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Neuroinflammation induced by the peptide amyloid-β (25–35) increase the presence of galectin-3 in astrocytes and microglia and impairs spatial memory

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ABSTRACT

Galectins are animal lectins that bind to β-galactosides, such as lactose and *N*-acetyllactosamine, contained in glycoproteins or glycolipids. Galectin-1 (Gal-1) and Galectin-3 (Gal-3) are involved in pathologies associated with the inflammatory process, cell proliferation, adhesion, migration, and apoptosis. Recent evidence has shown that the administration of Amyloid-β 25–35 ($A\beta_{25–35}$) into the hippocampus of rats increases the inflammatory response that is associated with memory impairment and neurodegeneration. Galectins could participate in the modulation of the neuroinflammation induced by the $A\beta_{25–35}$. The aim of this study was to evaluate the presence of Gal-1 and Gal-3 in the neuroinflammation induced by administration of $A\beta_{25–35}$ into the hippocampus and to examine spatial memory in the Morris water maze. After the administration of $A\beta_{25–35}$, animals were tested for learning and spatial memory in the Morris water maze. Behavioral performance showed that $A\beta_{25–35}$ didn't affect spatial learning but did impair memory, with animals taking longer to find the platform. On the day 32, hippocampus was examined for astrocytes (GFAP), microglia (Iba1), Gal-1 and Gal-3 via immunohistochemical analysis, and the cytokines IL-1β, TNF-α, IFN-γ by ELISA. This study's results showed a significant increase in the expression of Gal-3 in the microglia and astrocytes, while Gal-1 didn't increase in the dorsal hippocampus. The expression of galectins is associated with increased cytokines in the hippocampal formation of $A\beta_{25–35}$ treated rats. These findings suggest that Gal-3 could participate in the inflammation induced by administration of $A\beta_{25–35}$ and could be involved in the neurodegeneration progress and memory impairment.

1. Introduction

The use of the $A\beta_{25–35}$ peptide in animal models has contributed to the understanding of its effects on Aβ toxicity related mechanisms (Götz et al., 2011; Limón et al., 2011; Zussy et al., 2011). The administration of $A\beta_{25–35}$ into the temporal cortex, dorsal hippocampus or intracerebroventricular increases the nitric oxide pathway (Limón et al., 2009; Diaz et al., 2011), oxidative stress (Pérez-Severiano et al., 2004; Zussy et al., 2011), membrane lipid peroxidation (Zussy et al., 2013), glycosidic patterns changes (Limón et al., 2011; Lozano et al., 2017; Ramos-Martinez et al., 2018) and inflammation (Diaz et al., 2012), processes which may contribute to synaptic dysfunction and neuronal death. All these mechanisms, mainly the inflammation could be associated with a lesion in the dorsal hippocampal tissue, which is of great importance for spatial memory. The inflammatory response in the neurodegenerative process is unclear (Amor et al., 2010; Glass et al.,

Abbreviations: Gal-1, Galectin-1; Gal-3, Galectin-3; $A\beta_{25–35}$, Amyloid-β 25–35; CRD, Carbohydrate-recognition domain; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium binding adaptor molecule 1; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor alpha; IFN-γ, interferon γ; IL-1β, interleukin-1 beta; ELISA, enzyme-linked immunosorbent assay; LTM, long-term memory; MWM, Morris water maze; BSA, bovine serum albumin; PBS, phosphate buffered saline solution; DG, dentate gyrus.

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2010), leading to the question as, to whether inflammation has a causal role in disease pathogenesis. The inflammatory response is a process which involves the sequential activation of signalling pathways, leading to the production of both pro- and anti-inflammatory mediators (Glass et al., 2010). The immune system comprises the various glycan-binding proteins or lectins, including C-type lectins, siglecs, and galectins (Vasta et al., 2012). Understanding the cellular and molecular mechanisms that regulate the interactions between the immune system and the nervous system might be key to the prevention or delay of most late-onset central nervous system (CNS) diseases.

Galectins are a family of animal lectins with an affinity for β-galactosides defined by shared consensus amino acid sequences in the carbohydrate recognition domain (CRD) and an affinity for β-galactosides displayed on both N- and O-glycans on cell surface glycoconjugates (Liu and Rabinovich, 2005; Di Lella et al., 2011; Thiemann and Baum, 2016; Gabius and Roth, 2017). Galectins play an important role in the immune response via the recognition of the glycans, either in an extracellular or intracellular fashion (Almkvist and Karlsson, 2002; Rabinovich et al., 2002; Liu et al., 2012). By means of this activity, they promote cell growth, affect cell survival, modulate cell adhesion, and induce cell migration (Liu and Rabinovich, 2005; Di Lella et al., 2011; Vasta et al., 2012). Galectins through these mechanisms modulate a wide variety of immunologic responses while its expression increases in glial cells because of various neuroinflammatory stimuli (Liu and Rabinovich, 2005; Yang et al., 2008; Di Lella et al., 2011; Thiemann and Baum, 2016).

The two most extensively studied galectins are Galectin-1 (Gal-1) and Galectin-3 (Gal-3). Gal-1 is a proto-type galectin, comprising a single CRD that can dimerize. Gal-3 is a chimeric galectin, containing a C-terminal CRD and an extended N-terminal domain of about 120–160 amino acids that enables the molecule to form trimers or pentamers, giving galectin-3 CRDs relatively large space across which glycan ligands can be bound (Di Lella et al., 2011; Rabinovich and Croci, 2012; Thiemann and Baum, 2016). Galectins exhibit a wide array of functions in neuroinflammation, a widespread reaction that involves astrocytes and microglia (Starossom et al., 2012; Sirko et al., 2015). Gal-1 exerts activity in astrocytes and microglia, regulates proliferation and plays an important role after nervous system injuries (Elola et al., 2005; Camby et al., 2006; Starossom et al., 2012). Gal-1 is up-regulated in activated astrocytes to inhibit the proliferation in a dose-dependent manner, as well as attenuating, down-regulating the astrogliosis and increasing the production of brain-derived neurotrophic factor (BDNF), which is associated with the prevention of neuronal loss (Sasaki et al., 2004; Qu et al., 2011). In the central nervous system, Gal-1 has been reported to enhance the proliferation of endogenous neural stem cells in the developing brain and is mainly restricted to the subventricular zone (SVZ) and subgranular zone (SGZ), with the ability to integrate new neurons (Sakaguchi et al., 2006; Ishibashi et al., 2007; Imaizumi et al., 2011).

Gal-3 plays an important role in the inflammatory response (Henderson and Sethi, 2009), while its expression increases in microglial cells upon various neuroinflammatory stimuli, the ischemic injury process (Satoh et al., 2011a, 2011b; Lalancette-Hébert et al., 2012; Wesley et al., 2013; Burguillos et al., 2015) and mouse model of experimental autoimmune diseases (Lalancette-Hébert et al., 2007; Mok et al., 2007; Satoh et al., 2011a; Boza-Serrano et al., 2014). Gal-3 can be found in the cytoplasm, nucleus and membranes (Shimura et al., 2004). It functions as a mediator contributing to phagocytosis, cell proliferation and the regulation of apoptosis (Liu et al., 2002; Kim et al., 2003; Nakahara et al., 2005; Pasquini et al., 2011), under the stimuli of proinflammatory molecules, such as lipopolysaccharide (LPS) (Li et al., 2004) and interferon γ (IFN-γ) (Jeon et al., 2010). The Gal-3 expression has been located predominantly in Iba1-positive microglia and GFAP-positive astrocytes in the hippocampus (Doverhang et al., 2010; Satoh et al., 2011a; Yang et al., 2012). Gal-3 deficient mice are protected from injury, particularly in the hippocampus and striatum, suggesting that Gal-3 exerts its effects by modulating the inflammatory response in brain injury models (Doverhang et al., 2010).

In vivo studies with $A\beta_{25–35}$ suggest that inflammation plays a decisive role in neurodegeneration since it has been shown that this peptide induces the astrocytes and microglia activity. As activated astrocytes and microglia are thought to contribute to neurodegeneration, their number correlates to the damage sustained during the neurodegenerative process. The aim of the present work was to evaluate the expression of Gal-1 and Gal-3 in the neuroinflammation induced by the administration of the $A\beta_{25–35}$ peptide into the hippocampus. This study demonstrates that Gal-3 expression increases in astrocytes and microglia associated to the neuroinflammatory process induced by $A\beta_{25–35}$, so we propose it acts as an endogenous regulator in inflammatory conditions. These findings may contribute to the understanding of how Gal-1 and Gal-3 act as modulators under neuroinflammation conditions induced by the $A\beta_{25–35}$ peptide.

2. Materials and methods

2.1. Animals

Four-month old adult male Wistar rats (300–350 g, $n = 45$) were obtained from the *Claude Bernard* animal facilities at the *Benemérita Universidad Autónoma de Puebla*. The animals were housed in groups of five in transparent polycarbonate home cages measured 68 x 27 x 20 cm (Tecniplast, catalog 01288-1F) with stainless steel rack and feeder (Tecniplast, catalog 01288-5F), access to water and food ad libitum, at constant temperature (22 ± 2 °C) and humidity conditions, in a 12 h:12 h light-dark cycles (light turned on at 8 AM). They were then assigned randomly to groups. All procedures described in this study were established in accordance with the Official Mexican Standard (NOM-062-ZOO-1999) *Guide for Care and Use of Laboratory Animals*, the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL), and the National Institute of Health's Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and suffering ($n = 15$ per group).

