NOVEL APPROACHES FOR PROCOGNITION AND NEUROPROTECTION – SYNTHETIC AND HERBAL AGENTS

Botond Penke,Lívia Fülöp
Judit Hohmann
The University of Szeged, Hungary
Kriszta Zolnay
The Pharma Kígyó Ltd.

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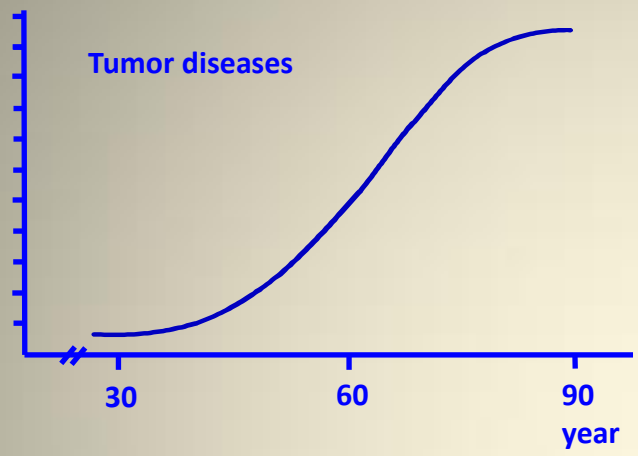
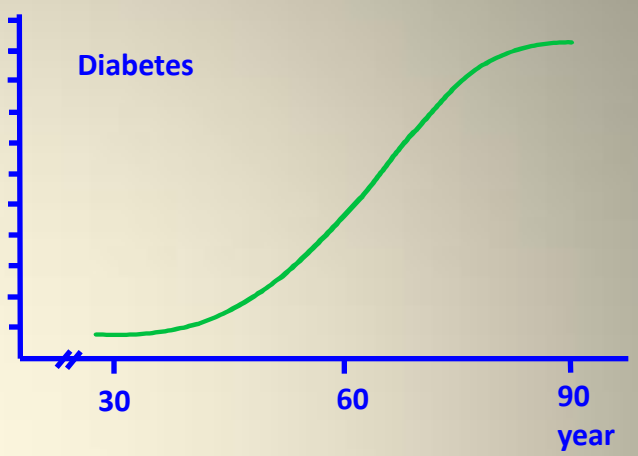
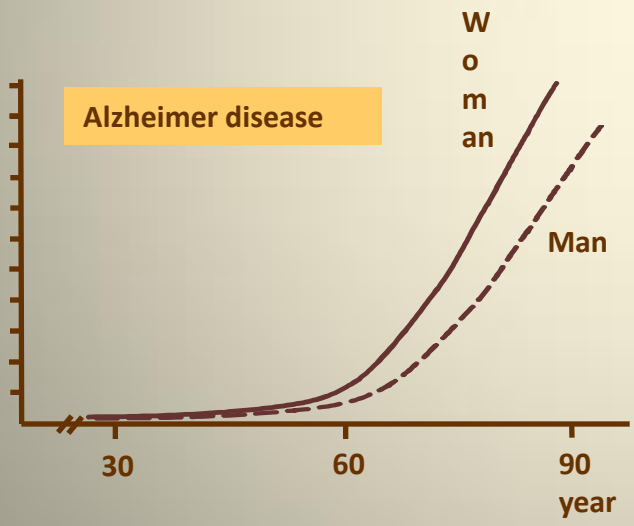
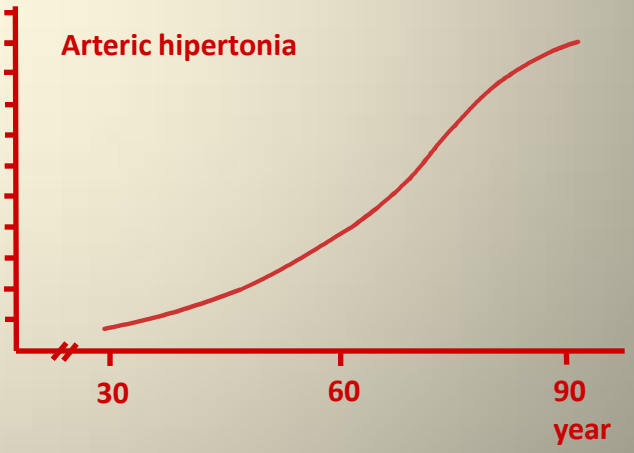
DISEASES COMING WITH AGEING









Growing life expectancy – growing social and family burden with aging diseases

DISEASES WITH COGNITIVE DISTURBANCES

Cognitive disorders are a category of mental health disorders that primarily affect the coupling of brain hemispheres, learning, memory, perception, concentration and problem solving

- Amnesia; Delirium
- Dementia (AD, PD, HD)
- Mood disorders: depression
- Psychotic disorders: schizophrenia

NEURODEGENERATIVE DISEASES

Alzheimer's D.
Parkinson's D.
Amyotrophic lateral sclerosis
Polyglutamine D. (Huntington D.)
Prion D. (CJD)
Lewy-body dementia
FTDP-17+Pick D.

INDENTICAL PATHOMECHANISMS IN NEURODEGENERATIONS?

