University of Szeged
Albert Szent-Györgyi Medical School
Theoretical Medicine Doctoral School

DEVELOPMENT OF NON-TRANSGENIC ANIMAL MODELS OF ALZHEIMER'S DISEASE AND INVESTIGATION OF DRUG CANDIDATES FOR ITS TREATMENT

Ph.D. thesis

Emőke Borbély

Supervisor: Lívia Fülöp Ph.D.
Department of Medical Chemistry,
Albert Szent-Györgyi Medical School,
University of Szeged

Szeged
2023

INTRODUCTION

The most common type of neurodegenerative disease is Alzheimer's disease (AD) with wide clinical heterogeneity. It is characterized by deficits in the learning process, severe memory loss, as well as complex behavioral changes. The number of AD patients increases with the growing life expectancy. AD has become one of the most important health and socioeconomic problems.

AD is neuropathologically characterized by severe synaptic loss and the presence of misfolded proteins, forming aggregates in cerebral brain tissue, mainly in the neocortex and in the hippocampus (HC). The extracellular amyloid plaques composed of β-amyloid (Aβ) peptide, and the intracellular neurofibrillary tangles (NFTs) formed by abnormally hyperphosphorylated tau proteins were at first observed postmortem in 1906 by Alois Alzheimer. The cerebral presence of these aggregates is considered the main pathological hallmark, and they are still required for the diagnosis of AD. Accumulation of Aβ is initiated already two or three decades before the onset of neurological symptoms and the diagnosis of AD. The appearance of Aβ deposits is followed by elevated tau levels in cerebrospinal fluid, significant brain atrophy, neuronal hypometabolism, and impaired memory function before the onset of symptoms.

There are two forms of AD: the dominantly inherited, early-onset or familiar form of AD (FAD, begins typically in the age of forties, and gives less than 5% of total cases), and the non-inherited, late-onset or sporadic form of AD (SAD, begins in the age of sixties, being the most prevalent form of age-related dementia with 95% occurrence). The principal risk factor of AD is age; the most endangered group is the population over-65 age. The clinical and histopathological symptoms of FAD are similar to those of SAD, and presumably, they share common pathomechanistic routes. To date, research is being conducted to clarify the etiology of the disease, so several hypotheses have been proposed to explain the pathomechanism of AD. According to one of these widely accepted theories, the amyloid cascade hypothesis (ACH), the development of the disease is caused by the excessive accumulation of the $A\beta_{1-42}$ protein produced by proteolytic cleavage of the amyloid precursor protein, and its reduced clearance.

Despite many years of research, the cause of AD is still unknown and there are no prophylaxis or curative treatments available. According to basic and clinical research results, therapeutic interventions may already be late in diagnosed AD patients to alleviate progression. Thus,

new, effective strategies and methods based on other molecular mechanisms instead of ACH are desperately required to detect, prevent, and treat AD in the pre-symptomatic stage.

AIMS

Based on the ACH, our research group aimed to establish such experimental models, in which potential drug candidates, designed and synthesized *in-house*, could be cost- and time-effectively tested *in vivo*. To achieve this, we formulated and validated different treatment protocols applying exogenous, toxic Aβ with different aggregation forms.

1. In one study, fibrillar $A\beta_{1-42}$ ($fA\beta_{1-42}$) was administered in the HC of rats. We examined the impact of a single injection of $fA\beta_{1-42}$ on spatial memory and on dendritic spine density in the HC of the treated animals. Rats were subjected to standard Morris water maze (MWM) tests *(IHC experiment)*.
2. The effect of Leu-Pro-Tyr-Phe-Asp-amide (LPYFDa), a potential drug candidate molecule was tested in a cooperative project. Our collaborators examined its effect in an injection model, in which $oA\beta_{1-42}$ was administered in wild-type mice in the presence of LPYFDa, and the animals were subjected to fear conditioning tests. In parallel with this, we aimed to evaluate spatial memory in a modified version of MWM. Instead of mice, we used rats, which were treated once with oligomeric $A\beta_{1-42}$ ($oA\beta_{1-42}$) and injected in the entorhinal cortex (EC). The neuroprotective impacts of LPYFDa on cognition and neuroinflammation were also studied in this model (*EC experiment).*

The change in the leading directions of AD-related drug research made us broaden our portfolio and find possible therapeutics with new pathomechanistic targets. We aimed to assess the role of adult neurogenesis and neuroinflammation in AD, and its connection to sigma-1 receptor (S1R)-dependent molecular processes.

3. In a third study, $oA\beta_{1-42}$ was administered intracerebroventricularly (ICV) in adult wild-type C57BL/6 mice, to induce early acute AD-like impairments in neurogenesis and to reveal its relationship with neuroinflammation *(ICV experiment)*. We attempted to restore normal functioning in the Aβ-treated animals by activating S1Rs with two different S1R agonists, PRE084 (2-(4-morpholinethyl)-1-phenyl cyclohexane carboxylate) and DMT (N, N-dimethyltryptamine).

