SYSTEMATIC CHARACTERIZATION OF A NON-TRANSGENIC $A\beta_{1-42}$ AMYLOIDOSIS MODEL: SYNAPTIC PLASTICITY AND MEMORY DEFICITS IN FEMALE AND MALE MICE

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1. INTRODUCTION

The accumulation of amyloid beta (Aβ) is one of the neuropathological hallmarks of Alzheimer's disease (AD). This accumulation leads to the disruption of the correct neural oscillatory synchronization in the hippocampus and an impairment of learning and memory processes (Palop and Mucke, 2016; Jeremic et al., 2021). Previous research from our group has shown an excitatory/inhibitory imbalance in male mice following intracerebroventricular (*icv.*) injection of $A\beta_{1-42}$ *in vivo* and *in vitro* (Sanchez-Rodriguez et al., 2017, 2020). However, the prevalence of AD is higher in women than men, and the available data from transgenic mice models of amyloidosis in both male and female mice present conflicting information.

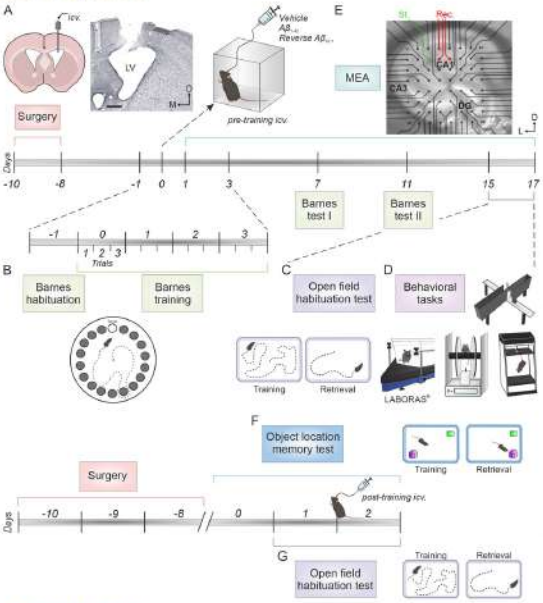
2. OBJECTIVES

Given the lack of data on the early stages of amyloidosis in female mice, the aim was to characterize the early stages of AD in both **male and female mice**:

I. At the behavioral level, by studying the effect of an *icv.* injection of $A\beta_{1-42}$ on **encoding and retrieval** hippocampal-dependent memory in male and female mice.

II. At the synaptic level, by studying the effect of an *icv.* injection of $A\beta_{1-42}$ on synaptic plasticity in male and female mice.