ER- STRESS-RELATED DISEASES

- Alzheimer's D.
- Parkinson's D.
- Amyotrophic lateral sclerosis
- Polyglutamine D. (Huntington D.)
- Prion D. (CJD)
- Lewy-body dementia
- FTDP-17+Pick D.
- Type 2 diabetes

PROTEIN CONFORMATION DISEASES

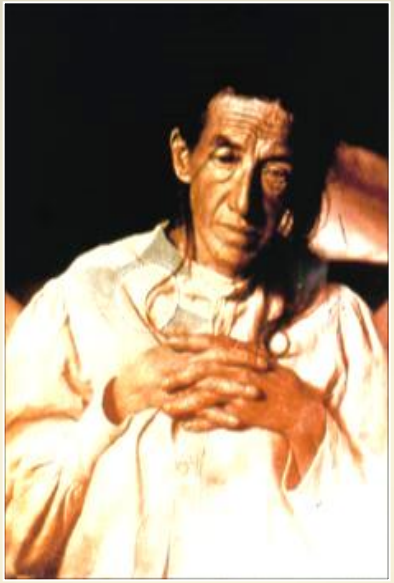
- Alzheimer's D. (Aβ, Tau)
- Parkinson's D. (α-syn)
- Amyotrophic lateral sclerosis
- Polyglutamine D. (Huntington D.)
- Prion D. (CJD)
- Lewy-body dementia (α-syn)
- FTDP-17+Pick D.
- Type 2 diabetes

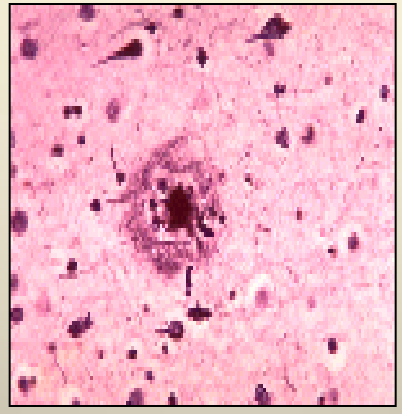
(J. Kim et al: Nature Rev. Drug Disc. 7, 1013-1030, 2008)

New target for neuroprotection: maintaning the protein processing system may help to stabilize the healty homeostasis of proteins.

DR. ALOIS ALZHEIMER (1864-1915)

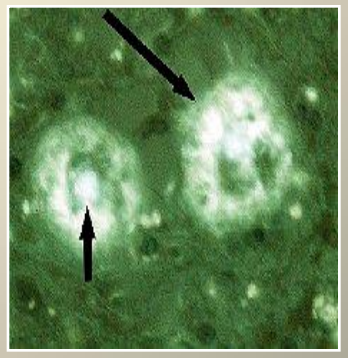
Auguste D (1906) 51 years old





Amyloid plaques

Neurofibrillary tangles



ALZHEIMER'S DISEASE – THE MOST FREQUENT DEMENTIA

Familial AD (early onset 1%): mutations in APP; PS1 or PS2 genes.

Sporadic AD (late onset, 99%): a multifactorial, polygenic neurodegeneration with wide clinical heterogeneity. Common side: β-amyloid accumulation

Heterogeneous background:

- Hypoxia, hypoglycemia, vascular disturbances,
- Energy-metabolism disturbances (astroglia malfunction)
- Neuroinflammation, microglia malfunction
- ER-stress, ER-mitochondrial cross talk disturbances, UPR
- Intra-and extracellular formation (acculamation of diseased-form Aβ)
- Synaptic malfunction and failure
- Prion-like spreading (directly cell-to-cell or by exosomes)

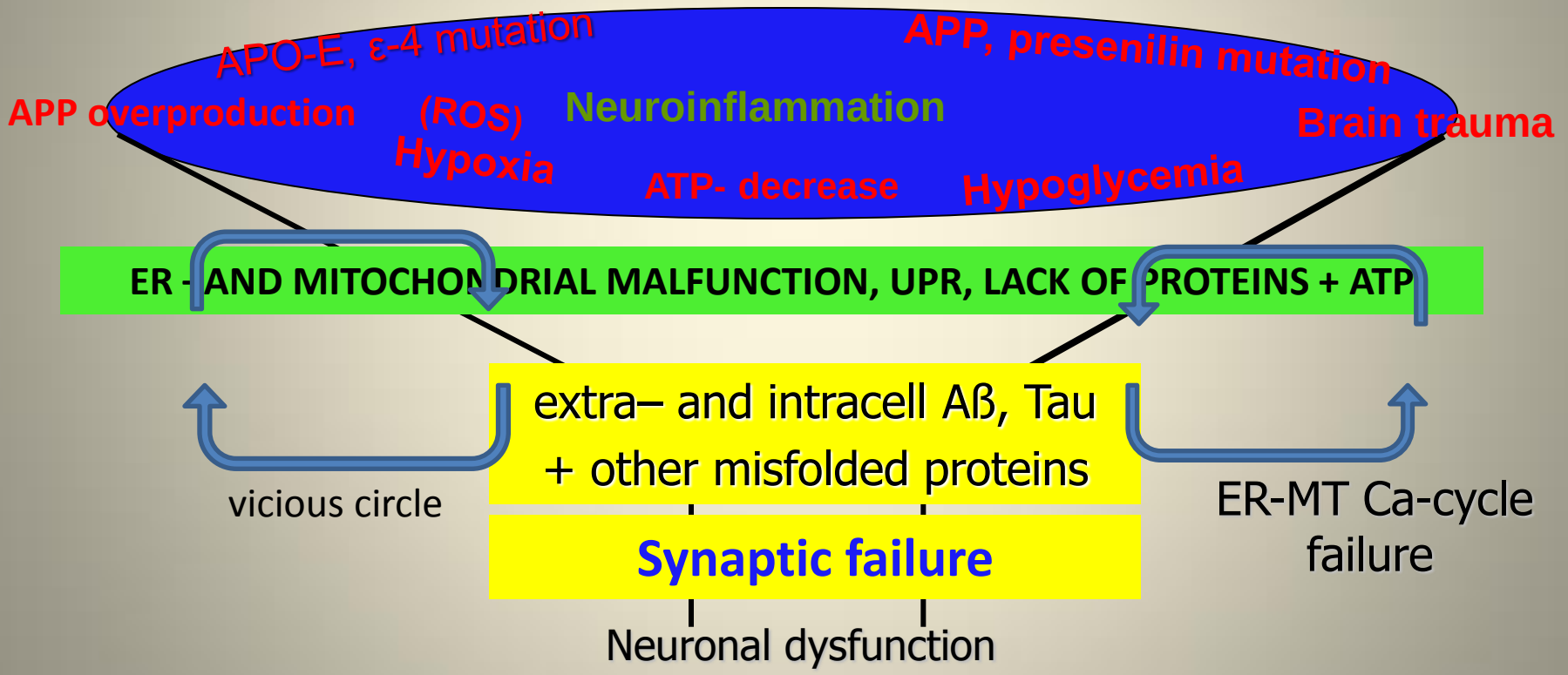
AD: CROSSTALK OF GENES AND ENVIRONMENTAL FACTORS