MATERIALS AND METHODS

Animals

Male Wistar rats (n=24, Charles-River, Germany), weighing 210-230 g at the beginning of the *IHC experiment*, male Wistar-Harlan rats (n=41, Charles-River, Germany), weighing 250-350 g, and aged 8-10 weeks at the beginning of the *EC experiment*, and male C57BL/6 wild-type mice (n=80) from *in-house* breeding, weighing 23-28 g and aged 12 weeks at the beginning of the *ICV experiment*, were used as subjects.

Description of the materials used for the treatments

For the preparation of the iso-A$\beta_{1\text{-}42}$ peptide, which is a chemically modified form of the natural sequence, an optimized synthesis protocol was developed *in-house*. In the *IHC experiment*, the fA$\beta_{1\text{-}42}$-containing samples were prepared as described by He *et al*. In the *EC experiment*, oA$\beta_{1\text{-}42}$ was prepared by applying a modified protocol of Stine *et al*. In the *ICV experiment*, iso-A$\beta_{1\text{-}42}$-containing samples were optimized by our protocol, then a stable aggregation state was prepared. The neuroprotective pentapeptide, LPYFDa was synthesized *in-house* by standard solid-phase peptide synthesis methodologies. The BrdU, DMT, and PRE084 were obtained from Sigma-Aldrich (Saint Louis, MO, USA) and Lipomed (AG, Switzerland).

Surgery, solutions, and drug administration

Rodents were anesthetized by an intraperitoneal (IP) injection of a mixture of ketamine and xylazine and were then placed into a stereotaxic apparatus. In all three experiments, Hamilton syringes were used for the injections.

In the *IHC experiment*, the solution was injected into the right HC unilaterally. Rats were randomly injected either with the fA$\beta_{1\text{-}42}$ (n=12) or with physiological saline (n=12).

In the *EC experiment*, the solution was injected into the EC at three bilateral sites. The rats were randomly assigned to groups: **1.** PBS, n=10; **2.** LPYFDa, n=10; **3.** oA$\beta_{1\text{-}42}$, n=10; and **4.** oA$\beta_{1\text{-}42}$ and LPYFDa (1:5 molar ratio), n=11.

In the *ICV experiment*, a single ICV injection of either oA$\beta_{1\text{-}42}$ or PBS was administered at the right side. To detect stem cells, mice were injected IP with BrdU 3 times, 24 h after the surgery. PRE084 and DMT were also administered IP daily between post-surgery days 7–12. Six groups of animals were developed: **1.** PBS-PBS, n=18; **2.** oA$\beta_{1\text{-}42}$-PBS, n=18; **3.** PBS-PRE084, n=11; **4.** oA$\beta_{1\text{-}42}$-PRE084, n=11; **5.** PBS-DMT, n=11; **6.** oA$\beta_{1\text{-}42}$-DMT, n=11.

Fixed platform Morris Water Maze (MWM) and modified Morris Water Maze (mMWM)

In the *IHC experiment*, spatial learning and memory were assessed by fixed platforms MWM on days 14 to 20 after IHC administration of $fA\beta_{1-42}$. Memory acquisition trials were performed daily, rats swam 4 times in a row per day in blocks, for 6 days. Twenty-four hours after the last acquisition trial, retention was assessed on a 120-sec probe trial, with the platform removed.

In the *EC experiment*, mMWM was carried out on the 15th day after the surgery. Two plastic platforms were used: a larger (d=10 cm), and a smaller (d=5 cm) hidden, escape island. On days 1 to 5 the larger, and on the 6th day the smaller platform was placed into the tank. During the experiments, two trials per day were performed from two different starting points, while the platform remained in the same position. One experiment lasted for six days. On days 1 to 4, animals were tested with the matching-to-sample protocol. In this phase, the large platform was relocated every day (from Q1 to Q4). During the first swim ('sample'), the animals had to learn the platform location. The second one served as the 'test' or 'matching' trial. On the 5th day, the large platform was submerged in the same quadrant, as the day before (Q4), while on the 6th day, the smaller platform was also located in Q4.

Histological examination and Western Blot (WB) analysis

In the *IHC experiment*, after the behavioral examinations, Golgi impregnation was carried out during which the native rat brains were cut into blocks. After their two-phase impregnation, 100 μm hippocampal sections were prepared. In further histological experiments, rodents were anesthetized with chloral hydrate and were perfused transcardially with PBS and paraformaldehyde (PFA). The brains were post-fixed in 4% PFA solution for a day and afterward, they were put in a mixture of 30% sucrose solution. In the *EC experiment*, four animals from each group were used. After the post-fixation, certain brain regions of EC were slotted, and coronal, 30 μm thick sections were cut by a freezing microtome. In case of the *ICV experiment*, two weeks after the administration of $oA\beta_{1-42}$, mice (n=8-8 from the PRE084- and DMT-treated, and n=15-15 from the control groups) were processed. Immunohistochemical analysis was carried out on 20 μM formalin fixed cryosections. For immunostainings, the slides were incubated with the primary antibody (Ab) followed by the corresponding biotinylated secondary Ab. The peroxidase reaction was carried out by

applying the Vectastain Elite ABC Kit system using 3,3'-diaminobenzidine as the substrate and $NiCl_2$ as an intensifier.