2.2. Preparation of aggregated $A\beta_{25–35}$

The $A\beta_{25–35}$ (Sigma Chemical Co., St. Louis, MO USA) was prepared from 1 mM stock solution. It was dissolved in isotonic saline solution (sodium chloride 0.9%) in order to obtain a final concentration of 0.1 μg/μL. The $A\beta_{25–35}$ solution was then incubated at 37 °C for 36 h before use in order to facilitate its aggregation as described earlier (Maurice et al., 1998; Stepanichev et al., 2008; Ortega et al., 2014). The isotonic saline solution was subjected to the same conditions to establish the control group.

2.3. Stereotaxic surgery

Animals were randomly assigned to three groups: Intact (n = 15), Vehicle (n = 15) and $A\beta_{25–35}$ (n = 15), the latter two for stereotaxic surgery. The coordinates to administrate into the CA1 subfield of the hippocampus were as follows: anteroposterior (AP) -4.0 mm from bregma; lateral (L), ± 2.8 mm from the midline; ventral (V), −2.4 mm below the midline. Coordinates were determined using the bregma as the point of reference (Paxinos and Watson, 1998). Animals were anesthetized with ketamine/xylazine (75:10 mg/kg i.p.) and placed on the stereotaxic surgery frame (Stereotaxic Instruments, Stoelting Co., Illinois, Wood Dale, USA). Each animal was bilaterally injected into the CA1 subfield of the hippocampus with 1 μL of $A\beta_{25–35}$ [0.1 μg/μL] solution or vehicle, infused at a rate of 0.2 μL/min using a syringe (Hamilton) and an infusion pump (Nanomite, Harvard Apparatus). After administration, a period of 5 min was left to elapse in order to the solution to diffuse, on completion of which, the syringe was removed. Both injections were carried out using conventional surgical procedures, after which all animals were returned to their cages and received

proper post-operative attention until full recovery.

2.4. Morris water-maze spatial-reference task

The Morris water maze (MWM) was run as previously described (Morris, 1984). The circular water pool (150 cm diameter and 80 cm high) was in a large test room, in which several cues were placed external to the maze. The pool was filled with water (22 ± 1 °C) to a height of 42 cm, then was divided into four conceptual quadrants and each point was designed as a starting position (N, S, E or W). A platform (20 cm diameter and 40 cm high) was set inside and kept in a constant position in the middle of SE quadrant, equidistant from the center and edge of the pool, submerged 2 cm below the water surface. The water was dyed with 0.1% white titanium oxide (TiO_2) in order to hide the platform. In every trial, rats were placed in the water facing the wall of the water pool, at one of the four possible starting locations. The order of the starting points was varied every day with no given sequence repeated on acquisition phase days. Rats underwent 4 trials per day at 50 min intervals, a procedure which was repeated for 5 training days. Each rat was given 90 s to search for the platform, to which it was gently guided if it failed to do so independently and was left to remain there for 30 s before being returned to its home cage. In the subsequent trials, if the rat was unable to find the platform within 90 s, the training session was finished and the maximum score of 90 s was assigned.

The memory test was undertaken 17 and 31 days after the administration of the $A\beta_{25–35}$ peptide, with all animals undergoing only two trials, as described above, but with the platform removed. Learning and memory trials were recorded using a video camera (Sony DCR-SR85) placed above the center of the pool. The variables measured were the time taken to find the platform (or the time taken to cross the site of the platform) and the time spent on the platform quadrant (or the quadrant in which the platform was located during training sessions).

2.5. Histological analysis

Next day after behavioral tests, rats were euthanized ($n = 6$, per group) by pentobarbital overdose. Animals were perfused intracardially first with 100–200 mL of isotonic saline solution and then with 100–200 mL of 4% paraformaldehyde in phosphate buffered saline solution (PBS) pH 7.4, 0.1 M. Immediately, brains were removed and post-fixed in the same fixative solution for 48 h. Subsequently, 40-μm thick coronal slices were taken from each brain at the level of the dorsal hippocampus, approximately −2.8 to −4.3 mm from the bregma using a vibratome (Leica VT1000 S), and then stored in a 10% sucrose solution until immunostaining procedures were carried out.

2.6. Immunohistochemistry and colocalization of GFAP, Iba1, Galectin-1 and Galectin-3

Immunostaining method was carried out on 40 μm sections taken from the dorsal hippocampus. The labeling was performed on free floating sections, that were in PBS containing 0.2% Triton X-100, after which the nonspecific binding sites were blocked and the tissues permeated with 2% bovine serum albumin (BSA, Sigma-Aldrich) in 0.2% Triton X-100 in PBS. The slices were incubated overnight at 4 °C with either primary antibody anti-GFAP (polyclonal rabbit; 1:250 dilution, Dako) in order to mark the astrocytes, or primary antibody anti-Iba1 (polyclonal rabbit; 1:500 dilution, Wako) in order to mark microglia. Afterwards, slices were incubated for 2 h at room temperature with rhodamine-labeled secondary antibody (polyclonal goat anti-mouse IgG, 1:200 dilution, Jackson ImmunoResearch) and, after being rinsed in PBS for 10 min, the nonspecific binding sites were blocked again with 2% BSA in PBS. The slices were incubated for 2 h at room temperature with primary antibody anti-Gal-1 (polyclonal mouse, 1:200 dilution, Santa Cruz Biotechnology, sc-28,248) or anti-Gal-3 (monoclonal mouse, 1:200 dilution, Santa Cruz Biotechnology, sc-32,790). The slices were incubated with a 1:250 dilution of fluorescein isothiocyanate (FITC)-labeled secondary antibody (polyclonal goat anti-mouse IgG Jackson ImmunoResearch) and then incubated with DAPI (1:10000 dilution, Sigma-Aldrich) for 20 min at room temperature and mounted on gelatin-subbed glass slides. They were then covered with Mowiol-DABCO resin (Sigma-Aldrich) and kept in darkness for 3–4 days. The immunostained cells were observed with a fluorescence microscope (Leica DM1000 LED) coupled to a mercury lamp and a 40× objective (Leica). DAPI was observed with blue filter to evaluate cell nuclei and determine the structure of the subfield. Gal-1 and Gal-3 were observed with green filter, and GFAP and Iba1 with red filter. The photomicrographs of the immunostained neurons in the CA1 of the hippocampus and dentate gyrus (DG) were evaluated in the dorsal hippocampal formation approximately −2.8 to −4.5 mm from the bregma, according to the Paxinos and Watson (1998). Tissue sections were observed with a DM1000 Leica microscope (400×) and digitized with a ProgRes C14plus camera and Progres Gryphax software. The positive cells were quantified using an ImageJ image-analysis system (National Institutes of Health, USA), in order to identify the immunoreactive cells per area (0.112 mm^2). The mean number of cells was calculated on 6 animals/treatment using 8 to 10 sections of dorsal hippocampus per animal, and then presented as the number of stained nuclei (mean ± SEM).

2.7. ELISA

Once behavioral experiments had been concluded, rats were decapitated ($n = 5$, per group) on the 32nd day after the administration of the $A\beta_{25–35}$ solution and their brains were immediately removed, washed in ice cold isotonic saline solution, and the dorsal hippocampus (3.5 mm × 4.5 mm × 3 mm) was then dissected. The extracted tissue from the dorsal hippocampus was homogenized in 1 mL of lysis buffer. The homogenate was centrifuged at 12,500*g* (4 °C) for 20 min, the supernatant was obtained and stored at −70 °C, after which it was used for protein and proinflammatory cytokine measurements. Protein concentration in the supernatant was measured by the Bradford protein assay. The concentration of IL-1β, TNF-α and IFN-γ in the homogenates of hippocampus was determined via sandwich immunoassay procedure, performed according to the manufacturer's protocol (Quantikine ELISA Rat IL-1β, TNF-α and IFN-γ, R&D Systems, USA). The lowest detection limits of these ELISA are in the range of 5 to 10 pg/mL.

2.8. Western blot analysis

Randomly chosen animals ($n = 4$, per group) were euthanized. The dorsal hippocampus (3.5 mm × 4.5 mm × 3 mm) was then dissected and tissue was homogenized in RIPA Lysis Buffer with a protease inhibitor cocktail (Sigma-Aldrich). The homogenates were centrifuged at 12,500*g* (4 °C) for 15 min, the supernatants transferred, aliquoted and frozen on dry ice. Protein concentration in the supernatant was measured by the Bradford protein assay. Samples containing 20 μg of total protein were loaded on 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto a PVDF membrane. After transferring to membrane, the nonspecific binding was blocked by Tris-buffered saline (TBS) containing 5% non-fat dry milk. The blots were then incubated with primary antibodies diluted in blocking buffer overnight at 4 °C. The following antibodies were used for western blot analysis: polyclonal mouse anti-Gal-1 (1:200 dilution, Santa Cruz Biotechnology, sc-28,248), monoclonal goat anti-Gal-3 (1:200 dilution, Santa Cruz Biotechnology, sc-32,790), monoclonal rabbit anti-β-actin (1:1000 dilution, Millipore). After washing the membranes twice with TBS, blots were incubated with corresponding goat anti-mouse horseradish peroxidase-conjugated IgG (1:3000 dilution, Millipore) at 23 ± 2 °C for 1 h, then visualized with Immobilon Western chemiluminescent HRP substrate (Millipore). The membranes were scanned and resulting digital images were analyzed by software ImageJ.

