhi et al., 2015)

To experimentally model early-stage AD symptoms, intraperitoneal injections of aluminum chloride (AlCl_3) were used, a commonly used approach for inducing neurodegenerative and neuroinflammatory changes in laboratory animals.

A total of 22 intraperitoneal AlCl_3 injections were administered to 3–4-month-old male rats:

AlCl_3 injection dose: 2.8 mL/kg body weight

pH: adjusted (approximately 4–5)

Injection schedule: daily, excluding weekends.

Mechanism of action of AlCl_3

- AlCl_3 activates tau kinases and inhibits tau phosphatases.
- It induces expression of amyloid precursor protein (APP).
- $\text{A}\beta_{1-42}$ protein accumulates.

Amniotic Extract Preparation Protocol:

1. Initial placental processing (tissue washing):

PBS + antibiotics (streptomycin, penicillin) + EDTA.

Identification and separation of the amnion and chorion layers.

Repeated washing of the separated layers.

2. Extraction:

A 1:5 suspension of purified amniotic tissue in PBS was prepared.

Extraction was performed on a shaker under cold conditions (4°C) for 48 h.

3. Collection and storage:

The material was centrifuged at $3,000 \times g$ for 90 min.

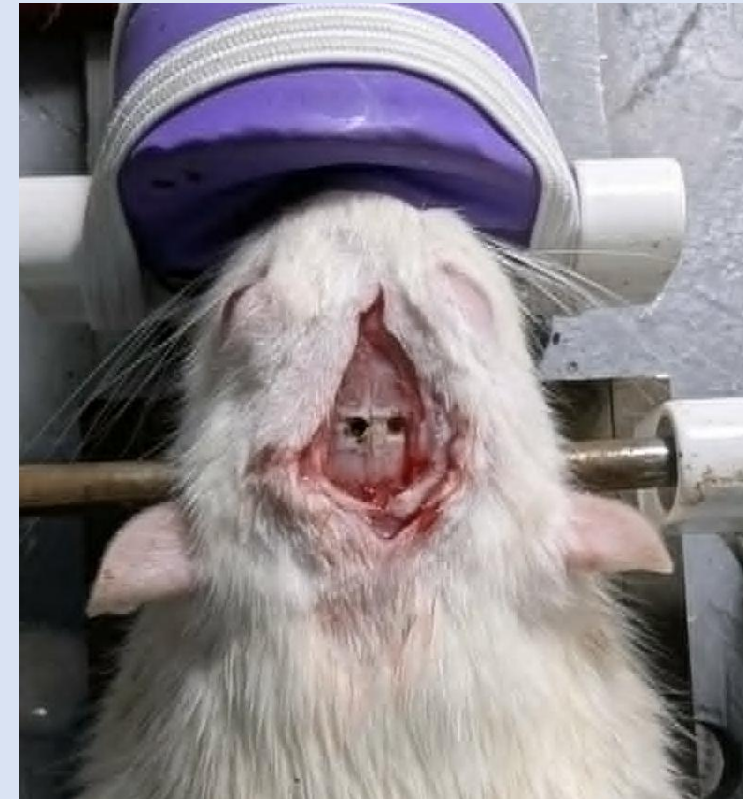
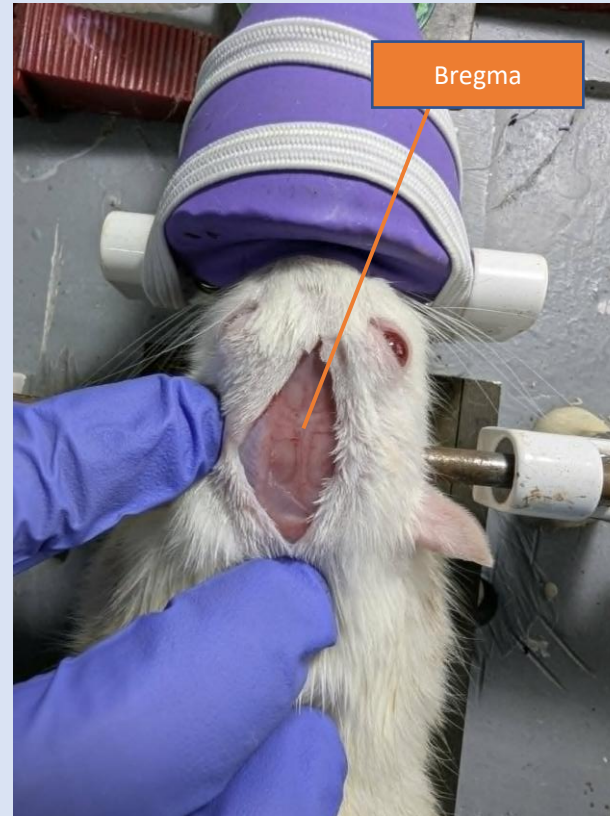
The resulting supernatant was stored at -40°C .

Protein content:

Bradford assay showed a protein concentration of 0.26 mg/mL in the extract.

Amniotic Extract Injection:

Amniotic extract was administered using a stereotaxic procedure. Trepanation site (coordinates: AP -2 mm, L 2 mm bilaterally, H 4 mm), targeting the lateral ventricles of the brain. A cranial burr hole was made to allow insertion of the microsyringe



Amniotic Extract Injection:

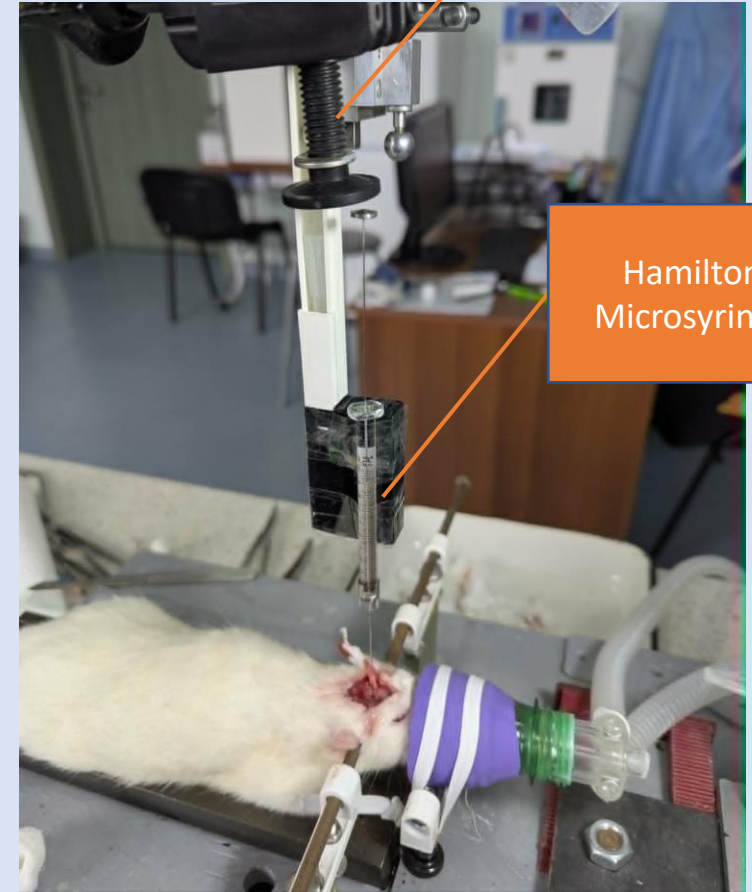
The prepared amniotic extract suspension was injected slowly and at a constant rate using a Hamilton microsyringe (5 μL per 10 min; 10 μL total). After injection, the microsyringe was kept in place for 5 min.

The amniotic extract microinjection procedure was performed twice, with a 2-day interval.