In the WB of *EC* and *ICV experiments*, EC of the rats (n=6 animals/group), cortical regions, and HC of mice (n=3 animals/group) were removed after cervical dislocation. After tissue homogenization and centrifugation, the protein samples were run on an SDS gel and transferred to a nitrocellulose membrane, then incubated with primary and secondary Ab. During the immunoblot analyses, the effect of $oA\beta_{1-42}$ on the expression of GFAP was detected. In the *ICV experiment*, we examined the effects of $oA\beta_{1-42}$ and PRE084 or DMT on the expression of S1R.

Quantification of the data

Golgi-impregnated sections were studied by inverse light microscopy, using oil-immersion objectives. A total of 25 pyramidal neurons from the dorsoventral hippocampal CA1 were examined from each of the 6 animals (25 dendritic shafts per animal were analyzed).

Immunohistochemical slides were scanned by a digital slide scanner (Mirax Midi, 3DHistech Ltd., Budapest, Hungary), equipped with a Pannoramic Viewer 1.15.4, a CaseViewer 2.1 program, and a QuantCenter, HistoQuant module. For quantifications, all sections derived from each animal were analyzed. The number of stem cells (BrdU+) and neuroblasts (doublecortin, DCX+) were assessed by the observers, and to calculate cell densities, we divided the total number of counted cells per animal with the dentate gyrus (DG)/HC area and represented them as cells/mm^2. In case of Neuronal Nuclei (NeuN), ionized calcium-binding adapter molecule 1 (Iba1), and GFAP, the densities (%) of neurons (NeuN+), microglia (Iba1+), and astrocytes (GFAP+) were calculated by the quantification software.

Statistical analysis

In the *IHC experiment*, behavioral data were analyzed by repeated measures one-way analysis of variance (rANOVA), followed by Fisher's LSD post-hoc tests for multiple comparisons. For the evaluation of the results of Golgi impregnation, a Student's t-test for independent samples was used. In the *EC and ICV experiments,* data obtained from the behavioral and immunohistochemistry analyses were evaluated with ANOVA, followed by Fisher's LSD post-hoc tests. The WB data were analyzed with Kruskal-Wallis nonparametric tests, followed by Mann-Whitney U tests for multiple comparisons. For the statistical analysis, SPSS software was used, and the results were expressed as mean ± (SEM). Statistical significance was set at $p \leq 0.05$.

RESULTS

IHC experiment

Injection of fAβ$_{1-42}$ induced deficits of spatial learning and memory in MWM and decreased dendritic spine density

MWM was used to test spatial learning and memory each day on days 14 to 20 after IHC administration of fAβ$_{1-42}$. Escape latency to find the platform was used as a measure for evaluating spatial memory. The results showed that the performance of both groups improved from day to day, reflecting the functioning of long-term memory. However, learning became slower each day in the fAβ$_{1-42}$-treated compared to the controls, as could be seen from escape latencies (Fig. 1A). A significant difference was detected between the groups also for the swimming distances (Fig. 1B). The Golgi staining method labels the neurons in the HC. Spine density was different between the two groups. In the fAβ$_{1-42}$-treated group, a decrease in density was detected, compared to the controls (Fig. 1C).

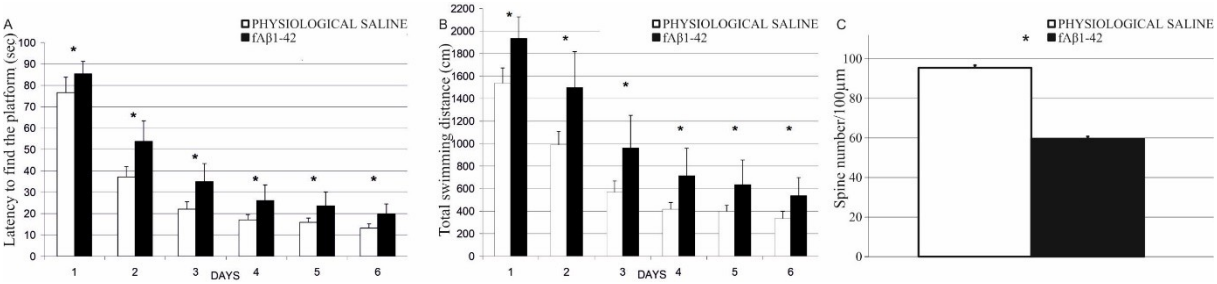

Figure 1. Results of IHC experiment. (A-B) In MWM, the performance of fAβ$_{1-42}$-treated animals impaired compared to the controls. (C) The amyloid treatment induced a decrease in spine density.