Genes: BIN1, ApoE4, ABCA7, SORL1, NME8, CR1, PICALM, PLD3, PTK2B, CD33, CASS4, EPHA1, APP, PS1, PS2, etc.

Epigenetic factors:

AGEING

Environment:
Hypoxia, Hypoglicemia, ROS, Brain traumas, Viruses, MT-ER toxins (herbicides perticides), etc.



Z. Datki, B. Penke, L. Fülöp: to be published

Neuron death → cell to cell spreading

THE TRANSMISSION OF PRION-LIKE PROTEINS

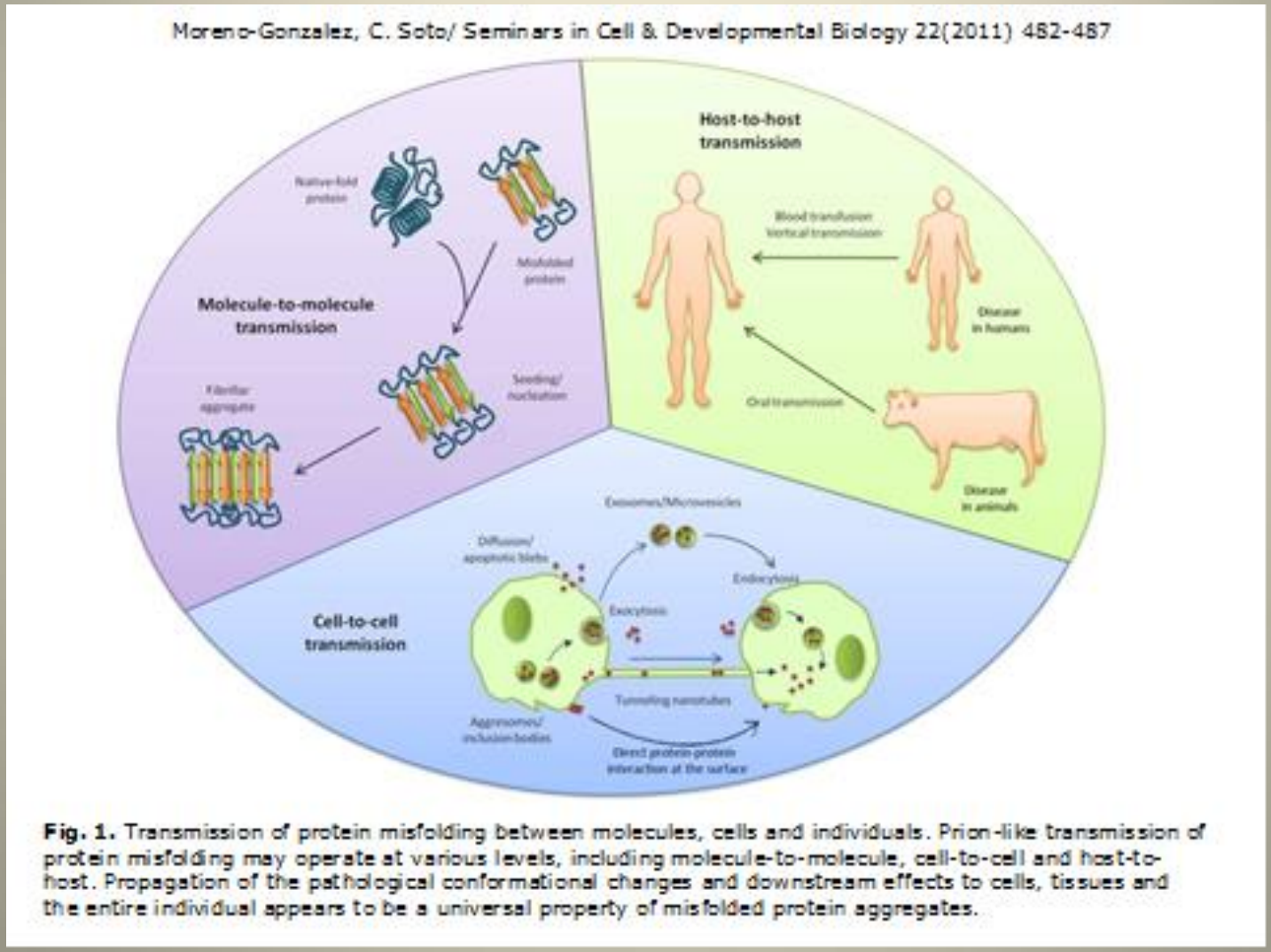


Fig. 1. Transmission of protein misfolding between molecules, cells and individuals. Prion-like transmission of protein misfolding may operate at various levels, including molecule-to-molecule, cell-to-cell and host-to-host. Propagation of the pathological conformational changes and downstream effects to cells, tissues and the entire individual appears to be a universal property of misfolded protein aggregates.