A total of 45 procedures were performed

Mechanical system for prolonged microinjection.

Hamilton
Microsyringe



Assessment of Cognitive Function, Working Memory, and Long-Term Memory:



Barnes Maze:

A test for assessing hippocampus-dependent (declarative) memory.

Used to assess spatial navigation, working memory, and long-term memory.

Assessment of Cognitive Function, Working Memory, and Long-Term Memory:

HABITUATION (Day 1):

The animal is introduced to the maze for the first time.

Goal: minimize stress.

LEARNING PHASE (4 days):

Measured:

- Time to enter the correct (target) hole (latency)
- Number of entries into incorrect holes

MEMORY TEST:

Conducted 1 h, 24 h, and 1 week after completion of training.

Long-term memory is assessed.

Measured:

- Number of entries into each hole
- Time spent at each hole

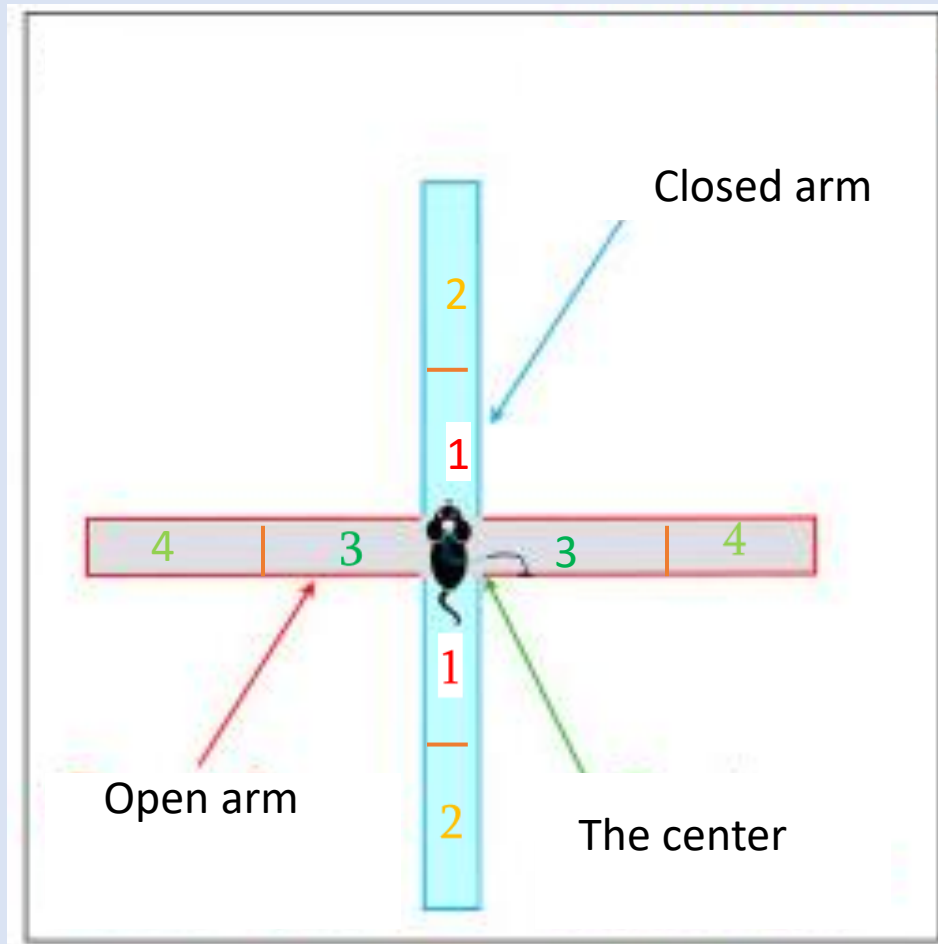
Results Analysis

Groups I vs II = Assessment of model-characteristic behavioral changes through spatial learning and memory testing.

Groups II vs III – evaluation of the therapeutic effect of amniotic extract using behavioral testing.

Assessment of Anxiety-Related Behavior and Risk-Assessment Indices

Anxiety-related emotional behavior and risk-assessment indices were evaluated using the Elevated Plus Maze (EPM) test.



Each rat was placed on the central platform with its head facing the center.
The animal was allowed to move freely for 5 min.
The time spent in the open and closed zones was recorded.
The number of entries into each zone and overall motor activity were also recorded

DiO Fluorescent Labeling of Hippocampal Sections and Dendritic and Synaptic Morphological Analysis.

Bevan, R. J., et al. (2024)

- Following anesthesia, the animal was decapitated, the brain was rinsed with cold HBSS, and the hippocampus was bilaterally dissected using a stereomicroscope.

Hippocampal tissue was sectioned at 200 μm using a McIlwain tissue chopper.

Sections were stained with DiO fluorescent dye at 2.5 mg/mL (30-min incubation).