EC experiment

OAβ$_{1-42}$ impaired, while LPYFDa improved the learning and memory functions when the smaller platform was used

In the *EC experiment*, we intended to test the effect of EC-administered oAβ$_{1-42}$ in our mMWM protocol, conducted with rats. LPYFDa, oAβ$_{1-42}$, or the combination of both substances were administered directly into the EC of rats, and mMWM was conducted 15 days after the injections. To assess the working memory or trial-dependent learning of the animals, the matching-to-sample paradigm was applied. During the 1st swims of days 1-6, statistical differences could not be detected in the latencies between the groups. In case of the 2nd swims of days 1-5, no differences were found between the groups either. Additionally, the pentapeptide was ineffective in this interval. However, upon the evaluation of the 2nd swims of the 6th day, a significant difference was found in the latencies to find the platform. The post-hoc analysis of the results revealed that the oAβ$_{1-42}$ group had a longer escape latency

compared to the other three groups, which confirms subtle learning and working memory deficits in oA$\beta_{1\text{-}42}$-treated rats, whereas LPYFDa seemed to have a positive effect on the impaired learning and memory capabilities (Fig. 2A). On the 6th day, a new searching strategy of the oA$\beta_{1\text{-}42}$-treated animals could also be observed. During the 2nd trial, they spent a significantly longer time in the annulus zone, searching for the platform, in comparison with the other groups. Besides, an improvement of the searching strategy could also be experienced, which was exerted by the protective LPYFDa (Fig. 2B).

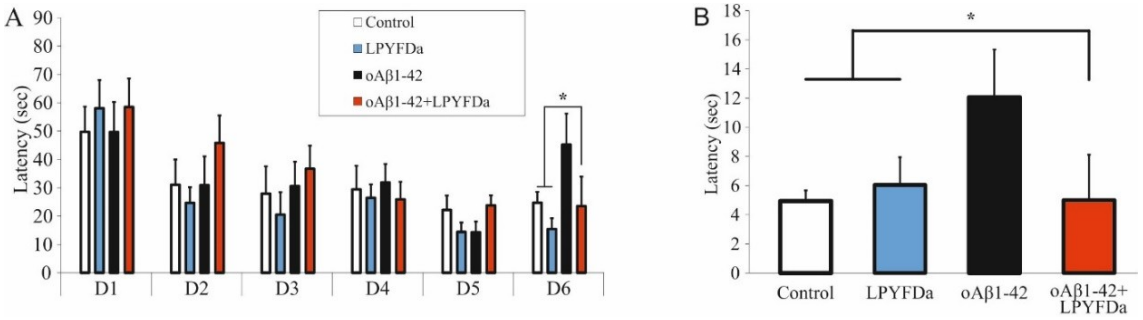

Figure 2. Behavioral results of LPYFDa experiments. **(A)** Escape latencies of the 2nd trials of the four experimental groups during the 6-day mMWM. **(B)** The total duration of searching in the annulus zone, in the 2nd trial, on the 6th day, using the smaller platform.

LPYFDa hindered the oA$\beta_{1\text{-}42}$-induced neuroinflammatory processes in the rat brain

To examine the effect of oA$\beta_{1\text{-}42}$ and LPYFDa on the activation of astrocytes, we applied immunohistochemical staining, and we found elevated numbers of hyperreactive astrocytes in the whole EC of the oA$\beta_{1\text{-}42}$-treated group compared to the other three groups (Fig. 3A). WB analysis also revealed significant differences between the GFAP intensity levels of the different experimental groups, and it corroborated the immunohistochemical results, as the intensity of GFAP was also significantly higher in the amyloid-treated group than in the other groups (Fig. 3B).

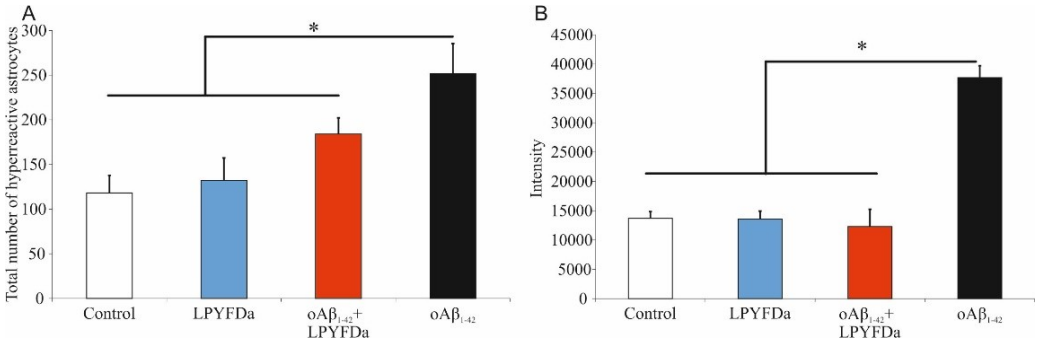

Figure 3. Histology and WB results of LPYFDa experiment. (**A**) Quantitative immunohistochemical results of the GFAP staining. (**B**) WB analysis of GFAP levels.

ICV experiment

Effects of PRE084 and DMT on adult neurogenesis in oAβ$_{1-42}$ and vehicle-treated mice

According to our results, the quantity of BrdU+ stem cells in the DG significantly differed among the six groups. OAβ$_{1-42}$ infusion significantly reduced the number of progenitor cells compared to the respective control group. Interestingly, significantly more severe negative changes were detected in animals treated with DMT. In those co-treated with both oAβ$_{1-42}$ and DMT, hardly any BrdU+ stem cells were detected in the SGZ. PRE084 treatment increased the amount of BrdU+ cells; the difference between the Aβ$_{1-42}$-infused groups was significant (Fig. 4A).