NEUROPROTECTIVE DRUGS AND COGNITIVE ENHANCERS

Improving mental functioning by altering the supply of neurons

- increasing circulation to the brain
- providing neurotransmitter precursors
- providing usable energy to the brain
- improving neuron functions
- preventing oxidative damage of brain cells

Neuroprotective substances:

Neurotrophins (BDNF, GDNF, etc.); antioxidants (EGCG and resveratrol);

AChE-inhibitors; mitochondria-directed AD/ALS medicines;

Ca^{2+} level↓ (Dantrolexe (RyR antagonist); Memantine, Xestospongin-C)

Chemical chaperones (PBA, TUDCA); ER-stress – UPR modulators (HSP-70 inducers, GSKZ 606414, KIRA6,)

Antibodies against toxic misfolded proteins

Cognitive enchancers: Nootropics (originally developed to combat specific disorders (ADHD))

- L-phenylalamine, L-tyrozine, L-glutamine
- Phoshatidyl-choline, ~ serine (ω -3, ω-6)
- Acetyl – l – carnitine
- Gingkolides (flavone glycorides), Huperzin-A, etc.
- Synthetics: amphetamine, methylphenidate, Tianeptine, Valproate, racetams, armodafinil, etc.

AD DRUG CANDIDATES IN THE PIPELINE – CLINICAL TESTING

1, Docosahexaenoic acid (DHA); two Phase 3 trials
2, Methylene blue (Rember), Phase 2.
3, PBT-2 (clioquinol); Phase 2.
4, Resveratrol; Phase 3
5, ACC-001; Phase 2.
6, AN-1792; Phase 2. (Discontinued)
7, Bapineuzumab (AAB-001); Phase 3.
8, Davunetide (AL-108); Phase 2. completed
9, Dimebon (latrepirdin); Phase 3. (completed, results negative)
10, Intravenous immunoglobulin (abbreviated IVIG, Gammagard); Phase 3.
11, Rosiglitasome (Avandin); Phase 3., completed, results negative
12, Semagacestat (LY 450 139); Phase 3. discontinued
13, Solanezumab (LY 2062430); Phase 3.
14, Tarenflurbil (Fluorizan); Phase 3. completed; results negative
15, Tramiprosate (Alzhemed); Phase 3. completed; results negative
16, Bexarotene

WHY THE FIRST AD DRUG CANDIDATES HAVE BEEN UNSUCCESFUL?

1, A very simplified disease model has been used for drug design (cholinergic, Glu-erg, extracellular plaque hypotheses, etc.)

2, Toxic, misfolded proteins have intracellular origin, and thus, drug targets are inside (endoplasmic ret., lysosomes, mitochondria, Golgi).

3, Antibody therapy: their concentration is too low in the brain and works only in the extracellular space.

4, Lack of suitable cell, tissue and animal models; translation problems

5, Phase II/Phase III studies: the patients' groups have been very heterogeneous

6, Very high expectations, high pressure from the side of the society, wrong decisions

Final consequences: AD is a heterogeneous, multifactorial disease, with a common final mechanism: intracellular formation of misfolded proteins.

The real AD drug targets are mostly intracellular proteins and mechanisms!

NEW ERA IN AD DRUG DESIGN – NEW POSSIBILITIES

AD has a prolonged preclinical phase, probably over 10-15 years.

Interaction between genetic and environmental factors play key role in the sporadic AD.

Genome analysis indicates genetic disposition for AD (e.g. ApoE4 genes).

Prevention and pre-clinical treatment of progressive cognitive impairment (MCI) may postpone AD onset.

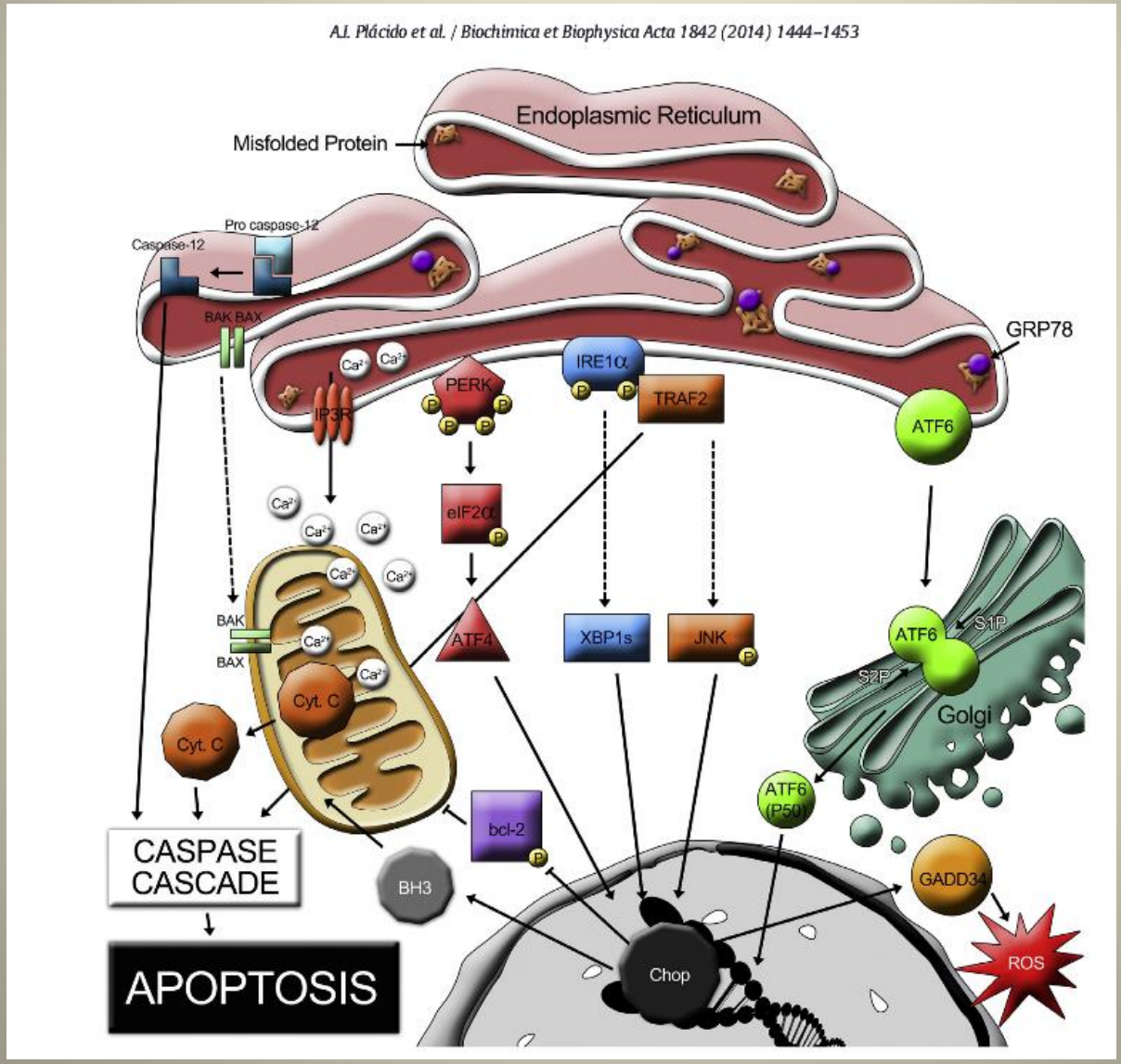
("It is never too early to take preventive measures from senility and dementia.")