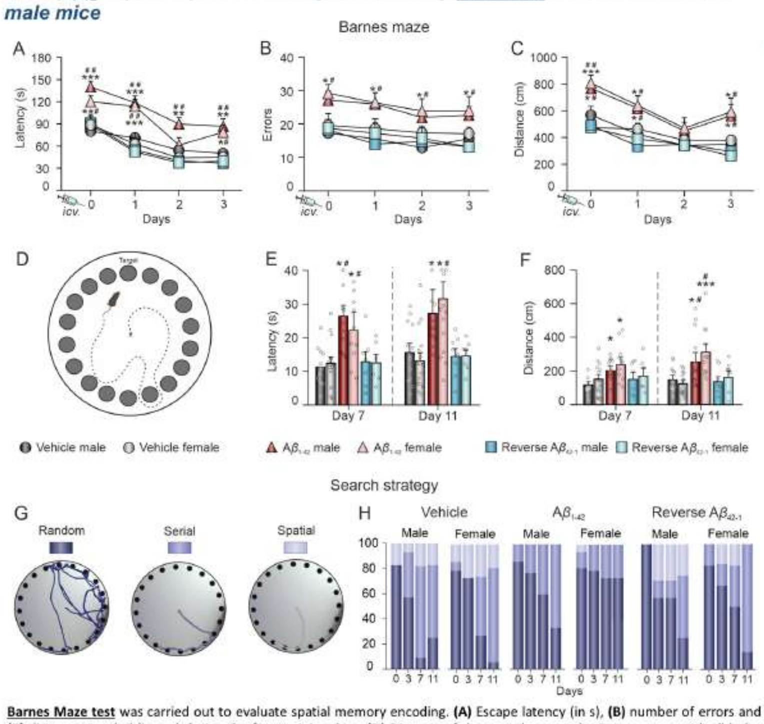
3. METHODS



Female and male C57BL/6 adult mice (12-24 weeks old; 20-30 g) were use. For *icv.* $A\beta_{1-42}$ administration, mice were implanted with a 26-G guide cannula targeting the left ventricle **(A)**. At the behavioral level, both experimental designs were used to assess the effect of Aβ1-42 on memory encoding (icv. injection pre-training) **(B;C)** and retrieval **(F;G)** (icv. injection post-training). **(D)** Between days 15 and 17 post-injection, mice went through a battery of behavioral tests to assess their overall state and spontaneous behaviors. **(E)** To assessment the effect of Aβ1-42 on synaptical level, coronal brain slices (300 µm) containing the dorsal hippocampus were obtained. Field excitatory postsynaptic potentials (fEPSPs) were evoked in the Schafer collateral pathway of the hippocampus and recorded in the stratum radiatum of the CA1 subfield using a multi-electrode array (MEA2100-Mini-System). For LTP induction, a high-frequency stimulation (HFS) protocol was used (five 1-sec-long 100-Hz trains, 30 sec intertrain interval).

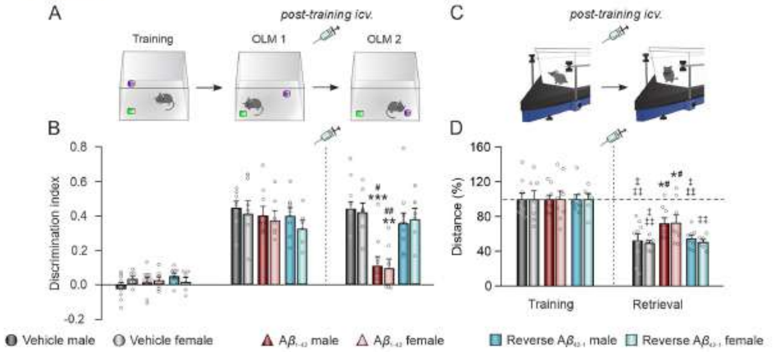
4. RESULTS

4.1. $A\beta_{1-42}$ impairs spatial learning and memory encoding in both female and male mice



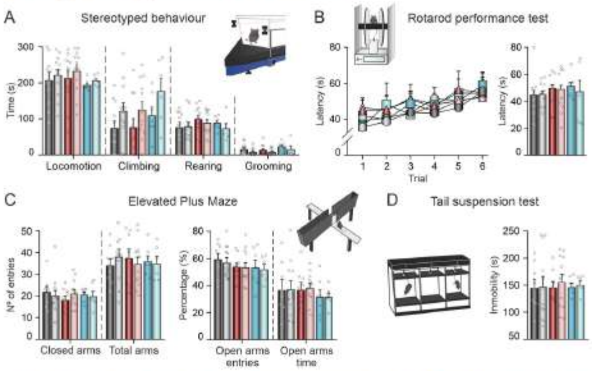
Barnes Maze test was carried out to evaluate spatial memory encoding. **(A)** Escape latency (in s), **(B)** number of errors and **(C)** distance traveled (in cm) during the four training days. **(D)** Diagram of the test phase on the Barnes maze with all holes closed. **(E)** Latency to find the last target hole and **(F)** distance traveled during the two tests. **(G)** Representative traces of the three possible search strategies. **(H)** Ratio of the use of each search strategy for all the experimental groups during training and test sessions. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle of the corresponding sex; # $p < 0.05$, ## $p < 0.01$ vs. reverse control, Aβ42-1, of the corresponding sex.

4.2. $A\beta_{1-42}$ impairs spatial and habituation memory retrieval in both female and male mice