To understand the effects of PRE084 and DMT on the maturation of granule cells, we quantified immature neurons in the DG. To label premature cells, we stained a microtubule-associated protein, DCX, which is expressed specifically in migrating neuronal precursors. The measured DCX densities were significantly different among the six groups. In those treated with oAβ$_{1-42}$-PBS and PBS-PRE084, the number of immature neurons was significantly higher compared to the control group. We also detected a significant difference between the oAβ$_{1-42}$-PBS and oAβ$_{1-42}$-PRE084 groups. DMT administration did not affect the number of premature neurons compared to PBS-PBS mice (Fig. 4B).

To detect and evaluate mature granule cells in the HC, we performed NeuN immunostaining. Again, significant differences were observed among the groups. In DMT-treated animals, significantly lower NeuN+ cell densities were evident in the HC compared to the PBS-PBS and oAβ$_{1-42}$-PBS groups (Fig. 4C).

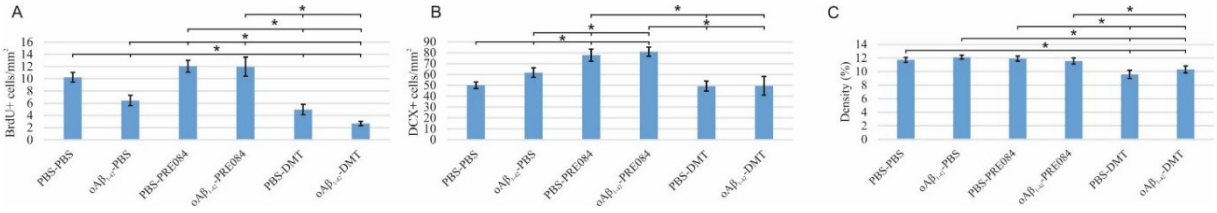

Figure 4. Results for **(A)** BrdU, **(B)** DCX, **(C)** NeuN immunolabeling.

Effects of PRE084 and DMT on neuroinflammation induced by oAβ$_{1-42}$

To identify activated microglia in the HC, we stained Iba1, expressed by microglia. Our results showed a significant difference in the density of Iba1+ microglia among the groups. OAβ$_{1-42}$ administration significantly increased the density of activated microglia compared to the vehicle-treated control groups. In the PBS-DMT group, the density of Iba1+ microglia was significantly reduced compared to PBS-PBS-treated animals. Still, none of the treatments was found to be able to alleviate the proinflammatory effect of oAβ$_{1-42}$ (Fig. 5A).

Significantly different GFAP+ cell densities were detected in the HC of the different groups. A significant increase in the rate of reactivated astrocytes was detected in the oAβ$_{1-42}$-PBS group compared to PBS-PBS-treated mice. Also, GFAP+ cell densities were significantly lower in all other groups compared to oAβ$_{1-42}$-PBS-treated mice. The stimulatory effect of oAβ$_{1-42}$ on astrocyte reactivation was alleviated by PRE084 and DMT administration (Fig. 5B).

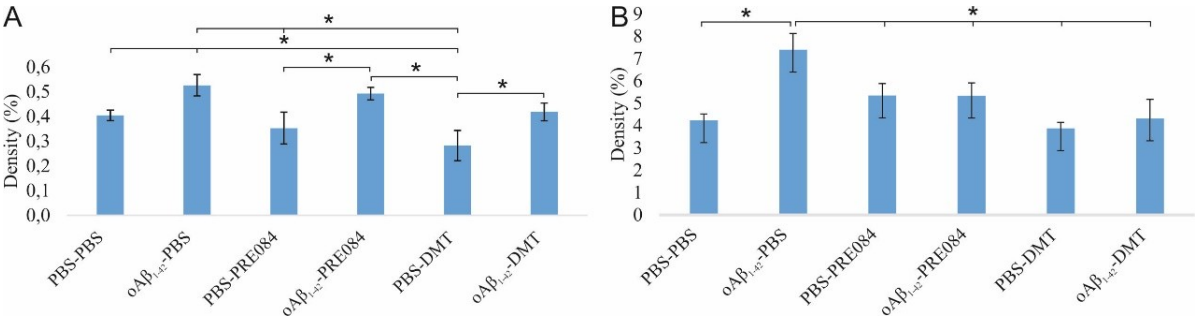

Figure 5. Results for **(A)** Iba1, **(B)** GFAP immunolabeling.

Alternation of S1R protein level as a result of the treatments

To determine the effects of oAβ$_{1-42}$ and PRE084 or DMT on the expression of S1R, a WB analysis was performed on HC and cerebral cortex samples. Our findings revealed a significant difference in the S1R levels among the groups. S1R protein levels were significantly elevated in all groups, except in PBS-DMT-treated animals, as compared to control subjects. In comparison with oAβ$_{1-42}$-PBS-treated mice, the oAβ$_{1-42}$-PRE084 and oAβ$_{1-42}$-DMT groups showed higher protein levels, while significantly lower levels of S1R were detected in PBS-PRE084 and PBS-DMT treated mice. As expected, the co-administration of oAβ$_{1-42}$ and either of the S1R agonists increased the S1R protein level compared to the respective control group. Notably, the expression of S1R was significantly increased in oAβ$_{1-42}$-PRE084-treated animals compared to the oAβ$_{1-42}$-DMT group (Fig. 6).