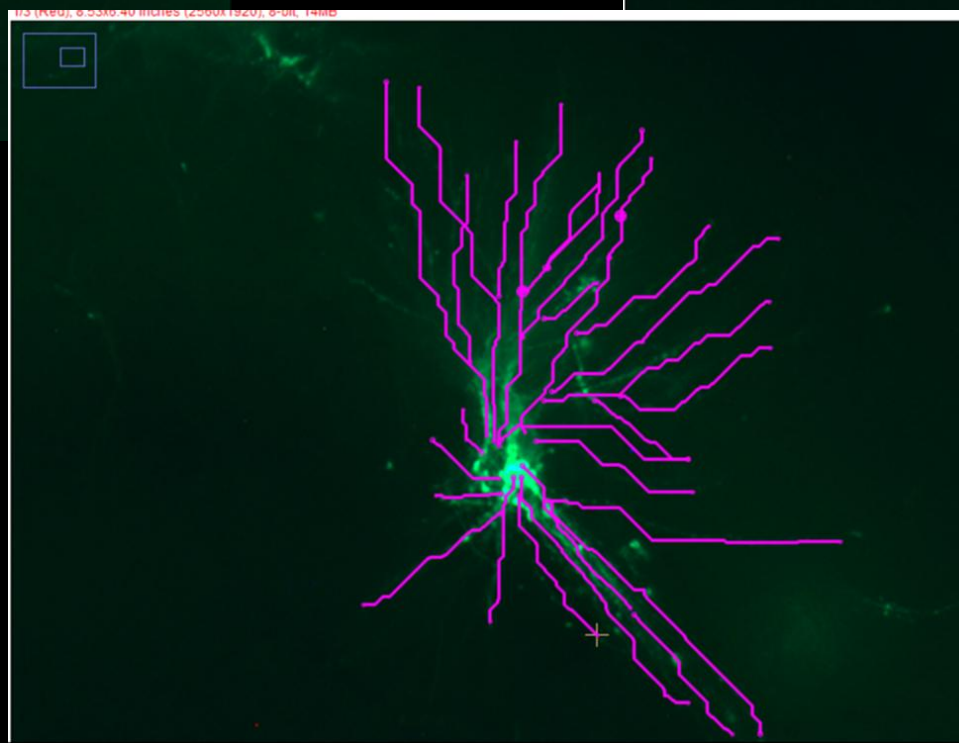
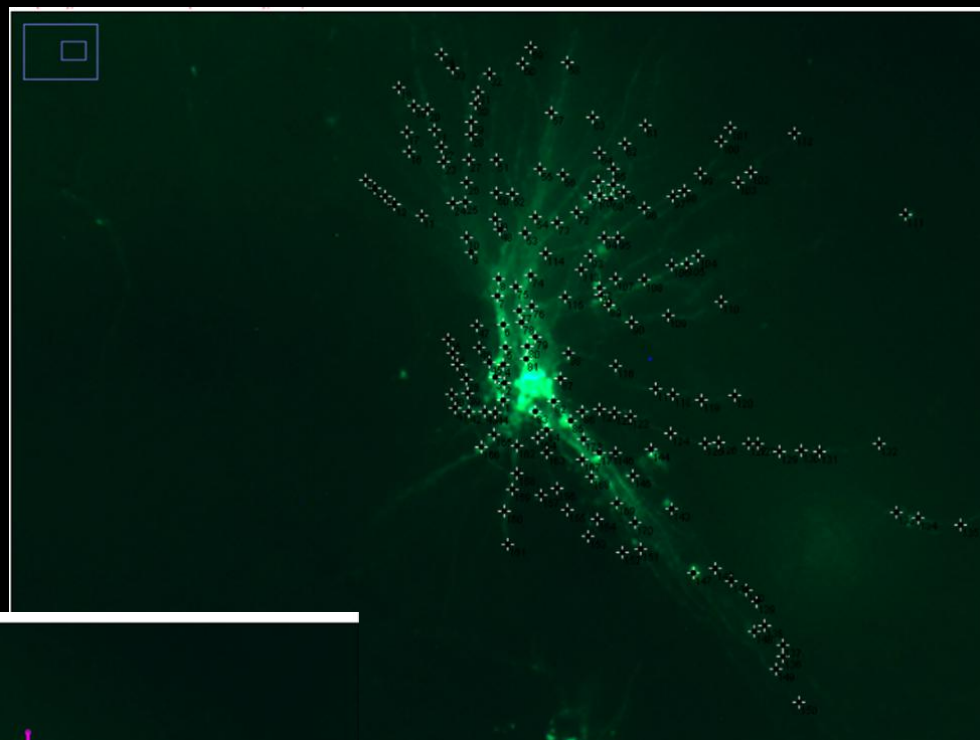
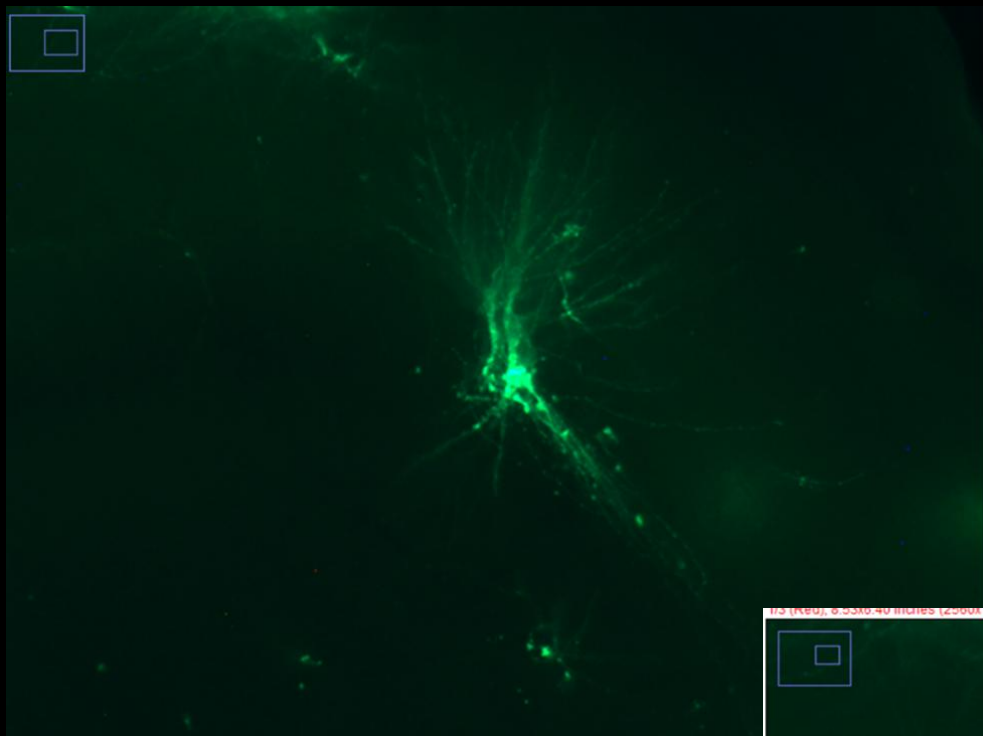
Washing: 5 times with HBSS at 5-min intervals.

Sections were fixed with 4% paraformaldehyde for 30 min.

Washing: 3 times with HBSS at 10-min intervals.

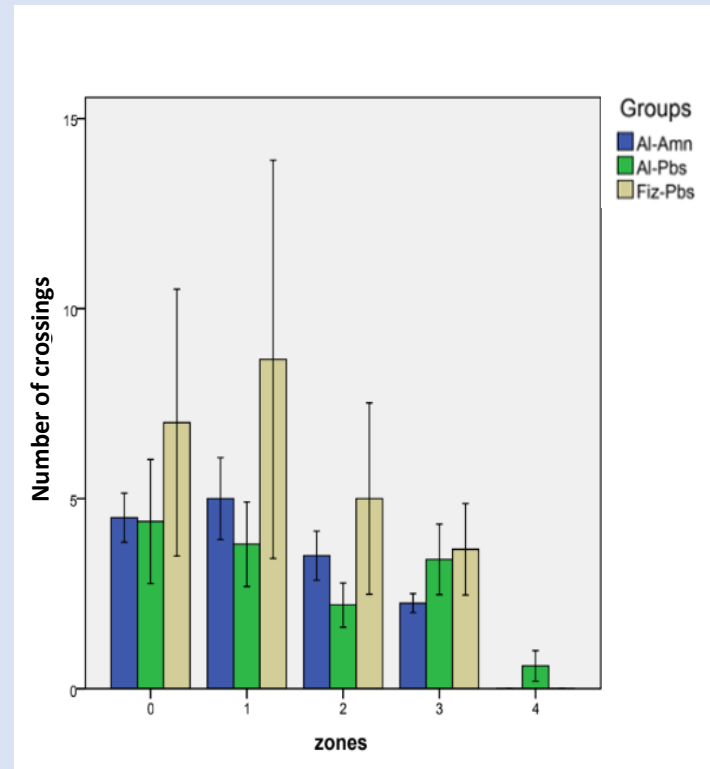
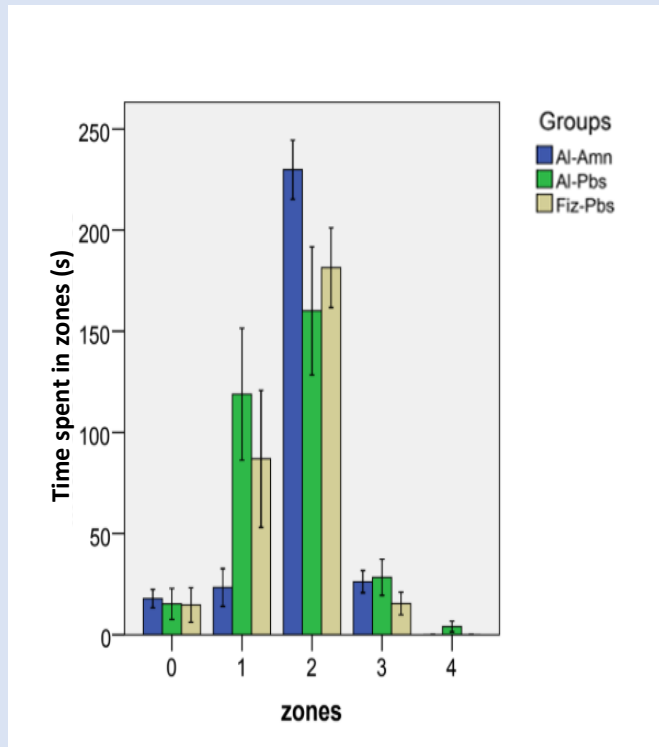
Samples were mounted on slides and immediately examined by fluorescence microscopy. Images were analyzed using ImageJ/Fiji to assess membrane and dendritic morphological parameters separately in the hippocampal CA1, CA3, and DG regions.