Targeted therapy: elimination of diseased-form toxic proteins
halting prion-like spreading

We use three different ways for neuroprotection:

1) Maintaining healthy homeostasis of proteins – by ER-chaperone induction;
2) Preventing intracellular Aβ formation – by modulation of APP endocytosis;
3) Use of neuroprotective and procognitive herbal substances.

ER-STRESS: PRO-SURVIVAL PATHWAYS
CHRONIC ER-STRESS: APOPTOSIS

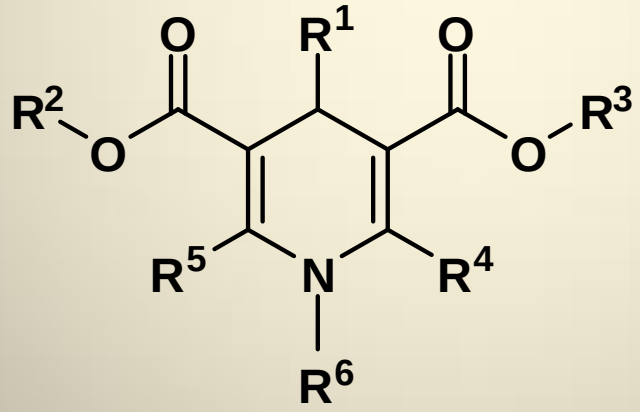


Multi-proteino-athy in AD:
Aβ
Tau
α-Syn
TDP-43

N°1 NOVEL DRUG CANDIDATE: AN ER-CHAPERONE INDUCER

The main aim is to restore and maintain healthy protein folding (protein homeostasis):

ER-chaperone inducers may modulate chronic endoplasmic reticulum stress and prevent apoptotic cell death by induction native folding.



Our novel drug candidates:
1,4 pyridine derivatives
LA series: LA101, LA 1011, etc.

Vígh L., Török Zs., Fülöp F., Penke B.
Hungarian Patent: P 1.200.680; 70 9 336 /GM/PCT/HU2012/00126

ANIMAL MODELS FOR PROCOGNITION AND NEUROPROTECTION

Behavior models for procognition:

- Attention (vigilance: Five-choice test)
- Reasoning and problem solving: attentional set-shifting
- Working memory span capacity
- Recognition memory: novel object recognition task
- Long-term memory and learning:

  Morris water maze
- speed of processing: Olfactory discrimination

Animal models for neurodegenerative diseases:

- Infusion models (intoxicating animals with Aβ, MPTP environmental toxins, etc.)
- Transgenic animals (Aβ, huntingtin, TDP-43, α-syn overproduction)
- Behavioral pharmacology for drug testing (NDR, Morris water maze, etc.)

The basic problem is the translation of results to human.

THE BIOLOGICAL EFFECTS OF LA1011

Efficacy in animal models *in vivo*

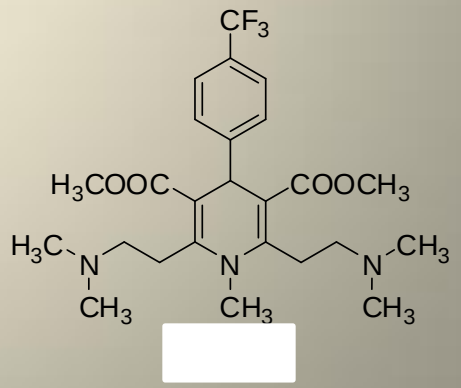
- *Neuroprotection*
  - Alzheimer's disease (APP_{swe} x PS1 mouse model)
  - ALS (B6SJL-Tg(SOD1*G93A)1Gur/J and B6.Cg-Tg(SOD1*G37R)42Dpr/J) mouse models)
  - Procognitive activity (6 month administration, mouse, MWM)
  - BBB penetration
- *Insulin resistance* (Zucker obese rat)
- *Anticancer activity* (mouse, B16 melanoma, C26 colon)
- *UV induced skin damage* (SKH-1 hairless mice)