2.9. Statistical analysis

All results are presented as the mean ± SEM of each group. A confidence interval of 95% and p value < .05 were considered statistically significant. Each physiological variable was analyzed to determine whether there were significant differences. Learning data analyzed were the latency to scape and the number of crossing on the target area, using a Two-way analysis of variance (ANOVA) of repeated measures, followed by Bonferroni post-test. In memory trial, the time taken to find the platform and the time spent on the platform quadrant were analyzed with a One-way ANOVA test followed by Bonferroni post-test. The number of GFAP, Iba1, Gal-1 and Gal-3 positive cells was analyzed using a One-way ANOVA followed by Bonferroni multiple comparison post-test. The analysis of cytokines IL-1β, TNF-α and IFN-γ were conducted using sandwich ELISA, with the cytokine expressed in μg/mL according to the Quantikine ELISA Rat assay procedure and analyzed using a One-way ANOVA and Bonferroni post-test. For Gal-1 and Gal-3 western blot images, ImageJ software was used for densitometric analysis and results were analyzed using a One-way ANOVA and Bonferroni post-test.

3. Results

3.1. The $A\beta_{25–35}$ peptide impairs spatial memory retrieval

As a first approach to understanding the role of the inflammatory response induced by the $A\beta_{25–35}$ peptide in spatial learning and memory, we used a sequence of experiments to demonstrate this hypothesis. Fig. 1 shows the schematic of sequences occurring after the administration of the $A\beta_{25–35}$ peptide [0.1 μg/1 μL] in the CA1 subfield of the hippocampus. The behavioral test was evaluated in the MWM from days 6–10 for the learning test, and on days 17 and 31 for the memory test. The animals were euthanized on Day 32, after which the dorsal hippocampus was analyzed for the expression of Gal-1 and Gal-3 by western blot; GFAP (astrocytes), Iba1 (microglia), Gal-1 and Gal-3 via either immunohistochemical examination; and ELISA for the cytokines IL-1β, TNF-α and IFN-γ.

The behavioral testing of the animals in this study was conducted from the sixth to the tenth day after the administration of $A\beta_{25–35}$ in the CA1 of the hippocampus. The latency to escape data for the spatial learning test in the MWM showed no differences between the $A\beta_{25–35}$ group, the vehicle group and the intact group in terms of the acquisition of information (Two-way ANOVA, $p > .05$) (Fig. 2A). The upper section of Fig. 2B shows the navigation paths taken by rats during localization of the escape platform in the learning test for the intact, vehicle and $A\beta_{25–35}$ groups. The number of crossings on the target quadrant reflects the closeness of the animals to the platform during spatial acquisition, and showed a decreased on the Day 7, for the vehicle group only (1.9 ± 0.16) compared with the intact group (2.7 ± 0.3) (One-way ANOVA, F (5,114) = 18.33, $p < .001$) (Fig. 2B). The time spent in the target quadrant (Supplement Fig. 1A) showed differences between the $A\beta_{25–35}$ group (14.5 ± 0.5) and the intact group (18.4 ± 1.6) on the Day 6 and differences between the vehicle group (7.1 ± 0.6) and the intact group (11.6 ± 1) on the Day 7 (Two-way ANOVA, F (4,48) = 2.32, p > .05) (Fig. 2A). The swimming speed (Supplement Fig. 1B) no showed differences between the $A\beta_{25–35}$, vehicle and the intact groups were found.

This study evaluates long-term memory (LTM) in the MWM on days 17 and 31 after the administration of $A\beta_{25–35}$ into the CA1 of the hippocampus. In the memory test on Day 17, the time taken to find the location of the platform (Fig. 2C) significantly increased for the $A\beta_{25–35}$ group (21.5 s ± 3.8) as compared to the vehicle (8.4 s ± 0.6) and intact group (10.3 s ± 1), whereas, by Day 31, the $A\beta_{25–35}$ group (25.78 s ± 1.6) showed a significantly greater increase as compared to the vehicle (8.9 s ± 0.5) and intact group (9.2 s ± 1) (One-way ANOVA F (5,114) = 18.33, $p < .001$). The upper section of Fig. 2D shows the navigation paths taken by rats in the memory test. The evaluation of the number of crossings of the target quadrant (Fig. 2D) revealed a decrease in the number of crossings by Day 17 for the $A\beta_{25–35}$ group (5.8 ± 0.3) compared to the vehicle (7.9 ± 0.3) and intact group (7.1 ± 0.3). By Day 31 crossings decreased in the $A\beta_{25–35}$ group (5.1 ± 0.3) compared to the vehicle (6.8 ± 0.4) and intact group (7.2 ± 0.3) (One-way ANOVA, F (5,114) = 9.907, $p < .001$). The time spent in the target quadrant (Supplement Fig. 1C) revealed a decrease in the Day 17 for the $A\beta_{25–35}$ group (21.8 ± 1.4) compared to the vehicle (31.4 ± 1) and intact group (28.6 ± 1.4). By Day 31 the time spent in the target quadrant decreased in the $A\beta_{25–35}$ group (21.7 ± 1) compared to the vehicle (29.5 ± 1.2) and intact group (29.5 ± 1.3) (One-way ANOVA, F (5,114) = 13.38, $p < .001$). The swimming speed (Supplement Fig. 1D) no showed differences between the $A\beta_{25–35}$, vehicle and intact groups were found. The $A\beta_{25–35}$ peptide induces an impairment in long-term memory retrieval, which is associated with compromised neuronal integrity.

3.2. The $A\beta_{25–35}$ peptide induces neuroinflammation in the dorsal hippocampus

The inflammatory response driven by astrocytes and microglia is a key element in neurodegenerative disorders. The administration of the $A\beta_{25–35}$ peptide in the CA1 of the hippocampus induced neuroinflammation associated with an increase in astrocytes, microglia and cytokines (Fig. 3). The quantitative analysis carried out in this study indicated that $A\beta_{25–35}$ peptide induces a neuroinflammatory process by increasing the number of cells positive to GFAP (astrocytes). In the CA1 subfield of the hippocampus (Fig. 3A), the number of cells positive to GFAP in the $A\beta_{25–35}$ group (48.2 ± 1) increased compared to the vehicle (23.6 ± 1) and intact group (22.9 ± 0.8) (One-way ANOVA, F (5,78) = 137.7, $p < .001$). In the DG (Fig. 3A), the number of cells positive to GFAP in the $A\beta_{25–35}$ group (61.3 ± 2.4) increased compared to the vehicle (23.3 ± 1.5) and intact group (26.5 ± 1.4) (One-way ANOVA, F (5,78) = 137.7, p < .001). Analysis of the number of cells positive to Iba1 (microglia) in the CA1 subfield of the hippocampus (Fig. 3B) showed that the $A\beta_{25–35}$ group (29 ± 0.5) increased compared to the vehicle (19.8 ± 0.7) and intact group (18.2 ± 0.4) (One-way ANOVA, F (5,79) = 44.34, p < .001). In the DG (Fig. 3B), the number of cells positive to Iba1 in the $A\beta_{25–35}$ group (25 ± 1)

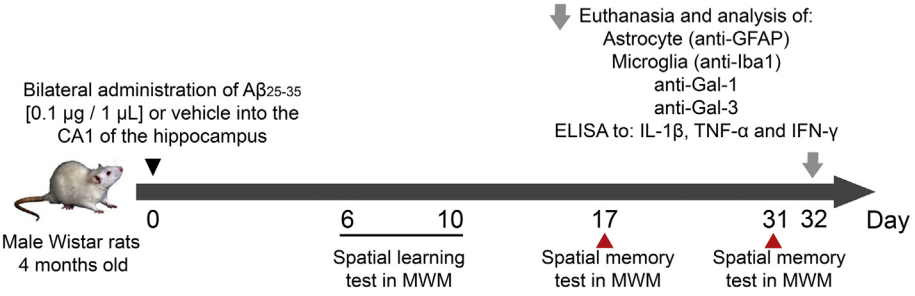

Fig. 1. Schematic of sequences after the administration of the $A\beta_{25–35}$ peptide [0.1 μg] into the CA1 subfield of the hippocampus. The behavioral test was evaluated in the Morris water maze (MWM) from days 6–10 for learning and on days 17 and 31 for memory. Animals were euthanized on Day 32, after which the analysis of GFAP (astrocytes), Iba1 (microglia), cytokines, Galectin-1 and Galectin-3 was undertaken.