The procedure was performed in darkness to prevent photodamage.



Results and Analysis

Assessment of Anxiety-Related Behavior and Risk-Assessment Indices – Elevated Plus Maze Test

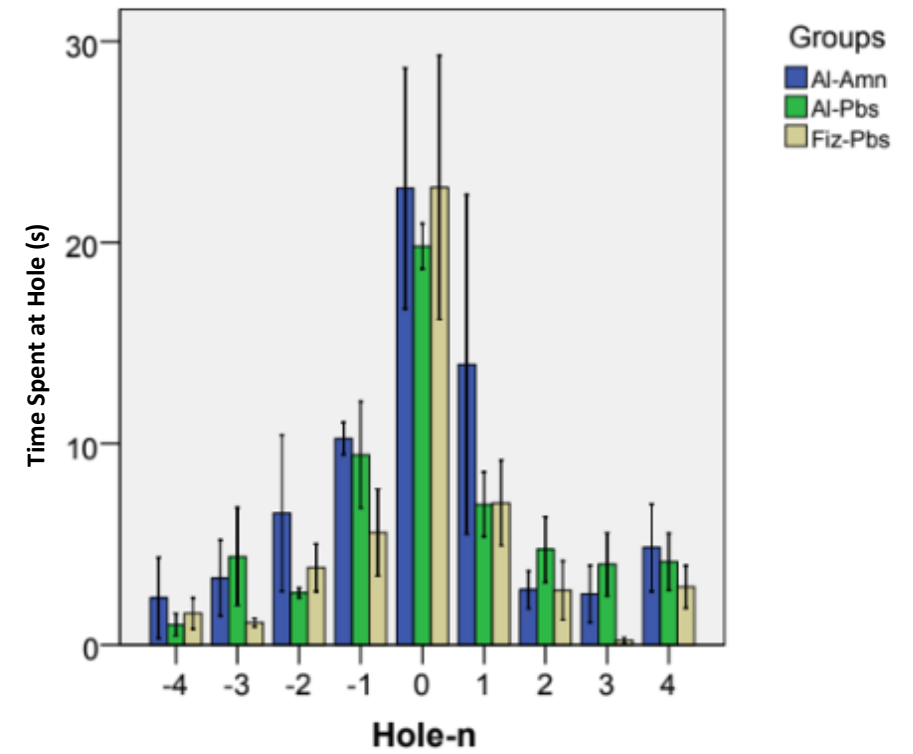
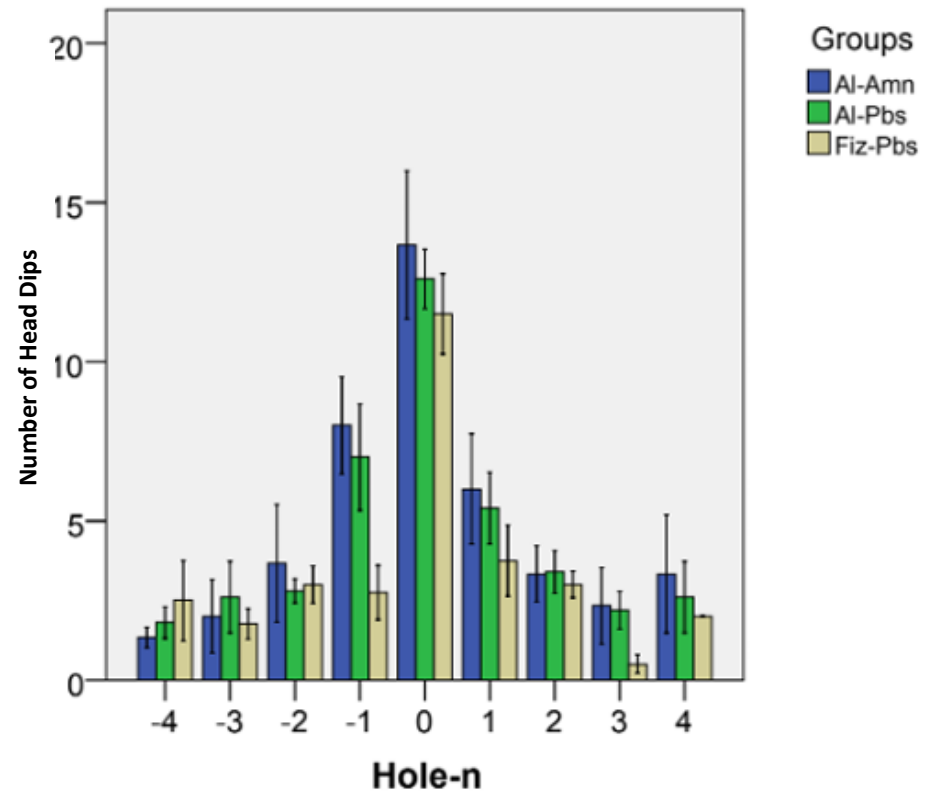


Based on these results, the relatively longer time spent in the open arm by the AL-PBS group may be attributable to impaired risk-assessment ability. Administration of amniotic extract may have contributed to the restoration of the animals' natural defensive responses and behavioral stabilization. (Takeuchi et al., 2011; Messier, 2001).