Toxicity

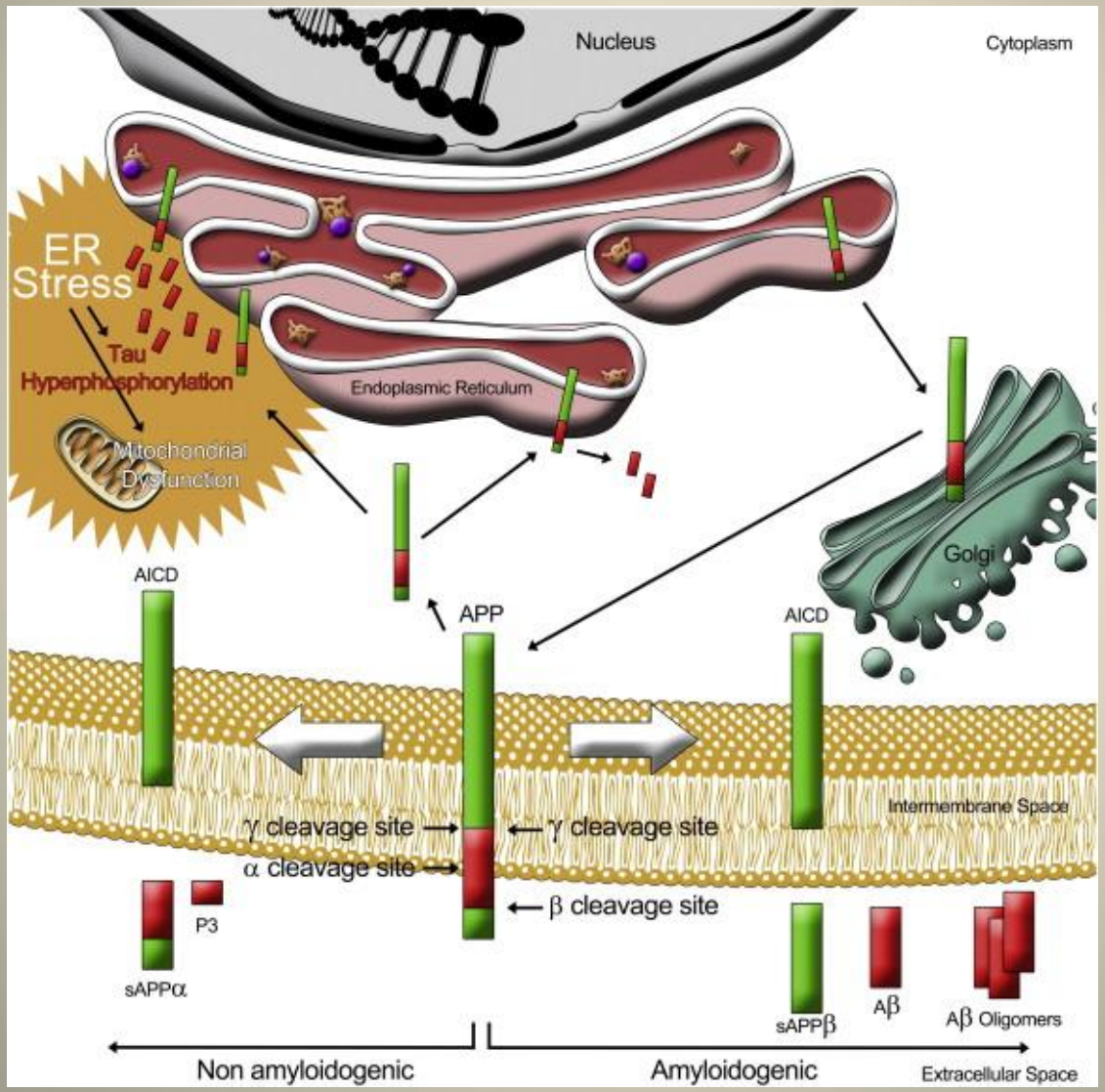
- *single dose* (mouse)
- *6 months effective dose* (mouse)

Ion channel profiling

- *L-type Ca-channel*
- *hERG channel*



N°2 NOVEL DRUG CANDIDATE: APP ENDOCYTOSIS MODULATOR BFR106



Al. Plácido BBA 2014.

PRECLINICAL STUDIES ON DRUG CANDIDATE BFR106

1) In vitro studies in SH-SY5Y cell culture: BFR-106 shows neuroprotective effect against β-amyloid.
2) In vivo electrophysiology studies in rat models: ip. administered BRF 106 protects neurons against β-induced firing.
3) In vivo LTP studies in rats: BRF 106 protects neurons against Aβ-induced decrease of LTP (theta oscillation coupled firing)
4) Ex vivo LTP studies in rat hyppocampal slices in organ bath
5) Behaviour and learning studies in an AD mouse model (APPxPS1 tg mice)
   (a 6-month experiment, ip. adminstration, daily 3mg/kg)
   - Open field activities
   - Morris water maze – almost perfect neuroprotection

6) Histological studies in APPxPS1tg mice after 6 month ip. BFR 106:
   - neuronal cell viability (cresyl violet)
   - dendritic spine density
   - amyloid accumulation

NOVEL DRUG CANDIDATE N°3: PROCOGNITIVE AND NEUROPROTECTIVE HERBAL SUBSTANCES

1. Ginseng – Panax ginseng – ginsenosides as phytochemicals
2. Ginkgo – Ginkgo biloba – ginkgolides; 240mg/day; only preventative
3. Gotn Kola – Centella asiatica – brain blood circulation/working memory
4. Green tea – Camellia sinensis – epigallocatechin gallate (EGCG), AChE↓
5. Bacopa – Bacopa monnieri – antioxidants
6. Ashwaganda – Withania somnifera – AD drug candidate; neurogenesis
7. Rhodiola – Rhodiola rosea – polyphenols, proanthocyanidins
8. Huperzia – Huperzia serrata – huperzin – A: AChE↓, NMDA-R antag.
9. Sage – Salvia officinalis – flavonoids, antioxidants, anti-inflam., AChE↓
10. Rosemary – Rosmarinus officinalis – antioxidants, 1,8 – cineole; ACh ↑
11. Lemon balm – Melissa officinilis – nicotinic muscarinic Ach agonists
12. Turmeric – Curcuma longa - curcumin