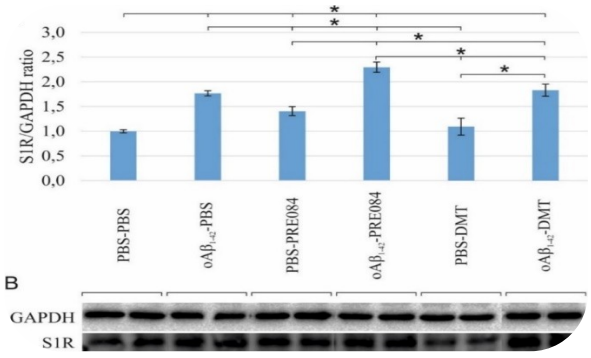

Figure 6. Results for the WB analysis.

DISCUSSION

In our research, we proved that murine models developed by the intraparenchymal and the ICV administration of oAβ_{1-42} or fAβ_{1-42} are valid and reproducible, thus useful paradigms for the detection of early behavioral and molecular symptoms of AD.

In case of the behavioral experiments, we suggested a modified MWM protocol, which can be properly used for the sensitive measurement of mild cognitive deficits caused by oAβ_{1-42} in a rat model. This method for rodents might successfully mimic the changing context in which AD patients have spatial learning and memory difficulties. The oAβ_{1-42}-treated rats showed mild spatial learning and memory impairments when the ratio of searching, and target areas were changed. The protective effect of the pentapeptide LPYFDa was also proven by behavioral and histological experiments.

In the ICV study, we established a model of early AD induced by oAβ_{1-42}, in which acute neuroinflammation, impaired neurogenesis, and elevated S1R levels were detected.

Adult neurogenesis is essential for CNS plasticity. During the treatment of AD, neurogenesis should be promoted, while neuroinflammation should be suppressed. S1R plays an important role in both processes. In our model, two S1R agonists were tested. DMT, binding moderately to S1R but presumably with a high affinity to 5-HT receptors, negatively influenced the early phase of neurogenesis. In contrast, the highly selective S1R agonist, PRE084 improved the proliferation and differentiation of hippocampal stem cells, manifesting in a quantitative increase of progenitor cells and immature neurons. Further experiments are required to investigate the main molecular pathways targeted by DMT, through which it affects neurogenesis and the survival of mature neurons. Moreover, DMT and PRE084 were found to significantly reduce oAβ_{1-42}-induced hyperreactive astrogliosis, however, none of these ligands had a remarkable effect on microglial activation. Therefore, further studies are needed to clarify the role of DMT and PRE084 in neuroinflammatory processes induced by Aβ_{1-42} and to assess the translatability of the results to human AD cases.

ACHIEVEMENTS

The major findings of the present study are as follows:

1. We induced AD-like symptoms by extracellular administration of oAβ_{1-42} or fAβ_{1-42} in two widely used rodent models.

- In the *IHC experiment*, we proved that fAβ_{1-42} decreased spatial learning ability in traditional MWM and reduced dendritic spine density in the rat HC CA1 region.
- In the *EC experiment*, we only observed a subtle but significant spatial deficit in rats when the modified version of MWM was used, i.e., the target was reduced. In this experiment, we noticed neuroinflammation, as we detected increased levels of hyperreactive astrocytes in the EC area.
- In the *ICV experiment*, we observed that AD-like cerebral hippocampal neurogenic and neuroinflammatory changes developed in mice by ICV administration of oAβ_{1-42}.

2. In our experimental models, we tested 3 substances, a neuroprotective pentapeptide (LPYFDa) and two S1R modulators (DMT, PRE084) regarding their effects on molecular processes.
    - In the *EC experiment*, LPYFDa could block the toxic effect of oAβ_{1-42}.
    - In the *ICV experiment*
        - DMT impaired, while PRE084 promoted the survival of progenitor cells.
        - PRE084 increased the number of premature cells, while DMT unaffected their quantity.
        - the density of mature granule cells was unaffected by PRE084 administration, while DMT induced a decrease in neuronal density.
        - oAβ_{1-42} stimulated microglia activation, and neither PRE084 nor DMT alleviated this effect, while DMT alone significantly decreased microglial density.
        - oAβ_{1-42} stimulated astrocyte reactivation, while the administration of DMT or PRE084 reduced this effect.
        - S1R protein level was elevated by oAβ_{1-42} treatment, as well as by the co-administration of oAβ_{1-42} and PRE084 or DMT.

ACKNOWLEDGEMENTS

I would like to thank the following persons without whom I would not have been able to complete and make it through my Ph.D. degree.