Barnes Maze Test

Assessment of Cognitive Function, Short-Term, and Long-Term Memory

1 h



Barnes Maze Test

Assessment of Cognitive Function, Short-Term, and Long-Term Memory

1 h

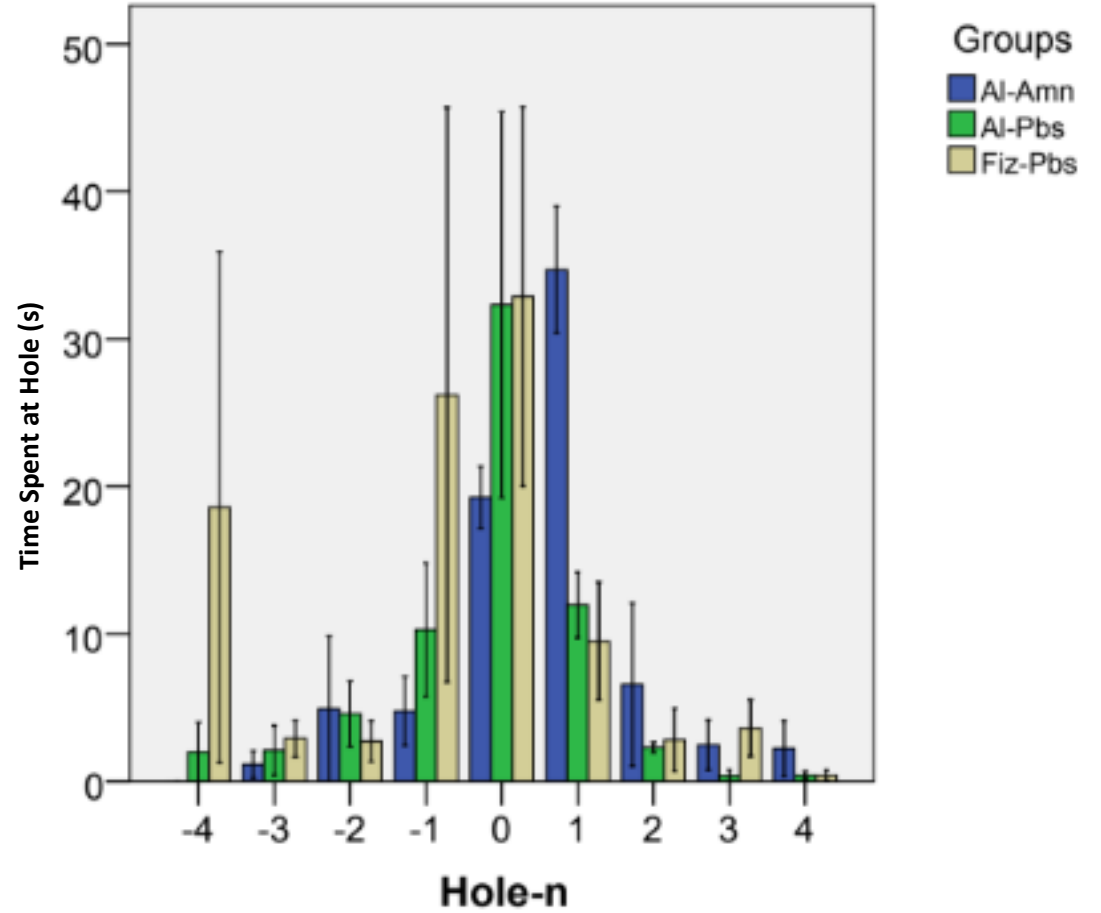
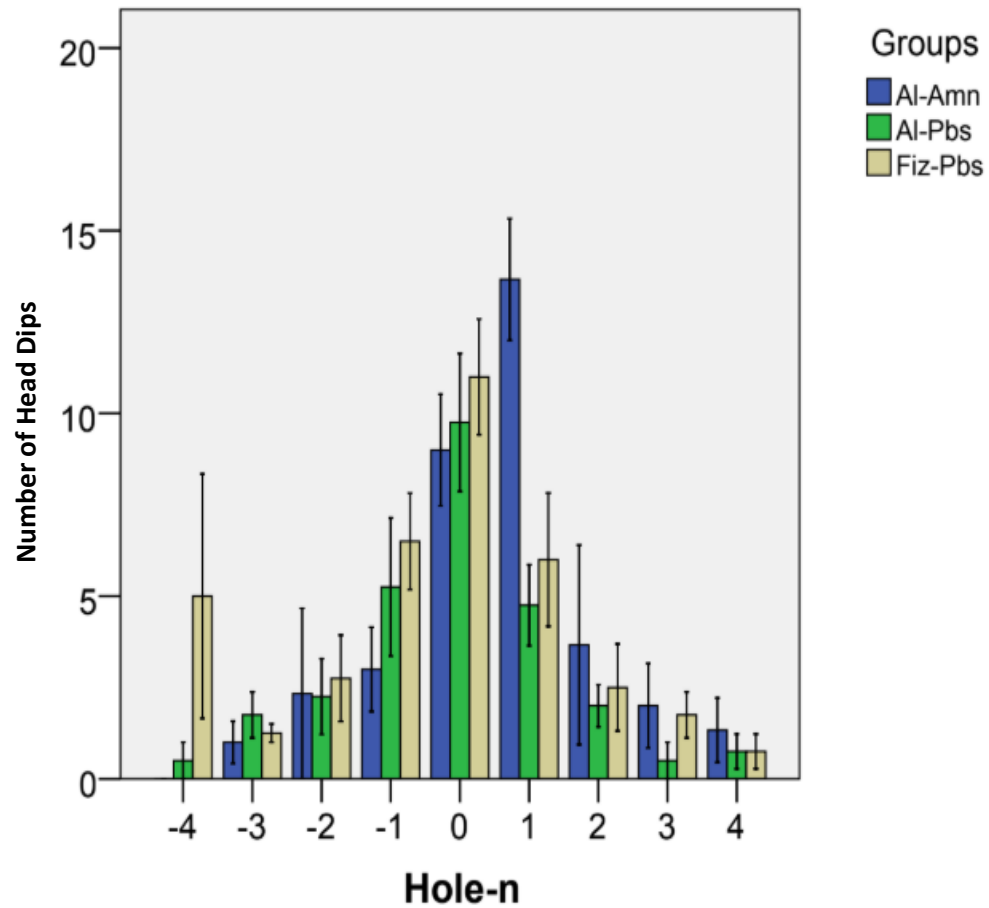
Notably, activity in all three groups was primarily concentrated around the target hole, whereas interest in the peripheral (−4 and +4) holes was low, indicating successful acquisition of the task. Accordingly, the aluminum chloride-induced early-stage AD model did not show a marked impairment of short-term memory at the 1-h interval