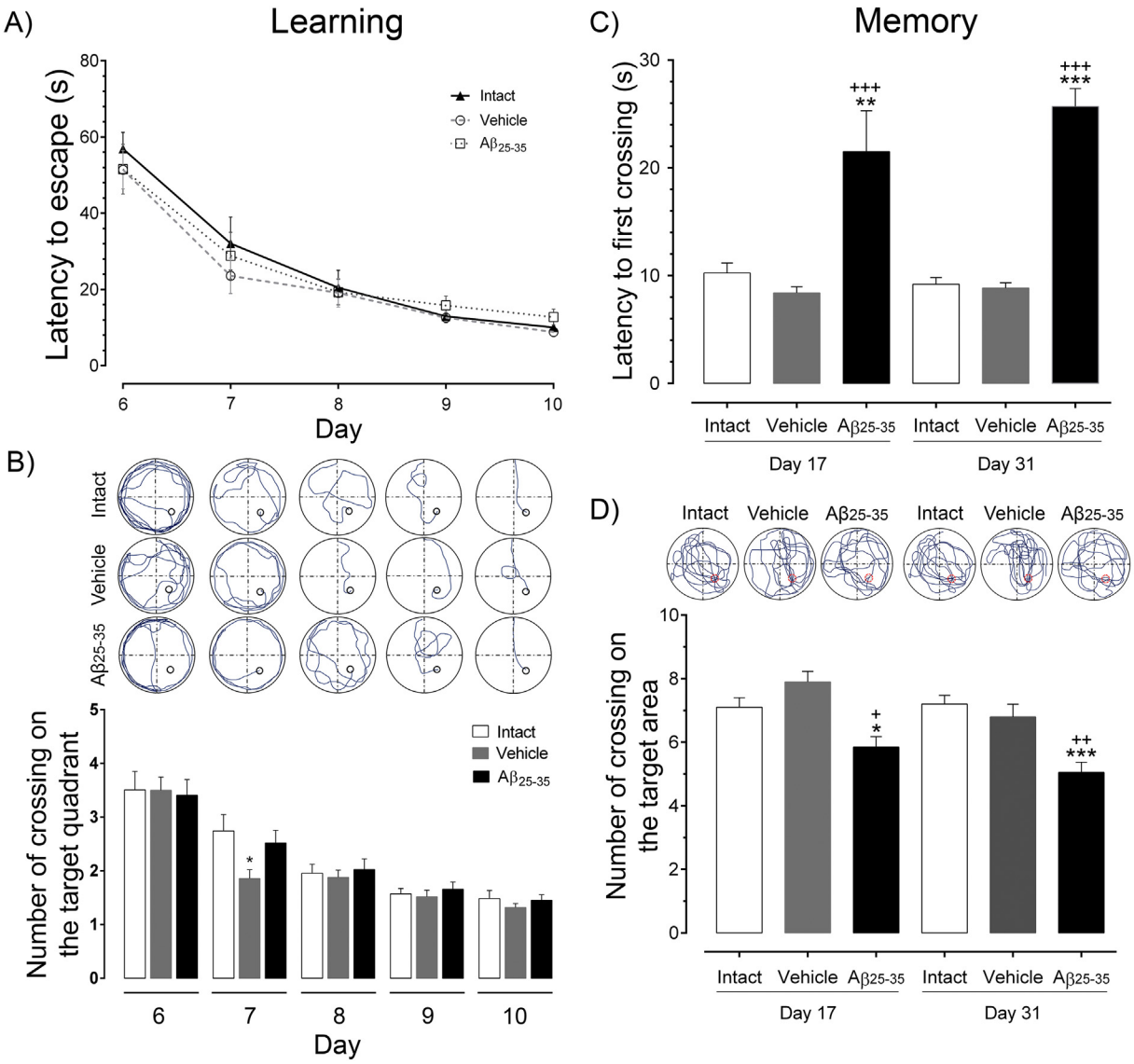

Fig. 2. The administration of the $A\beta_{25-35}$ peptide into the CA1 subfield of the hippocampus impairs long-term memory. A) Shows the time taken to find the platform, which is evaluated as latency to escape during the learning test. B) Shows the number of crossings on the target quadrant (SE) during the learning test (One-way ANOVA with a Bonferroni post-test: $p > .05$). The memory task was undertaken on days 17 and 31 in the MWM. C) Shows the latency time for crossing the target site, while D) shows the number of crossings of the target quadrant (One-way ANOVA with a Bonferroni post-test: vs Intact, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$; vs Vehicle, $^{+}p < .05$, $^{++}p < .01$, $^{+++}p < .001$).

increased compared to the vehicle (20.7 ± 0.6) and intact group (19.4 ± 0.8) (One-way ANOVA, F (5,79) = 44.34, p < .001).

The concentration of cytokines was determined via the sandwich immunoassay technique in homogenates of the dorsal hippocampus of rats 32 days after the administration of the $A\beta_{25-35}$ peptide or vehicle, and after behavioral tests. The levels of IL-1β (Fig. 3C) increased in the $A\beta_{25-35}$ group (364.6 pg/mL ± 21) compared to the vehicle (152.3 ± 15) and intact group (151.2 pg/mL ± 29) (One-way ANOVA F (2,9) = 30.34, p < .001). This increase in IL-1β is associated with the induction of neuroinflammation after administration of the $A\beta_{25-35}$ peptide. The cytokine TNF-α (Fig. 3D) significantly increased for the $A\beta_{25-35}$ group (215.7 pg/mL ± 21) compared to the vehicle (55.7 ± 6) and intact group (71.55 pg/mL ± 20) (One-way ANOVA F (2,12) = 26.31, p < .001), which were tested at the same times. IFN-γ (Fig. 3E) concentration showed an increased in $A\beta_{25-35}$ (71 pg/mL ± 3.5) and vehicle groups (52 pg/mL ± 4.3) compared to the intact group (37 pg/mL ± 1.1) (One-way ANOVA F (2,12) = 26.63, p < .001).

3.3. The $A\beta_{25-35}$ peptide increased the expression of Galectin-3 in the dorsal hippocampus

Galectins exhibit a wide array of functions in neuroinflammation, different reactions involve astrocytes and microglia. The inflammatory response after administration of the $A\beta_{25-35}$ peptide in the CA1 of the hippocampus increased the expression of galectin-3 (Fig. 3H). The expression of Galectin-1 and Galectin-3 was determined by western blot analysis in the dorsal hippocampus of rats 32 days after the administration of the $A\beta_{25-35}$ peptide or vehicle (Fig. H). The expression of Gal-1 (Fig. 3F) in the dorsal hippocampus did not change in the $A\beta_{25-35}$ group (1.2 ± 0.12) compared to the intact group (0.85 ± 0.17) (One-way ANOVA, F (2,6) = 2.24, $p > .05$). However, the expression of Gal-3 increased, associated with the induction of neuroinflammation after administration of the $A\beta_{25-35}$ peptide. Gal-3 (Fig. 3G) significantly increased in the $A\beta_{25-35}$ group (1.53 ± 0.1) compared to the vehicle (1.2 ± 0.05) and intact group (1.01 ± 0.16) (One-way ANOVA, F (2,6) = 5.71, $p < .05$).

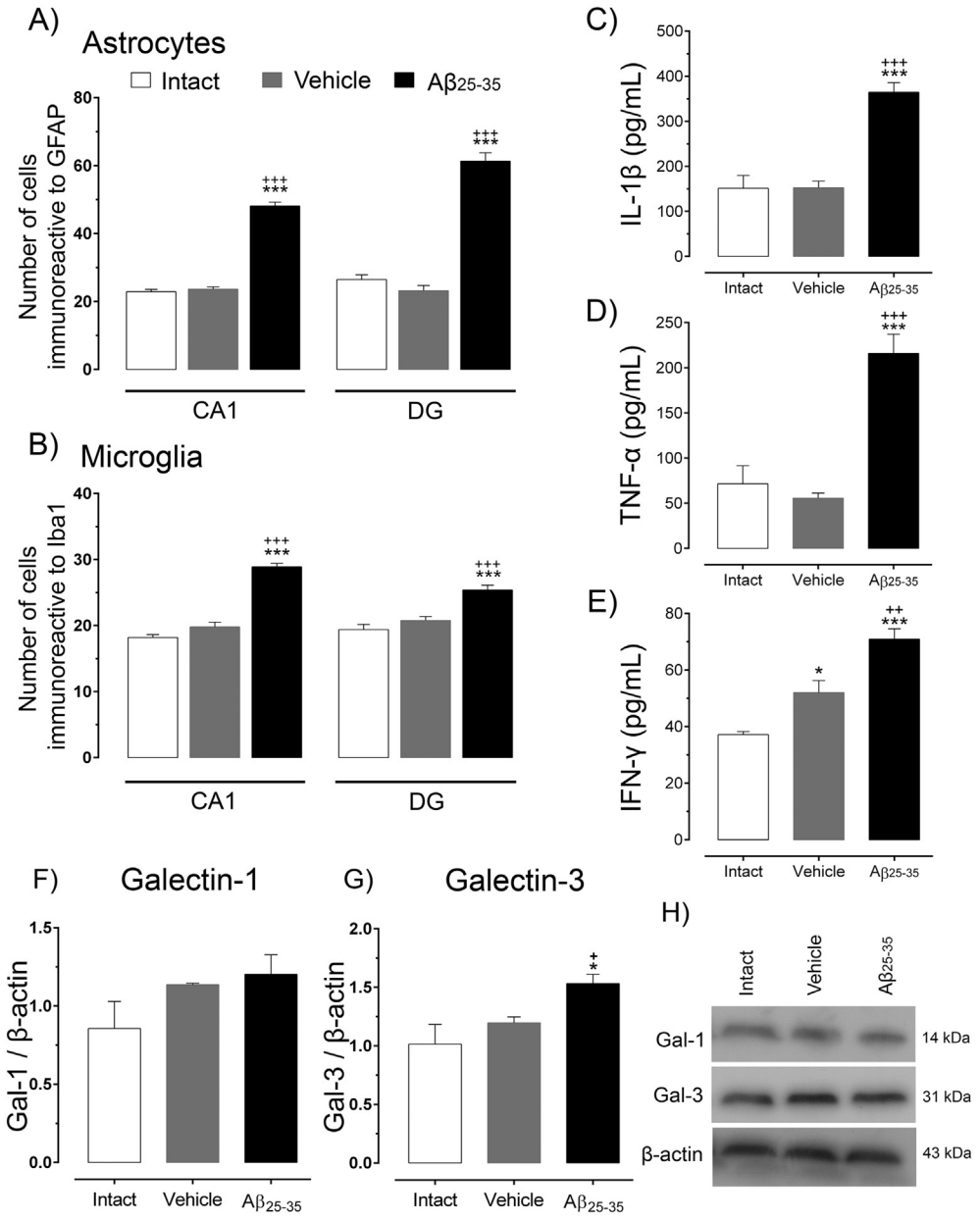

Fig. 3. The administration of the $A\beta_{25–35}$ peptide into the CA1 subfield of the hippocampus induced neuroinflammation. The number of cells positive to A) GFAP and B) Iba1 in the CA1 of the hippocampus and DG correlated to the astrocyte and microglia activity at Day 32. The concentration of IL-1β in C), TNF-α in D), IFN-γ in E) by ELISA and expression of galectin-1 in F) and galectin-3 in G) was evaluated 32 days after administration of the $A\beta_{25–35}$ peptide (One-way ANOVA with a Bonferroni post-test: vs Intact, *p < .05, **p < .01, ***p < .001;; vs Vehicle, $^{++}$p < .01, $^{+++}$p < .001).