I am thankful to my first supervisors and mentors, Prof. Dr. József Toldi and Dr. Katalin Kóródi, for initiating my scientific career.
I am especially grateful to Prof. Dr. Botond Penke and Dr. Zsuzsa Penke-Verdier for their professional mentorship, scientific guidance, encouragement, and support throughout my Ph.D. studies.
I would like to express my special thanks to my last supervisor and mentor, Dr. Lívia Fülöp who made this work possible. I am grateful for her significant support.
I wish to thank all the members of the laboratory, especially Titanilla Szilágyi-Szögi for her excellent help and friendship.
I thank the members of the Department of Medical Chemistry for their help.
Finally, I am extremely grateful to my entire family for their untiring support.
This research was supported by the following grants:
European Union Seventh Framework Programme Food Grant 211 696 (LipiDiDiet) as well as by the Hungarian research grant TÁMOP 4.2.2/A-11/1/KONV-2012-0052.
National Research, Development, and Innovation Office (GINOP-2.3.2-15-2016-00060) and by the Hungarian Brain Research Program I and II - Grant No. KTIA_13_NAP-A-III/7, and 2017-1.2.1-NKP-2017-00002.

1 PUBLICATIONS RELATED TO THE Ph.D. THESIS

I. **LPYFDa neutralizes amyloid-beta-induced memory impairment and toxicity.** Journal of Alzheimer's Disease 2010 Jan;19(3):991-1005.
Ivica Granic, Marcelo F. Masman, Cornelius Mulder, Ingrid M. Nijholt, Pieter J.W. Naude, Ammerins de Haan, Emőke Borbély, Botond Penke, Paul G.M Luiten, Ulrich L.M. Eisel. (IF: 4,261)

II. **Simultaneous Changes of Spatial Memory and Spine Density after Intrahippocampal Administration of Fibrillar Aβ1–42 to the Rat Brain.** BioMed Research International, 2014 June 23; Article ID 345305, doi:10.1155/2014/345305,
Emőke Borbély, János Horváth, Szabina Furdan, Zsolt Bozsó, Botond Penke, and Lívia Fülöp (IF: 1,579)

III. **Impact of two neuronal Sigma-1 receptor modulators, PRE084 and DMT, on neurogenesis and neuroinflammation in an $A\beta_{1-42}$-injected, wild-type mouse model of AD.** Int J Mol Sci. 2022 Feb 24;23(5):2514. doi: 10.3390/ijms23052514.
Emőke Borbély, Viktória Varga, Titanilla Szögi, Ildikó Schuster, Zsolt Bozsó, Botond Penke, Lívia Fülöp (IF: 5,924)

2 PUBLICATIONS AND PATENTS NOT RELATED TO THE Ph.D. THESIS

IV. **A combined electrophysiological and behavioural study for the assessment of activity-dependent changes in mice.** Kóródi K, Bite A, Borbély E, Kovács G, Nagy A, Süle Z, Toldi J. Acta Biol Hung. 2002;53(1-2):85-94.

V. **Overexpression of Hsp27 ameliorates symptoms of Alzheimer's disease in APP/PS1 mice. Cell Stress Chaperones.** Melinda Erzsébet Tóth, Viktor Szegedi, Edina Varga, Gábor Juhász, János Horváth, Emőke Borbély, Balázs Csibrány, Róbert Alföldi, Nikolett Lénárt, Botond Penke, Miklós Sántha 2013 Nov;18(6):759-71.

VI. **Effects of the Pentapeptide P33 on Memory and Synaptic Plasticity in APP/PS1 Transgenic Mice: A Novel Mechanism Presenting the Protein Fe65 as a Target.** Szögi T, Schuster I, Borbély Emőke, Gyebrovszki A, Bozsó z, Gera j, Rajkó R, Sántha M, Penke B, Fülöp L. Int. J. Mol. Sci, 2019 Jun 22;20(12):3050. doi: 10.3390/ijms20123050.

VII. **Post-diaminobenzidine Treatments for Double Stainings: Extension of Sulfide-Silver-Gold Intensification for Light and Fluorescent Microscopy.** Török I, Seprényi G, Pór E, Borbély E, Szögi T, Dobó E. J Histochem Cytochem. 2020 Aug;68(8):571-582. doi: 10.1369/0022155420942213.

VIII. **Neuroinflammatory processes are augmented in mice overexpressing human heat-shock protein B1 following ethanol-induced brain injury.** Dukay B, Walter FR, Vigh JP, Barabási B, Hajdu P, Balassa T, Migh E, Kincses A, Hoyk Z, Szögi T, Borbély E, Csoboz B, Horváth P, Fülöp L, Penke B, Vígh L, Deli MA, Sántha M, Tóth ME. J Neuroinflammation. 2021 Jan 10;18(1):22. doi: 10.1186/s12974-020-02070-2.

IX. **Hippocampal Sclerosis in Pilocarpine Epilepsy: Survival of Peptide-Containing Neurons and Learning and Memory Disturbances in the Adult NMRI Strain Mouse.** Mátyás A, Borbély E, Mihály A. Int J Mol Sci. 2021 Dec 24;23(1):204. doi: 10.3390/ijms2301020.

X. **Examination of Longitudinal Alterations in Alzheimer's Disease-Related Neurogenesis in an APP/PS1 Transgenic Mouse Model, and the Effects of P33, a Putative Neuroprotective Agent Thereon.** Szögi T, Borbély E, Schuster I, Bozsó Z, Sántha M, Tóth ME, Penke B, Fülöp L. Int J Mol Sci. 2022 Sep 8;23(18):10364. doi: 10.3390/ijms231810364.PMID: 36142277.