Barnes Maze Test

Assessment of Cognitive Function, Short-Term, and Long-Term Memory

24 h



Barnes Maze Test

Assessment of Cognitive Function, Working, and Long-Term Memory

24 h

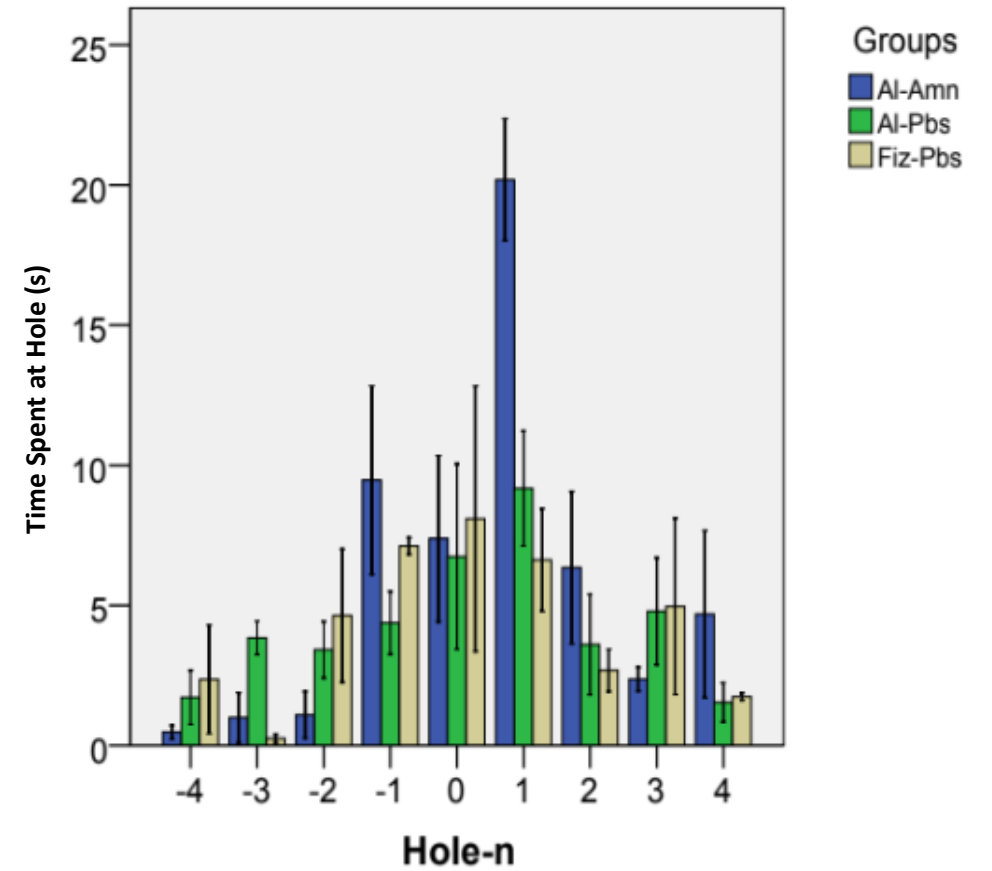
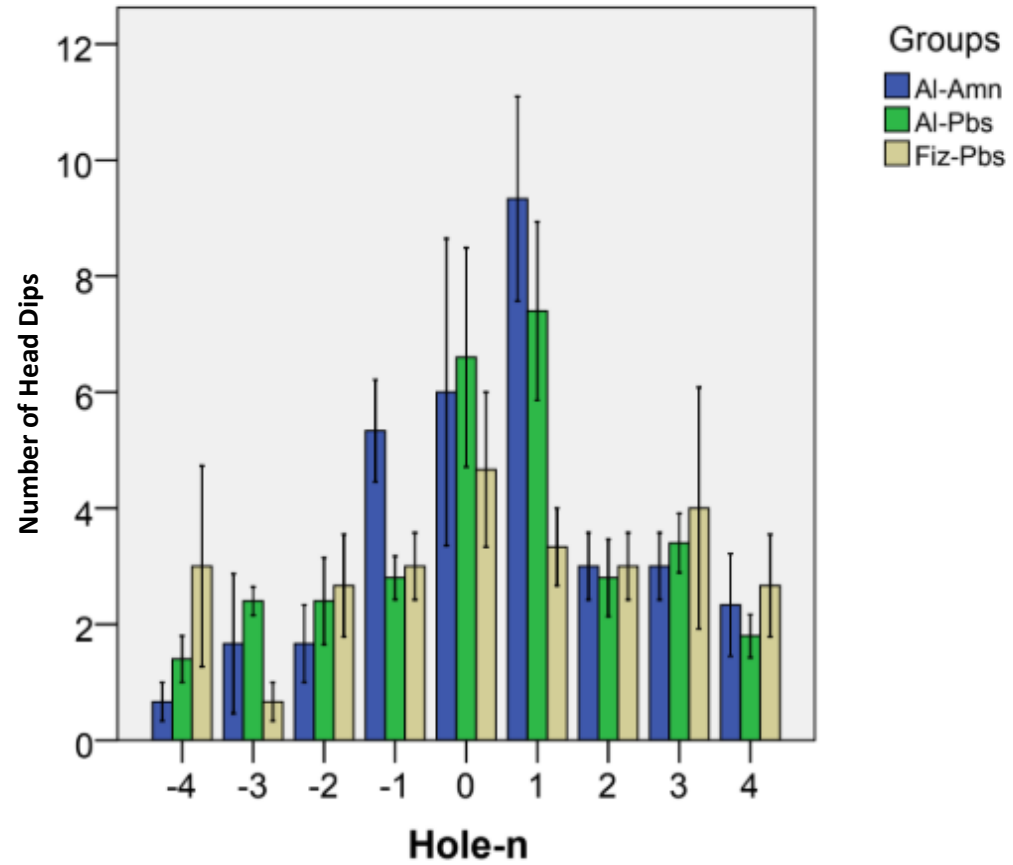
Although the mean values show a trend toward better preservation of spatial memory in the Al-Amn group, substantial overlap of the error bars prevents unambiguous interpretation of these differences.



Barnes Maze Test

Assessment of Cognitive Function, Short-Term, and Long-Term Memory

168 h



Barnes Maze Test

Assessment of Cognitive Function, Short-Term, and Long-Term Memory

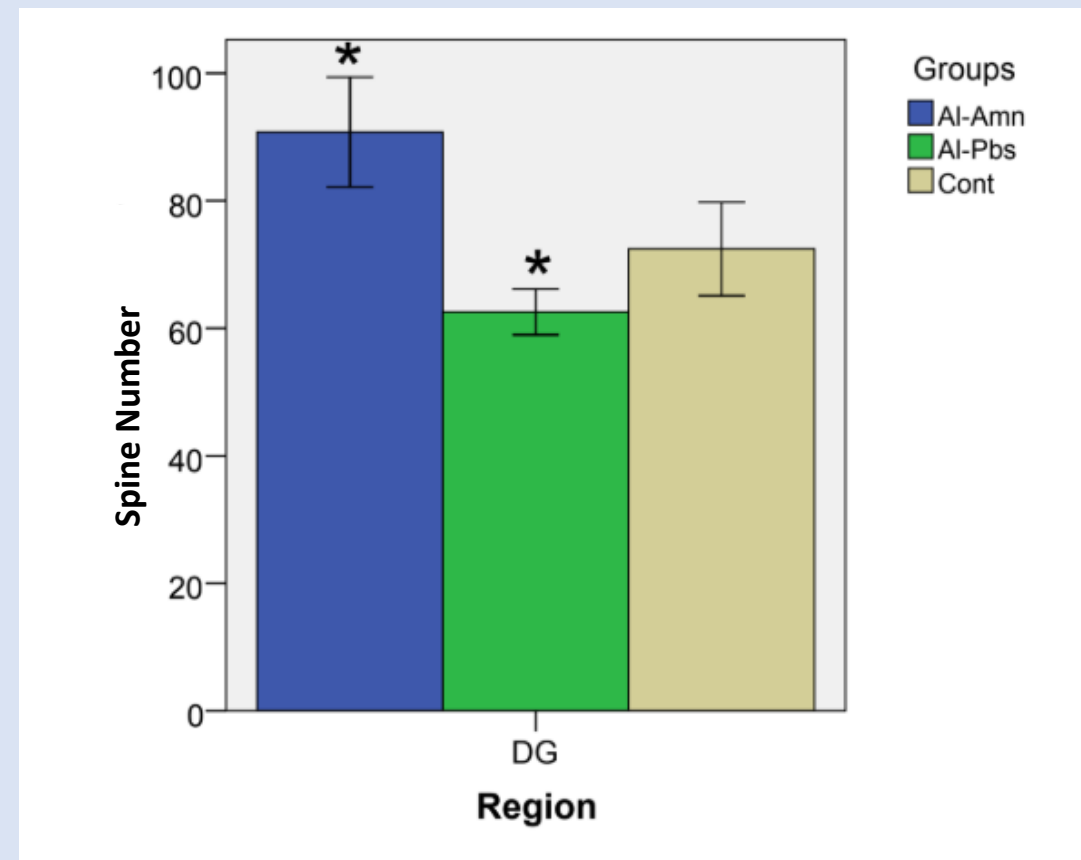
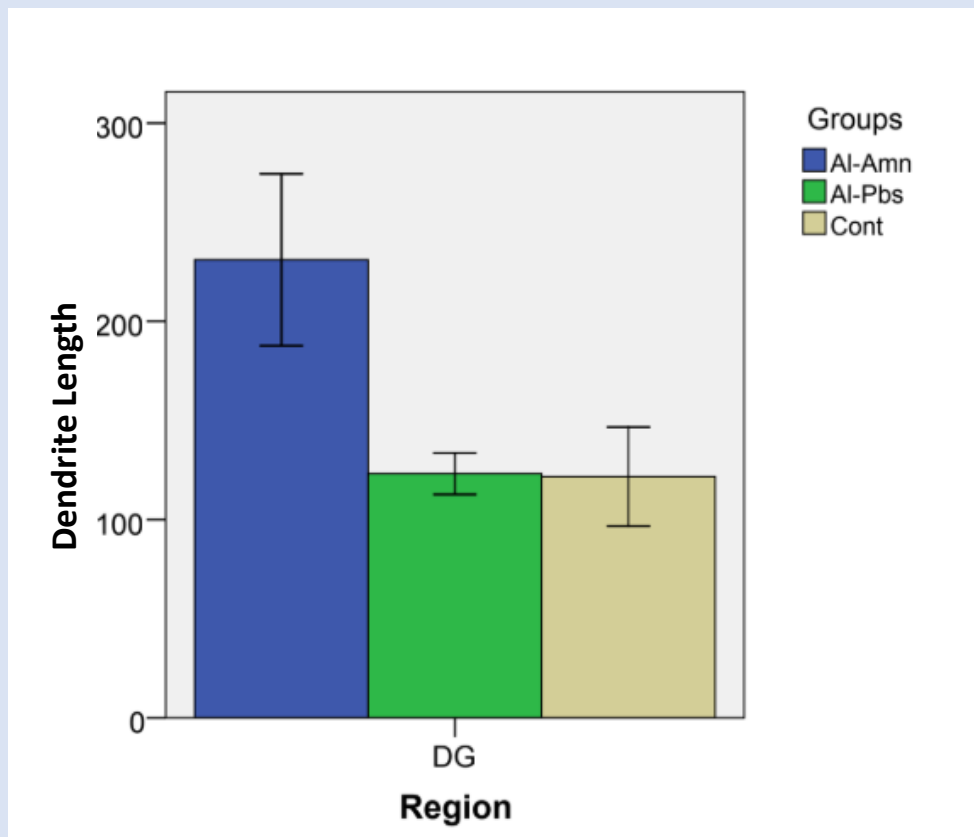
168 h