3.4. The $A\beta_{25–35}$ peptide induced astrocytes activation and Galectin-3 expression

The inflammatory response driven by astrocytes and microglial cells is a key element in neurodegenerative disorders. Galectins participate in the regulation of immune tolerance and inflammation by interacting with glycosylated receptors on the surface of immune cells. This study sought to ascertain whether the $A\beta_{25–35}$ peptide induces a neuroinflammation process and changes in the expression of Gal-1 and Gal-3 in the hippocampal cells at Day 32 post-surgery. Gal-1 and Gal-3 are known to be involved in the inflammatory response and their expression is increased in microglial cells, due to the protein-glycan interaction occurring in the regulation of innate and adaptive immune responses. The evaluation of immunoreactivity of GFAP with Gal-1 and Gal-3 it would facilitate the understanding of their participation in the neuroinflammation induced by the administration the $A\beta_{25–35}$ peptide in the CA1 of the hippocampus.

The experimental groups showed a low immunoreactivity to Galectin-1 as shown in the photomicrographs (Fig. 4A). The analysis of the total number of cells positive to Gal-1 in CA1 subfield of the hippocampus (Fig. 4B) and DG (Fig. 4C) didn't significant differences between the experimental groups (One-way ANOVA, $p > .05$). The number of colocalized cells immunoreactive to GFAP-Gal-1 in the CA1 (Fig. 4B) and DG (Fig. 4C) didn't show significant differences in the $A\beta_{25–35}$ group compared to the intact group (One-way ANOVA, $p > .05$). However, $A\beta_{25–35}$ peptide increased immunoreactivity to Galectin-3 and colocalization with GFAP in the CA1 and the DG (Fig. 5A, left). Analysis of the total number of cells positive to Gal-3 in the CA1 subfield of the hippocampus (Fig. 5B) showed an increase in the $A\beta_{25–35}$ (31.2 ± 1.3) and vehicle groups (13.3 ± 0.9) compared to the intact group (8.53 ± 0.6). The number of cells immunoreactive to GFAP-Gal-3 colocalized in the CA1 subfield of the hippocampus

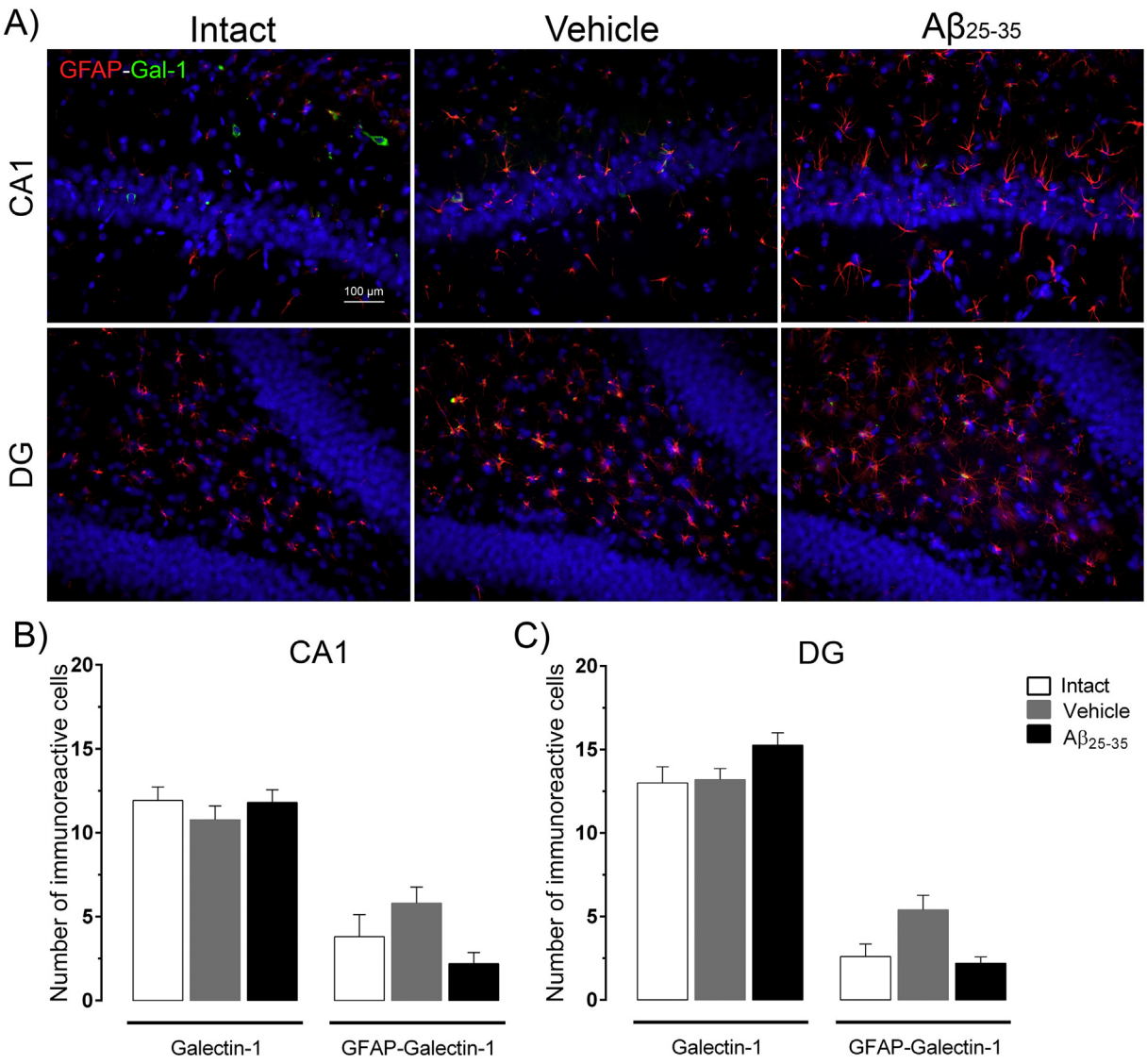

Fig. 4. The administration of the $A\beta_{25-35}$ peptide into the CA1 subfield of the hippocampus didn't modify the expression of Gal-1 associated with astrocytes activation in the CA1 and DG. A) Shows the photomicrographs of astrocytes (GFAP, red) colocalized with Gal-1 (green) at Day 32. $A\beta_{25-35}$ peptide didn't change the number of colocalized GFAP-Gal-1 cells in B) the CA1 of the hippocampus and C) the DG (One-way ANOVA with a Bonferroni post-test: $p > .05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 5B, right) indicated a significant and consistent increase in the $A\beta_{25-35}$ group (18.8 ± 1.5) compared to the vehicle (9 ± 0.9) and intact group (8.2 ± 0.4) (one-way ANOVA, $F(5,54) = 80.35$, $p < .001$). The total number of cells positive to Gal-3 in the DG (Fig. 5C, left) showed an increase in the $A\beta_{25-35}$ group (27.3 ± 3.3) compared to the vehicle (13.6 ± 2) and intact group (13.4 ± 1.1). In the DG, the number of cells colocalized to GFAP-Gal-3 (Fig. 5C, right) showed a significant increase in the $A\beta_{25-35}$ group (20.6 ± 3.3) compared to the vehicle (9.6 ± 0.7) and intact group (7 ± 0.4) (One-way ANOVA, $F(5,30) = 11.5$, $p < .001$). The obtained results indicate that $A\beta_{25-35}$ peptide increases Gal-3 expression, associated with astrocyte activation.

3.5. The $A\beta_{25-35}$ peptide induced microglia activation and galectins expression

The administration the $A\beta_{25-35}$ peptide in the CA1 of the hippocampus induced an increase in immunoreactivity of Iba1 (microglia) and no changes the expression of Gal-1. In the photomicrographs, the $A\beta_{25-35}$ group showed an increase in immunoreactivity to Iba1 and but not of Gal-1 in the CA1 and the DG (Fig. 6A). The results of this study indicate that the $A\beta_{25-35}$ peptide induces a neuroinflammatory process by increasing the number of cells positive to Iba1 (microglia). Analysis of the total number of cells positive to Gal-1 and colocalized Iba1-Gal-1 in the CA1 subfield of the hippocampus (Fig. 6B) and the DG (Fig. 6C) did not show difference between experimental groups (One-way ANOVA, $p > .05$).