XI. **Peptides and peptidomimetics for the therapy of neurodegenerative diseases and use thereof.** Fulop L., Penke B., Zarandi M., Bozso Zs., Berkecz R., Janaky T., Martinek T., Datki Zs.,Szegedi V.,Soos K., Penke Zs, Borbely E. (2015) Hungarian Patent Notification, HU2014000207A2.

POSTERS

I. **Behavioural evaluation of a simple, non transgenic model of Alzheimer's disease** Rosta JM, Borbely E, Penke Zs, Penke B, Toldi J 7th International Conference, AD/PD 2005, Sorrento, Italy, March 9-13.

II. **Protective effect of putative drug candidates on the amyloid-beta oligomer-induced astrocyte activation in rat entorhinal cortex** D Simon; E Borbely; E Sipos; K Soós; B Penke; L Fülöp Neurodegenerative Diseases: Alzheimer's and Parkinson's Diseases: Advances, Concepts and New Challenges Prague, Czech Republic 2009.03.11. – 15.

III. **Intracerebroventricular administration of the synthetic AB1-42 to the rat brain. Connection of spatial memory and spine density** E. Borbély, J. Horváth, Á. Kasza, Zs. Frank, S. Furdan, G. Fűr, T. Szögi, K. Németh, L. Fülöp, Z. Bozsó, Z. Penke, B. Penke, 75th Anniversary of Albert Szent-Györgyi's Nobel Prize Award, Szeged, Hungary, 2012.

IV. **Amyloid plaques confer neuroprotection against exogenous Aβ** I. Gureviciene, Á. Kasza, Z. Frank, E. Mugantseva, Z. Datki, E. Borbély, J. Horváth, L. Khirug, B. Penke, H.Tanila, SfN Annual Meeting, New Orleans, LA, 2012.

V. **Administration of the synthetic Aβ1-42 to the rat brain: comparison of intracerebroventricular and intrahippocampal injection model in spatial memory** E. Borbély, Á. Kasza, J. Horváth, Zs. Frank, Sz. Furdan, G. Fűr, T. Szögi, K. Németh, L. Fülöp, Zs. Bozsó, Z. Penke, B. Penke IBRO International Workshop 2012, Szeged, Hungary, January 19-21.

VI. **Intracerebroventricular and intrahippocampal administration of the synthetic aβ1-42 to the rat brain. connection of dendritic spine density and spatial memory.** E. Borbély, J. Horváth, Sz. Furdan, G. Fűr, T. Szögi, Zs. Bozsó, B. Penke, L. Fülöp**,** 11th International Conference, AD/PD 2013, Florance, Italy, March 6-10.

VII. **Novel Method for *In Vivo* 3D Monitoring of the Hippocampal Neurodegeneration in Sleep Deprived Hairless Mice** E. Mozes, Zs. Datki, B. Penke, E. Borbely, J. Horvath, L. Fulop 11th International Conference, AD/PD 2013, Florance, Italy, March 6-10.

VIII. **Krónikusan injektált védőanyag hatásának vizsgálata a térbeli memóriára és a dendrittüske sűrűségre APP/PS1 transzgenikus egerekben** Furdan Szabina, **Borbély E.**, Horváth J., Penke B., Fülöp L. XIII. Alzheimer-kór Konferencia és a Magyar Neuropathológiai Társaság 2013. éves Kongresszusa, 2013, Debrecen, Magyarország, Szeptember 20-21.

IX. **Az alvásmegvonás hatásának *in vivo* vizsgálata egér hippokampuszban** Mózes E., Borbély E., Horváth J., Furdan Sz., Datki Zs., Penke B., Fülöp L. XIII. Alzheimer-kór Konferencia és a Magyar Neuropathológiai Társaság 2013. éves Kongresszusa, 2013, Debrecen, Magyarország, Szeptember 20-21.

X. **Effects of chronically injected neuroprotective compound on spatial memory and hippocampal dendritic spine density in APP/PS1 transgenic mice** Sz. Furdan, E. Borbély, J. Horváth, B. Penke, L. Fülöp IBRO International Workshop 2014, Debrecen, Hungary, January 16-17.

XI. **Application of fear conditioning and fear extinction assay on anxious (Ax) and non-anxious (nAx) mice** Á. Szántai, E. Borbély, G. Müller, L. Fülöp, V. Szegedi IBRO International Workshop 2014, Debrecen, Hungary, January 16-17.

XII. **Effects of the neuroprotective pentapeptide, p33 on the neurogenesis in APP/PS1 mice** T. Szögi, E. Borbély, D. Tüdős, B. Penke, L. Fülöp 13th International Conference on Alzheimer's and Parkinson's, AD/PD 2017, Vienna, Austria Mar 29 – Apr 2.

XIII. **Longitudinal examination of the neurogenesis in transgenic Alzheimer's disease mice model** T. Szögi, E. Borbély, D. Tüdős, B. Penke, L. Fülöp 13th International Conference on Alzheimer's and Parkinson's, AD/PD 2017, Vienna, Austria Mar 29 – Apr 2.