In the AI-PBS group, activity was distributed more broadly across different holes, whereas in the AI-Amn group, exploratory behavior was more concentrated around the target area. This trend may indicate a potential beneficial effect of amniotic extract on the preservation of spatial memory.

However, partial overlap of the error bars across all groups and high data variability continue to limit interpretation of these differences

Morphological Analysis of Hippocampal Neurons

DG Region – Granule Cells



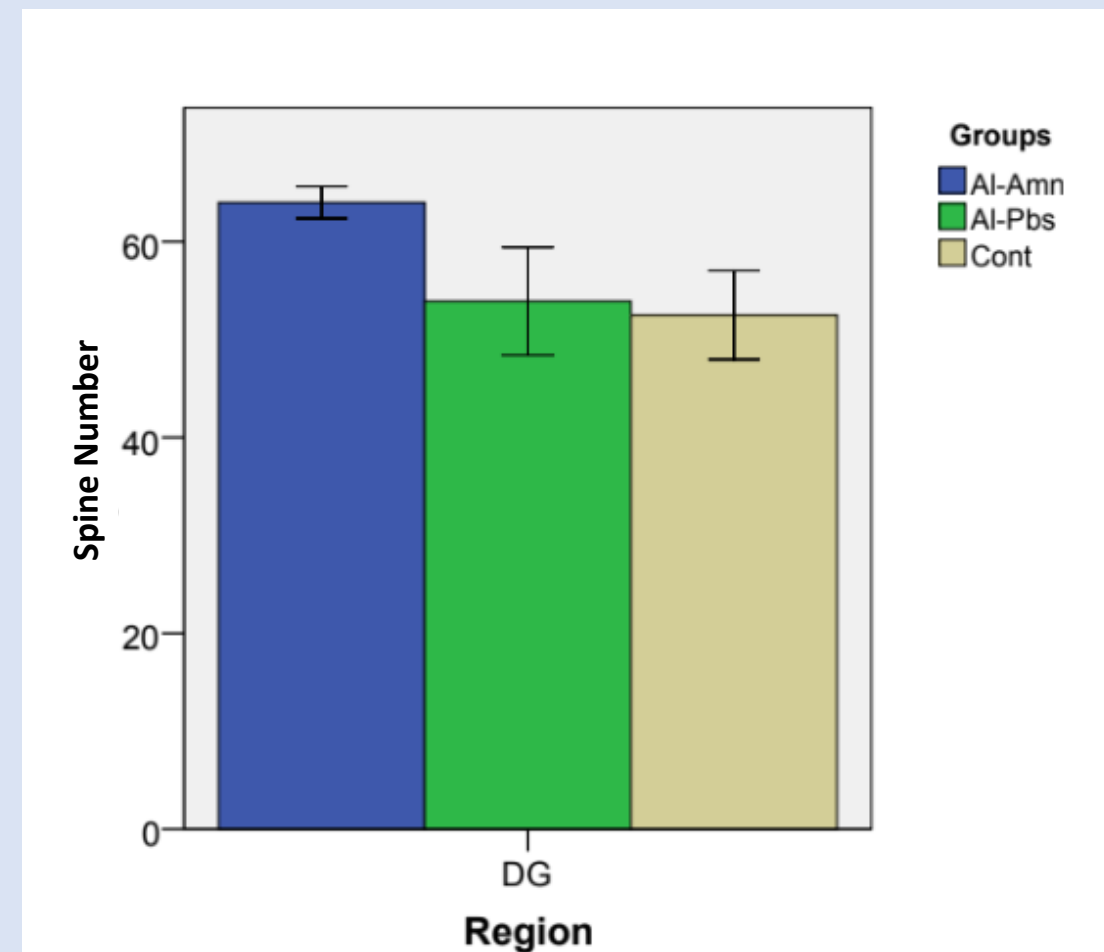
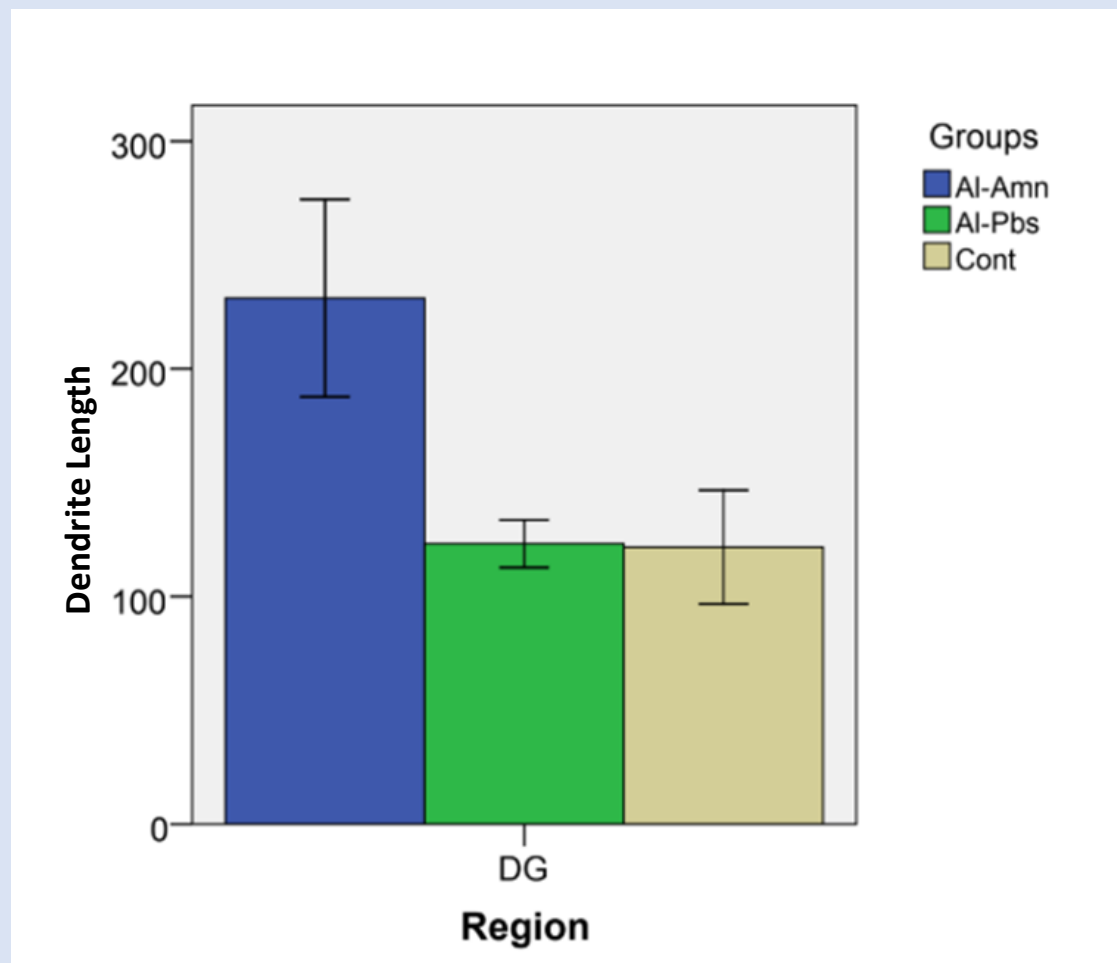
Morphological Analysis of Hippocampal Neurons

DG Region – Granule Cells

An increase in dendritic spine number was observed and was statistically significant, suggesting improved synaptic plasticity. The change in neuronal length was not statistically significant, although an increasing trend was observed.