The $A\beta_{25-35}$ peptide increased immunoreactivity to Galectin-3 and colocalization of Iba1-Gal-3 in the CA1 subfield of the hippocampus and the DG (Fig. 7A). Analysis of the total number of cells positive to Gal-3 in the CA1 subfield of the hippocampus (Fig. 7B, left) showed an increase in $A\beta_{25-35}$ (31.2 ± 1.2) and vehicle groups (13.3 ± 1) compared to intact group (8.5 ± 0.6) (One-way ANOVA, $F(5,41) = 41.7$, $p < .01$). The number of cells immunoreactive to Iba1-Gal-3 colocalized in the CA1 subfield of the hippocampus (Fig. 7B, right) indicated an increase in the $A\beta_{25-35}$ group (13 ± 1.1) compared to the vehicle (3.6 ± 0.2) and intact group (2.0 ± 0.5) (One-way ANOVA, $F(5,49) = 5.9$, $p < .01$). The number of cells immunoreactive to Gal-3 in the DG (Fig. 7C, left) increased in the $A\beta_{25-35}$ group (27.3 ± 3.3) compared to the vehicle (13.6 ± 2) and intact group (13.4 ± 1.2).

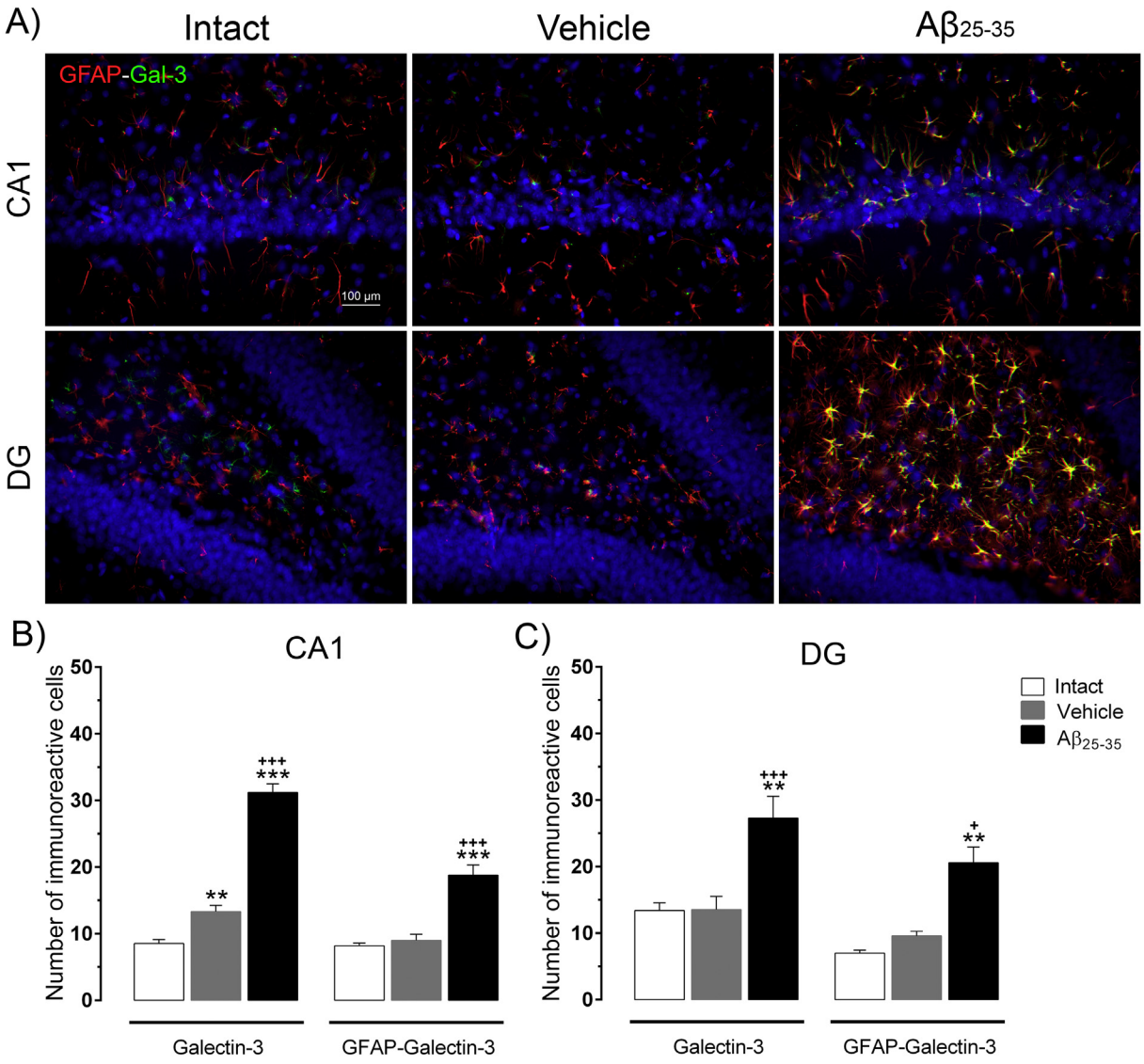

Fig. 5. The administration of the $A\beta_{25–35}$ peptide into the CA1 subfield of the hippocampus increased the expression of Gal-3 associated with astrocyte activation in the CA1 and DG. A) Shows the photomicrographs of astrocytes (GFAP, red) colocalized with Gal-3 (green) at Day 32. $A\beta_{25–35}$ peptide increased the number of colocalized GFAP-Gal-3 cells in B) the CA1 of the hippocampus and C) the DG (One-way ANOVA with a Bonferroni post-test: vs Intact group, $^{**}p < .01$, $^{***}p < .001$; vs Vehicle group, $^{+}p < .05$, $^{+++}p < .001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The total number of cells colocalized to Iba1-Gal-3 in the DG (Fig. 7C, right) showed a significant increase in the $A\beta_{25–35}$ group (11 ± 1.1) compared to the vehicle (1.1 ± 0.4) and intact group (0.3 ± 0.15) (One-way ANOVA, $F(2,25) = 4.36$, $p < .05$). Results indicate that $A\beta_{25–35}$ peptide increases the expression of Gal-3 in microglial cells, which may be related to cognitive impairment.

4. Discussion

Understanding the mechanisms of Aβ mediated toxicity is a prerequisite for the development of efficient and safe therapeutic approaches to treat Alzheimer's disease (AD) (Götz et al., 2011; Heneka et al., 2015). Misfolded and aggregated proteins bind to pattern recognition receptors on microglia and astroglia that trigger an innate immune response characterized by the release of inflammatory mediators, which contribute to disease progression and severity (Glass et al., 2010; Heneka et al., 2014; Bronzuoli et al., 2016). The use of the $A\beta_{25–35}$ peptide in animal models has contributed to understanding the relationship between inflammation and detrimental effects on cognitive ability (Limón et al., 2011; Zussy et al., 2011; Diaz et al., 2012). The lectin-glycan interaction contributes to the initiation, execution, and resolution of the inflammation, playing an essential role in the maintenance of tissue homeostasis and the response to injury (Rudd et al., 2001; Johnson et al., 2013). Galectins are considered as modulators in the activation of astrocytes and microglial cells through the crosslinking of specific glycoconjugates (Sasaki et al., 2004; Boza-Serrano et al., 2014). The balance between Gal-1 with anti-inflammatory properties and Gal-3 with pro-inflammatory properties could modulate the neuroinflammation induced by the $A\beta_{25–35}$ peptide and its relationship with neurodegenerative disorders. Understanding the interactions within the central nervous system between Gal-1, Gal-3 and the neuroinflammation might be key to prevent or delay neurodegeneration.

The results of this work demonstrate that the administration of the $A\beta_{25–35}$ peptide deteriorates the cognitive abilities evaluated in the MWM. The acquisition of new information from Day 6 to 10 in the MWM was similar for all groups (Fig. 2A). The number of crossings and the navigation paths taken by rats (Fig. 2B) were correlated with spatial learning. However, in the memory test conducted on days 17 and 31,