Bacopa

Huperzia

Rhodiola

Rosemary

ESTABLISHMENT OF A RESEARCH CENTER FOR R&D OF NEUROPROTECTIVE AND PROCOGNITIVE HERBAL SUBSTANCES

The main aims of the new research center:

1) Utilization of herbal medicines for neuroprotection and procogniton (medical device)
2) Standardization of delivery of herbal substances (right place, right concentration, right period of time)

R&D studies:

- Isolation and structure determination of novel herbal substances
- Pharmacologic studies (pure compounds and plant extracts)
  - In vitro experiments in cell lines for neuroprotection
  - In vivo studies in rodent models of neurodegenerative diseases
  - In vivo studies for procognitive effects
- ADME studies
- Acute and chronic toxicity (?)
- Mechanism of action studies
- Application of novel drug delivery systems (solid/ lipid nanoparticles, matrix systems, hydrogels, core-shell nanoparticles for controlled release) Hungarian Patent Application: N°1300744 (2013)

DEPARTMENT OF PHARMACOGNOSY ACTIVITIES

- Crude, fractionated or purified extracts for pharmacological screening
- Bioactivity-guided fractionation and isolation of active compounds
- Structure elucidation of pure compounds
- Development of analytical method for extract standardization
- Quality control of (purified) extracts applied in pharmacological studies

RESEARCH GROUPS, FACILITIES IN INSTITUTE OF MEDICAL CHEMISTRY, SZEGED UNIVERSITY

1. Molecular Simulation and Drug Design
2. Synthetic and Analytical Chemistry
3. Protein Interactions and NMR Methods
4. Cell/tissue Culture and Fluorescence Methods
5. Proteomics Laboratory
6. Electrophysiology Laboratory
7. Behaviour and Histology Laboratory
8. Transgenic Mice Facility

SUMMARY – PUTATIVE R&D PROJECTS FOR COOPERATIONS

THERE ARE NEW WAYS AND NOVEL TREATMENT POSSIBILITES FOR NEUROPROTECTION AND PROCOGNITION.

1) **Project N°1**: Neuroprotection with prevention of ER-stress and protein misfolding (e.g. LA1011).

Tasks: continue and close preclinical studies for MOA; perform toxicity studies; dossier; Phase I.

2) **Project N°2**: Neuroprotection with prevention amyloid formation and APP trafficking (e.g. BFR 106)

Tasks: continue and close preclinical studies ;MOA;

chronic toxicity studies; dossier; Phase I.

3) **Project N°3**: Establishment of a herbal research center for neurodegenerative – procognitive studies

Tasks: a new R&D center foundation, on the basis of the research laboratories of The University of Szeged, The Pharma Kígyó Ltd and cooperating partners

SUMMARY – TAKE-HOME MESSAGE

There is a new era in cognitive enhancers, neuroprotection and neurorepair.

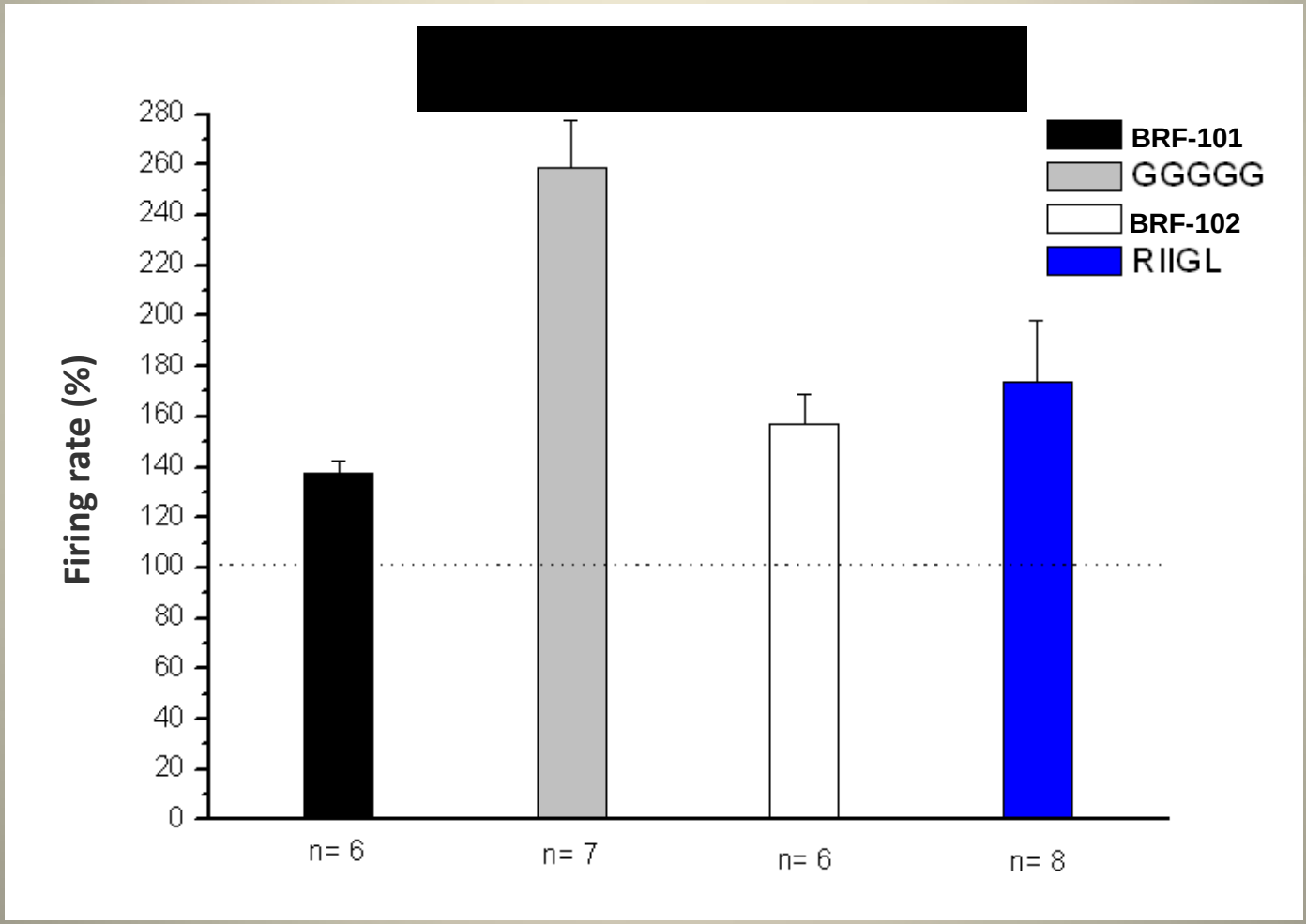
The main aim is the prevention of neurodegenerative processes protecting neurons e.g. by nourishing and detoxifying. Clear evidence of protective effects in humans requires long-term administration of substance and monitoring over a period of years.