Morphological Analysis of Hippocampal Neurons

DG Region – Mossy Cells



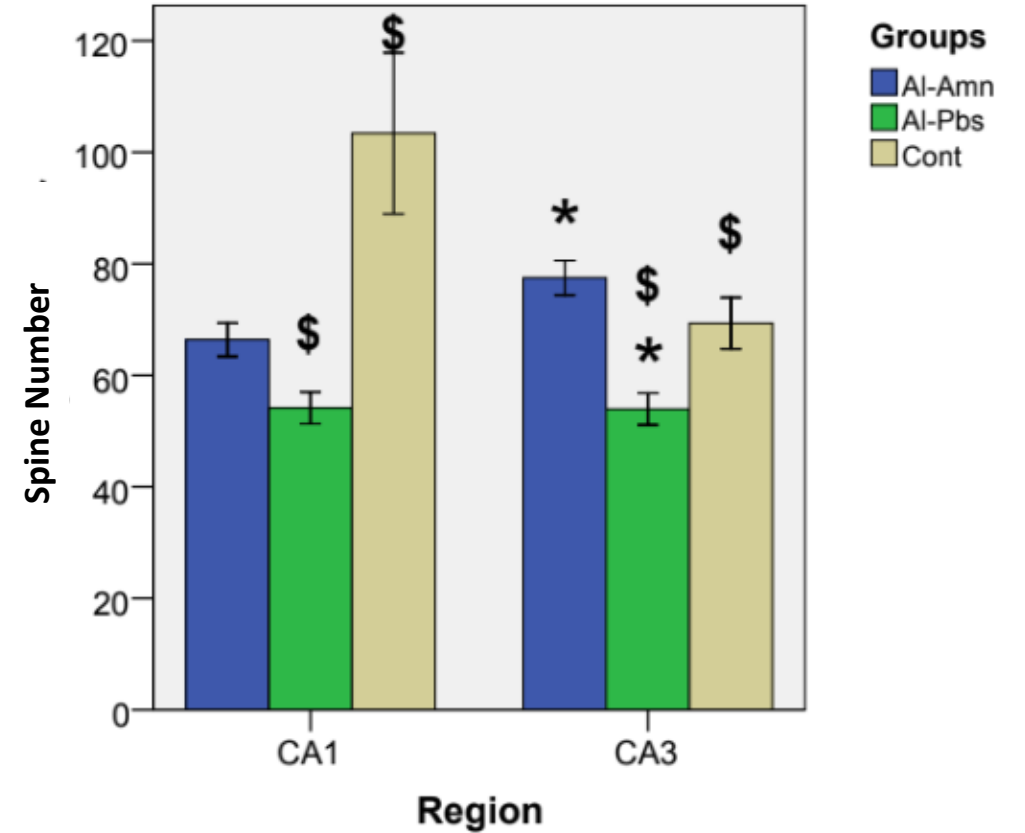
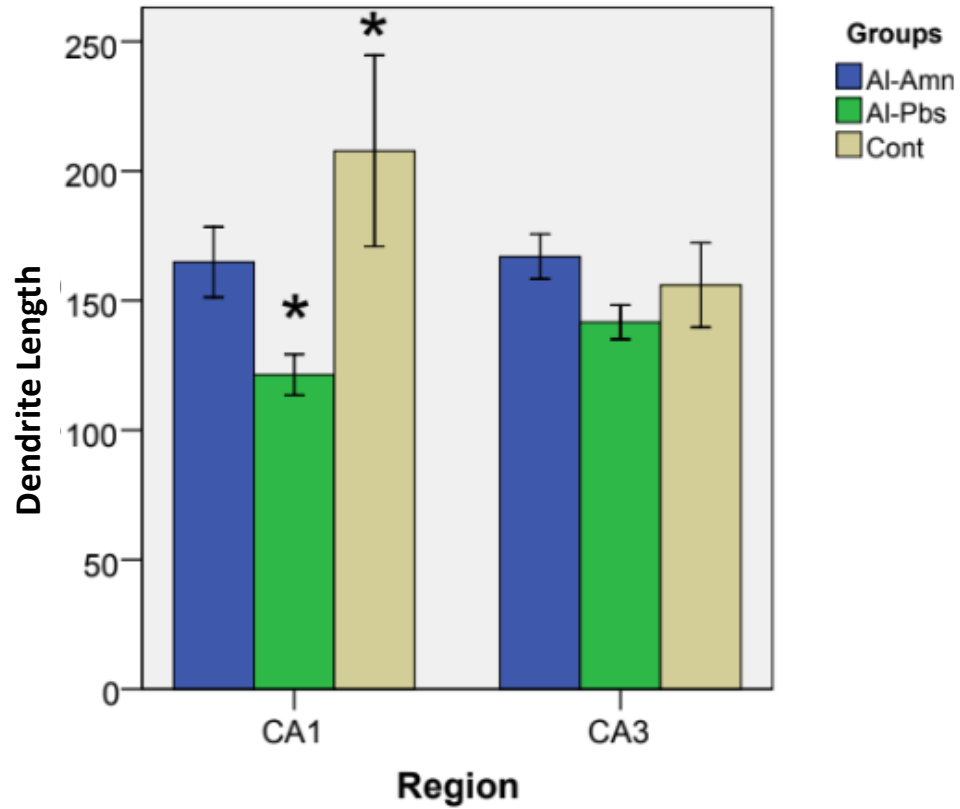
Morphological Analysis of Hippocampal Neurons

DG Region – Mossy Cells

ANOVA of neuronal length and spine number in DG mossy cells showed no statistically significant differences among the groups.

Morphological Analysis of Hippocampal Neurons

CA1 and CA3 Regions – Pyramidal Cells



Morphological Analysis of Hippocampal Neurons

CA1 and CA3 Regions – Pyramidal Neurons

Morphological analysis of the CA1 region showed no apparent effect of amniotic extract on either dendrite length or spine number.

In the CA3 region, the most pronounced change was observed in dendritic spine number. The AL-AMN group showed the highest value, whereas the AL-PBS group showed the lowest.

Conclusion

- Amniotic extract showed an effect in the anxiety-related behavioral test, reflected in restoration of risk-assessment and decision-making abilities in the treated group. This finding is consistent with the increased number of spines in hippocampal granule neurons, which may reflect recovery of their functional properties.
- Although no differences were observed in the short-term (1 h) and intermediate-term (24 h) memory paradigms, in the long-term (1-week) paradigm the treated group maintained a pronounced preference for the target hole and its surrounding area, unlike the other groups. This may indicate a potential positive effect of the treatment on memory and cognitive function. This finding is further supported by increased spine numbers in pyramidal and granule neurons in the treated groups.

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Thank you for your attention!