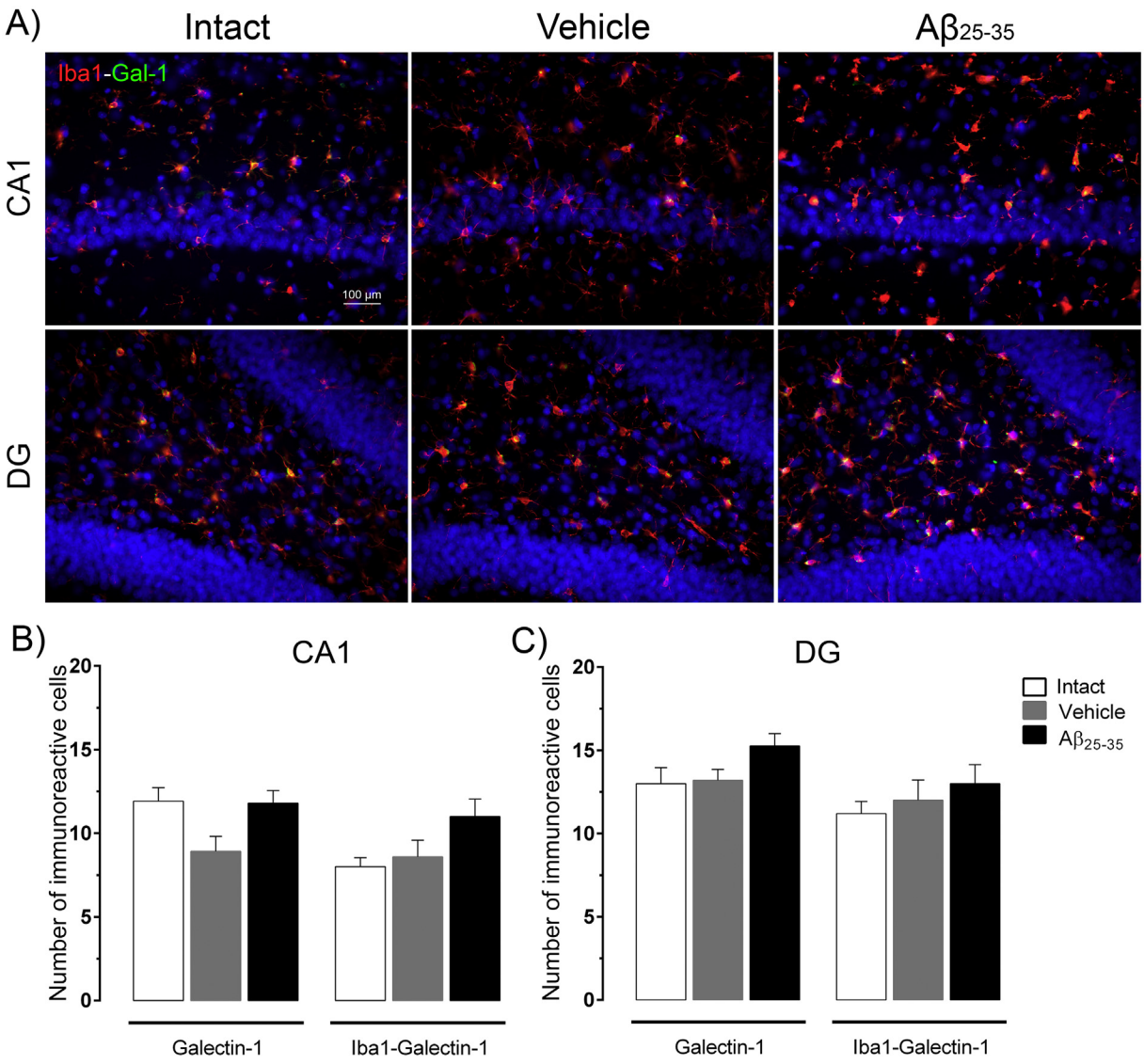

Fig. 6. The administration of the $A\beta_{25–35}$ peptide into the CA1 subfield of the hippocampus did not change the expression of Gal-1 associated to microglia activation in the CA1 and DG. A) Shows the photomicrographs of microglia (Iba1, red) colocalized with Gal-1 (green) at Day 32. $A\beta_{25–35}$ peptide did not change the expression of Gal-1 and the number of colocalized Iba1-Gal-1 cells in B) the CA1 of the hippocampus and C) the DG (One-way ANOVA with a Bonferroni post-test: $p > .05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the $A\beta_{25–35}$ group showed an increase in the latency to first crossing over the place where the platform was located (Fig. 2C), and a decrease in the number of crossings on the target quadrant (Fig. 2D) compared to the intact group. The long-term memory formation is accompanied by the crucial phase of consolidation. The consolidation of the memory is sensitive to disruption, and its maintenance requires protein synthesis to transform newly learned information into stable strengthened synapsis (Bailey et al., 2004; Lamprecht and LeDoux, 2004; Frost et al., 2010). The hippocampus plays a crucial role in spatial representation and is responsible for an adequate performance in spatial learning tasks. It contributes to the consolidation of memory, with its integrity required to recognize, codify, store, and recover acquired spatial information (Broadbent et al., 2004). $A\beta_{25–35}$ impairs retrieval of information, by changes in the structure and formation of new dendritic spines, therefore weakening neuronal communication (Ramírez et al., 2018). This stablishes a relationship between hippocampal lesion via the $A\beta_{25–35}$ peptide and spatial memory impairment recorded in the MWM task. The $A\beta_{25–35}$ peptide causes cognitive deficit by facilitating several synaptic damage mechanisms (Lazcano et al., 2014; Ramírez et al., 2018), and inducing the activation of the nitric oxide (NO) pathway (Limón et al., 2009), oxidative stress (Butterfield et al., 2001), membrane lipid peroxidation (Butterfield et al., 2002; Gunn et al., 2016), mitochondrial dysfunction (Canevari et al., 1999), alterations in membrane permeability (Lin et al., 2001), excitotoxicity (Parameshwaran et al., 2008; Esposito et al., 2013), cholinotoxicity (Patricio-Martínez et al., 2016) and inflammation (Rosales-Corral et al., 2004; Diaz et al., 2012). These effects might thus contribute to dorsal hippocampus dysfunction and memory impairment.

The inflammation induced by the $A\beta_{25–35}$ peptide is a possible cause of neurodegenerative events, through the activation of astrocytes and microglia and the secretion of various cytokines and chemokines (Zussy et al., 2011; Diaz et al., 2012). However, the $A\beta_{25–35}$ induced inflammatory process associated with the neurodegeneration and cognitive deficit in the long term is currently unclear. The findings of this research show that $A\beta_{25–35}$ increased immunoreactivity to GFAP (Fig. 3A) and Iba1 (Fig. 3B) in the CA1 subfield of the hippocampus and the DG. The $A\beta_{25–35}$ peptide causes the chronic activation of microglia and reactive astrocytes, contributing to neuronal damage in the dorsal hippocampus, while the imbalance of the inflammatory process could promote neuronal death. The inflammatory response is characterized

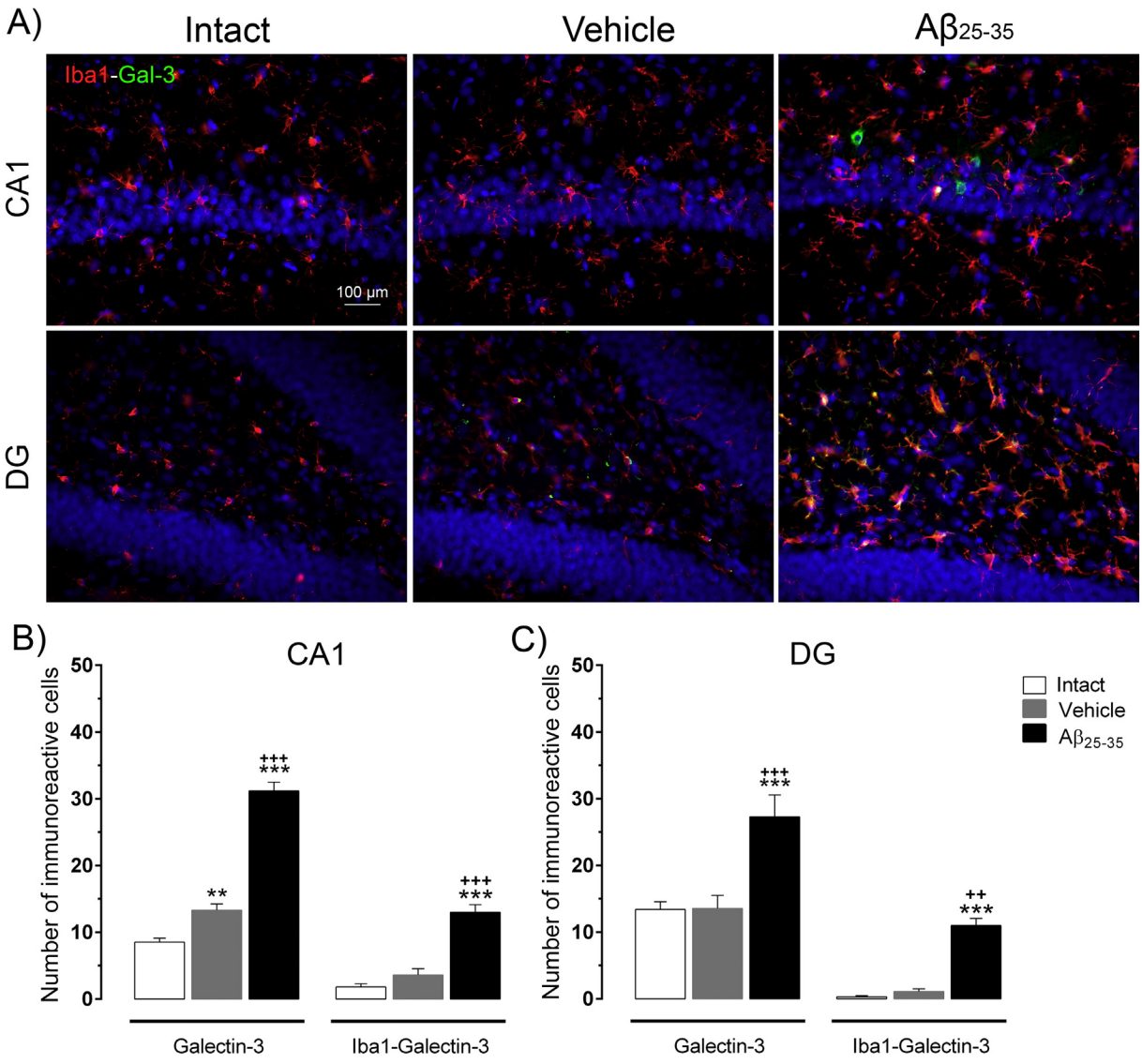

Fig. 7. The administration of the $A\beta_{25-35}$ peptide in the CA1 region of the hippocampus activated microglia and induced the expression of Gal-3 in the CA1 and the DG. A) Shows the photomicrographs of astrocytes (Iba1, red) colocalized with Gal-3 (green) at Day 32. $A\beta_{25-35}$ peptide increased the number of colocalized GFAP-Gal-3 cells in B) the CA1 of the hippocampus and C) the DG (One-way ANOVA with a Bonferroni post-test: vs Intact group, $^{**}p < .01$, $^{***}p < .001$; vs Vehicle group, $^{++}p < .01$, $^{+++}p < .001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

by the coordinated activation of various signalling pathways that regulate the expression of both pro- and anti-inflammatory mediators (Vallabhapurapu and Karin, 2009). Cytokines are regulators of the intensity and duration of the inflammatory process, promoting the activation of various signalling pathways in response to cellular stress (Rubio-Perez and Morillas-Ruiz, 2012; Fuster-Matanzo et al., 2013; Di Benedetto et al., 2017). Therefore, this research studied the effect of $A\beta_{25-35}$ on the concentration of the cytokines IL-1β (Fig. 3C), TNF-α (Fig. 3D) and IFN-γ (Fig. 3E), which play an important role in the modulation of neuroinflammation. The results indicate a significant increase of these cytokines in the dorsal hippocampus of animals treated with the $A\beta_{25-35}$ peptide compared to the intact group. Many researchers report that IL-1β, TNF-α and IFN-γ play a central role in modulating the inflammatory response.