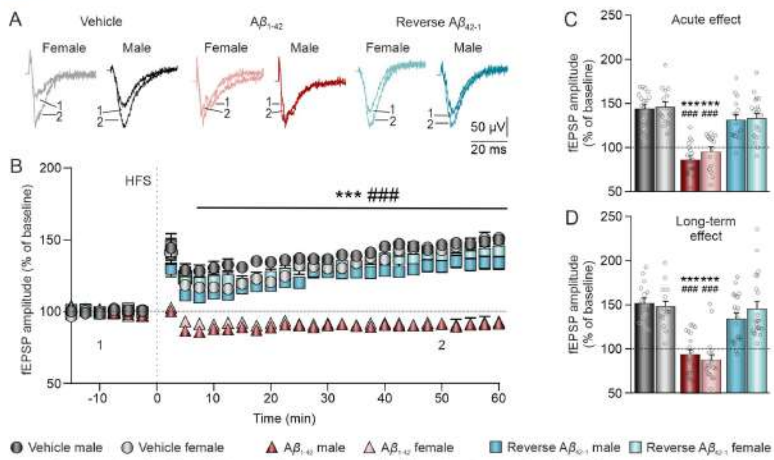
(A) For the **object location memory** (OLM) test, the location of an object was changed between the training and each memory tests. Treatment was administered *icv.* between OLM1 and OLM2 to evaluate memory retrieval. **(B)** Discrimination index for each experimental group in each trial. **(C)** **Open field habituation test** was used, administering the treatment between the training and the retrieval sessions to evaluate the retrieval of exploratory habituation memory. **(D)** Distance traveled during both sessions. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle of the corresponding sex; # $p < 0.05$, ## $p < 0.01$ vs. reverse control, Aβ42-1, of the corresponding sex; ‡ $p < 0.05$, ‡‡ $p < 0.01$. ‡‡‡ $p < 0.001$ vs. training session.

4.3. $A\beta_{1-42}$ administration does not induce alterations in locomotor activity, anxiety, and depression-like behavior



Behavioral tasks to evaluate general health state were carried out on days 15 - 17 post-*icv.* Injection. **(A)** Stereotyped behaviors were assessed measuring the time spent locomotion, climbing, rearing, and grooming. **(B)** Latency to fall off the Rotarod during the six trials (left) and the whole session (right). **(C)** Anxiety levels were assessed by the percentage (%) of entries and time spent on open arms in an elevated plus maze. **(D)** Depression-like behavior was assessed by measuring the immobility time.

4.4. $A\beta_{1-42}$ inhibits ex vivo hippocampal LTP and induces LTD in both female and male



To study **synaptic plasticity**, fEPSPs amplitude was recorded for 15 min as a baseline before using a high-frequency stimulation (HFS) protocol and for 60 min after LTP induction. **(A)** Representative averaged traces of fEPSPs recorded in the CA1 area, collected during the BL (1) and ≈50 min post-HFS (2) **(B)** Time course of LTP evoked in the CA1 area after HFS. Recordings were obtained from day 1 to 17 post-*icv.* Injection **(C-D)** Bars illustrate fEPSPs amplitude of the last 10 min of the recording, to show acute (**C**; 24-48 h post-*icv.* injection) *vs.* long-term (**D**; 3-17 days post-*icv.* injection) effects on LTP. *** $p < 0.001$ *vs.* vehicle of the corresponding sex; ### $p < 0.001$ *vs.* reverse control, $A\beta_{42-1}$, of the corresponding sex.

5. CONCLUSIONS

I. At the behavioral level, a single $A\beta_{1-42}$ *icv.*-injection impairs both spatial and non-associative habituation memory when administered before and after learning, thus affecting both memory encoding and retrieval in female and male mice.

II. At the synaptic level, a single $A\beta_{1-42}$ *icv.*-injection impairs long-term synaptic plasticity in both female and male mice. This effect can be seen both acutely and long-lasting.

These results validate our murine model for the study of acute amyloidosis, which opens the possibility of investigating the early stages of the pathogenesis and treatment of AD in both women and men.

REFERENCES

1. Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci. 2010;13(7):812-8.
2. Jeremic D, Jiménez-Díaz L, Navarro-López JD. Past, present and future of therapeutic strategies against amyloid-β peptides in Alzheimer's disease: a systematic review. Ageing Res Rev. 2021;72:101496.
3. Sánchez-Rodríguez I, ..., Navarro-López JD, Jiménez-Díaz L. Activation of G-protein-gated inwardly rectifying potassium (Kir3/GirK) channels rescues hippocampal functions in a mouse model of early amyloid-β pathology. Sci Rep. 2017;7(1):14658.
4. Sánchez-Rodríguez I, Djebari S,..., Jiménez-Herrera R, Iborra-Lázaro G, et al. Hippocampal long-term synaptic depression and memory deficits induced in early amyloidopathy are prevented by enhancing G-protein-gated inwardly rectifying potassium channel activity. J Neurochem. 2020;153(3):362-76.

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