The pro-inflammatory cytokines, including TNF-α and IL-1β, or Toll-like receptors (TLR) signalling, trigger the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Li et al., 2004). The NF-κB pathway plays a central role in the inflammation and activation of the adaptive immune response through the expression of the target genes of pro-inflammatory cytokines, chemokines, and cell adhesion molecules (Pasparakis, 2009). Interestingly, Gal-1 expression is up-regulated during the inflammatory response (Ilarregui et al., 2009), suggesting a critical role of this endogenous lectin in promoting resolution of inflammation and a possible functional link between this transcription factor and Gal-1 expression (Toscano et al., 2006; Barrionuevo et al., 2007; Norling et al., 2008; Lawrence, 2009; Toscano et al., 2011). During neuroinflammation, IFN-γ induces an M1 phenotype in microglial cells, which is characterized by high levels of the major histocompatibility complex class II (MHC II), CD86 and inducible nitric oxide synthase (iNOS) expression in a STAT1-dependent manner, as well as production of proinflammatory cytokines and chemokines, such as TNF-α and CCL2 (Butovsky et al., 2006; Kawanokuchi et al., 2008). The IFN-γ pathway predominantly activates the Janus associated kinase/signalling transducer and the activator of the transcription-1 (JAK/STAT) signalling pathway. Out of the seven STAT family members, STAT1 is the main downstream effector of IFN-γ. Upon phosphorylation by JAKs, STAT1 homodimerizes and translocate to the nucleus, where it initiates the transcription of IFN-γ-stimulated genes (ISGs).

The working group that conducted this study has previously showed

that the $A\beta_{25–35}$ peptide induces glycosidic changes, associated with the neurodegenerative process (Limón et al., 2011; Lozano et al., 2017; Ramos-Martinez et al., 2018). Understanding the interactions between the immune system and the nervous system might be key to preventing or delaying neurodegenerative diseases. This paper proposes that the glycosylation of cell surface proteins can be critical in the protein-glycan interactions involved in the cellular signalling that takes place for the regulation of innate and adaptive immune responses (van Kooyk and Rabinovich, 2008; Rabinovich et al., 2012). The task of decoding glycan information is assigned, in part, to a great variety of mammalian glycan-binding proteins or lectins, including selectins, pentraxins, siglecs and galectins (Vasta et al., 2012). Galectins are characterized by their ability to recognizing glycans that contain *N*-acetyllactosamine (Galβ1,4GlcNAc) sequences, displayed in both N- and O-glycans of the cell surface glycoconjugates (Hirabayashi et al., 2002; Liu et al., 2002; Yang et al., 2008). Gal-1 and Gal-3 play an important role in the modulation of inflammation, by cross-linking cell-surface glycoconjugates, which, like many other receptor-ligand systems, can trigger a cascade of transmembrane signalling events (Yang et al., 2008). Through this mechanism, galectins are able to modulate a wide variety of immunological processes, including apoptosis, activation, cell adhesion and cytokine secretion in astrocytes and microglia (Sirko et al., 2015). However, in neurodegenerative events, the participation of galectins in the modulation of the immunological response remains largely unknown.

This study showed that the expression of Gal-1 in astrocytes (Fig. 4) and microglia (Fig. 6) didn't show significant changes between experimental groups in the hippocampal formation. Therefore, after the damage induced by $A\beta_{25–35}$ peptide an overactivation of the glial cells occur. However, the expression of Gal-1 didn't change which could be associated with an anti-inflammatory response. The expression of Gal-1 in astrocytes induces a reaction in brain injury by the release of neurotrophic factors that mediate the inflammatory response and the tissue remodeling post-lesion (Sasaki et al., 2004; Sofroniew and Vinters, 2010). In vivo models, Gal-1 promoted apoptosis in Th1 cells, the expression of IL-10 and the downregulation of pro-inflammatory cytokines, result in limiting the immune response (Toscano et al., 2006, 2011). Astrocytes undergoing a reactive response are neuroprotective under certain conditions, while excessive astrogliosis is detrimental and contributes to neuronal damage (Farina et al., 2007; Sofroniew and Vinters, 2010). During the first response occurring post-lesion, the expression of Gal-1 induces astrocyte differentiation and modulates their proliferation. In differentiated astrocytes, Gal-1 greatly enhances the up-regulation of BDNF (Sasaki et al., 2004). Moreover, the Gal-1-glycan interactions contribute to neuroprotection by deactivating classically activated microglia and inducing an alternative M2 microglial phenotype of alternative activation by modulating the mitogen-activated protein kinase p38 (p38MAPK), cAMP response element binding (CREB), and the NF-κB signalling pathways (Starossom et al., 2012).

An increase in the expression of Gal-3 in astrocytes (Fig. 5) and microglia (Fig. 7) was observed in the hippocampal formation following damage induced by the $A\beta_{25–35}$ peptide on Day 32. This study proposes that Gal-3 could be contributing to modulating the inflammation and neurodegeneration in the CA1 subfield of the hippocampus and DG. The expression of Gal-3 was up-regulated in the activated microglia and astrocytes that confer strong chemotactic properties and contribute to the phagocytosis involved in inflammatory processes (Walther et al., 2000; Yan et al., 2009). The expression of Gal-3 can be found in the cytoplasm, nucleus, and membranes (Shimura et al., 2004) and increases in the glial cells involved in the inflammatory response (Satoh et al., 2011a, 2011b; Lalancette-Hérbert et al., 2012; Wesley et al., 2013). The different subcellular localizations of Gal-3, together with its possible post-translational modification, are likely to affect the function of Gal-3 and explain why rather contradictory effects have been reported, such as the pro- versus anti-apoptotic (Nakahara et al., 2005) and the pro- versus anti-inflammatory (Jeon et al., 2010). Gal-3 plays an important role in cell proliferation and the regulation of apoptosis, as well as in pathological processes as an inflammatory mediator (Liu et al., 2002). A recent study showed that Gal-3 increases both the expression and secretion of astrocytes and microglia by activating the JAK-STAT signalling pathway in response to IFN-γ (Jeon et al., 2010), suggesting that Gal-3 exerts its effects by modulating the inflammatory response in brain injury models (Yang et al., 2012). Moreover, it has been suggested that Gal-3 displays cytokine-like properties in brain-resident immune cells, regulating immune and inflammatory responses (Li et al., 2008; Dhirapong et al., 2009; Henderson and Sethi, 2009). The effect of the $A\beta_{25–35}$ peptide in this study is related with the results found in the treatment of microglia and astrocytes cultures with Gal-3, which markedly increased the expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IFN-γ) but did not alter the expression of anti-inflammatory mediators (e.g. transforming growth factor-β and IL-10) (Jiang et al., 2009; Radosavljevic et al., 2012; Shin, 2013). Moreover, Gal-3 is involved in the proinflammatory response triggered by some microglial receptors such as α-synuclein (Boza-Serrano et al., 2014). Gal-3 could be inducing a long-term inflammatory response in this amyloid-induced neurodegenerative process by interacting with immune receptors as Toll-like 4 (TLR-4). That could prolong the inflammatory response in the brain (Burguillos et al., 2015). Recent reports have demonstrated that Gal-3 is released by activated microglia in response to proinflammatory stimuli and can act as an endogenous ligand for TLR4, thus eliciting a proinflammatory M1 response in the brain. Gal-3 can act through binding to TLR4 carbohydrates increasing the microglial inflammatory response (Burguillos et al., 2015; Yip et al., 2017). After administration of the $A\beta_{25–35}$ peptide, Gal-3 could play a decisive role in the expansion and enforcement of the inflammatory response and might potentially contribute to the long-term inflammatory response.

The balance between pro- and anti-inflammatory signalling is critical to maintaining immune homeostasis under physiological conditions and controlling inflammation in different pathological settings. Gal-1 and Gal-3 play an important role in the modulation of the neuroinflammatory process. After damage induced by the $A\beta_{25–35}$ peptide, the expression of the galectins is essential to maintaining an effective level of immune response without causing tissue damage. However, chronic and progressive damage by the $A\beta_{25–35}$ peptide disrupted the neuroinflammation that promotes neurodegenerative disorders. These findings open a new panorama related to the neuroinflammation induced by the administration of the $A\beta_{25–35}$ peptide, which is associated with a deficit in the retrieval of information learned in the MWM. This study clearly shows that the neuroinflammation induced by $A\beta_{25–35}$ peptide increased the expression of Galectin-3, which could modulate astrocyte and microglia activity.

Conflict of interest

None of the authors has conflict of interest relevant to this research.

Acknowledgments

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