Most of neurodegenerative diseases are protein conformational and simultaneously ER-stress related disorders. Induction of chaperones (MPS-70, ER-chaperones) provide an effective method of neuroprotection (Our novel compound: CA 1011 proved ot be neuroprotective in vivo in an AD mouse model)

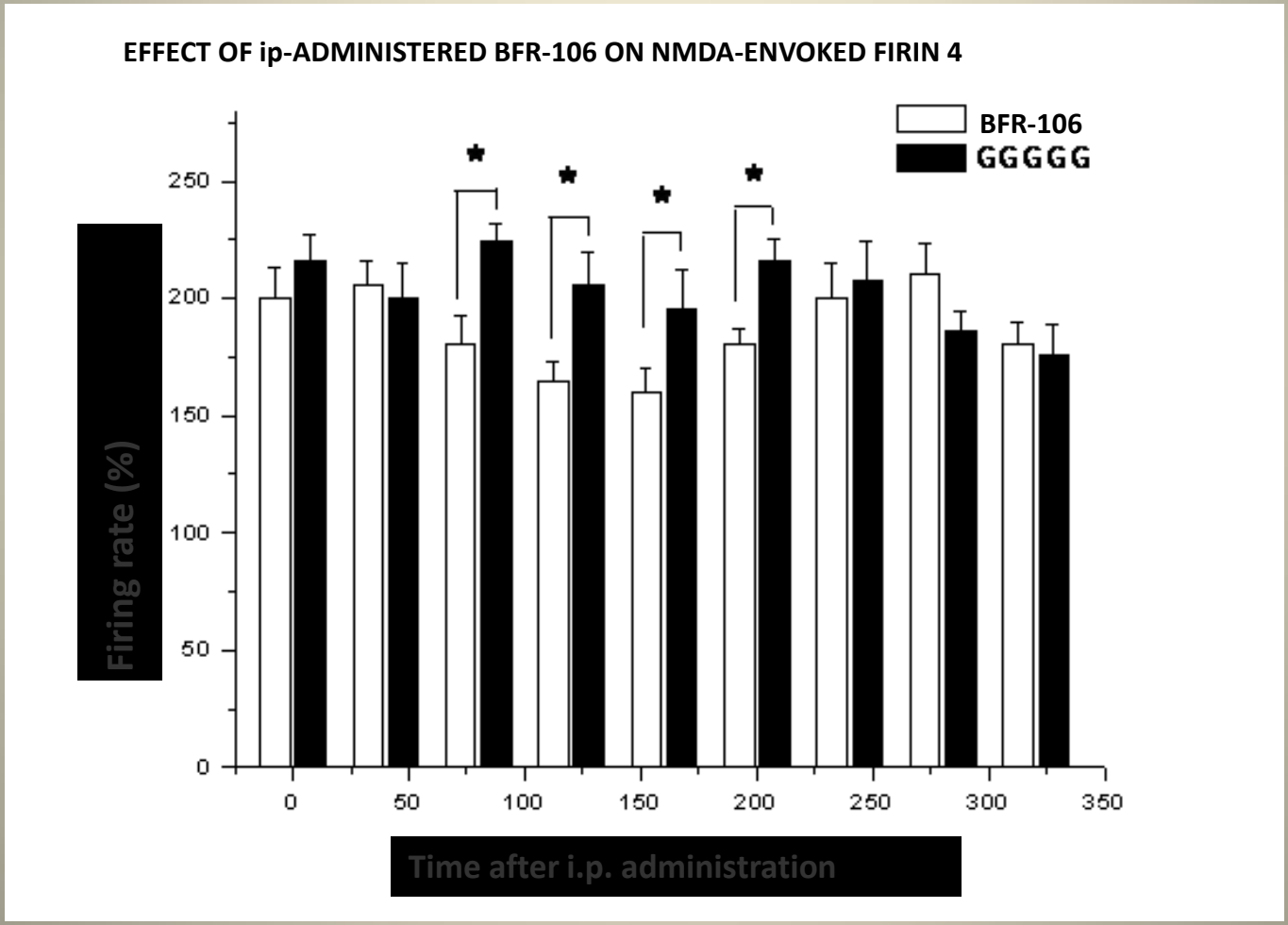
Modulation of β-amyloid formation by peptido-mimetics (like a novel drug canditate „apape”) was effective in neuroprotection (in vivo, in an AD mouse model).

Herbal substances have the ability to regenerate neuronal network. The active components of herbs should be identified and studied for their mechanism of action.

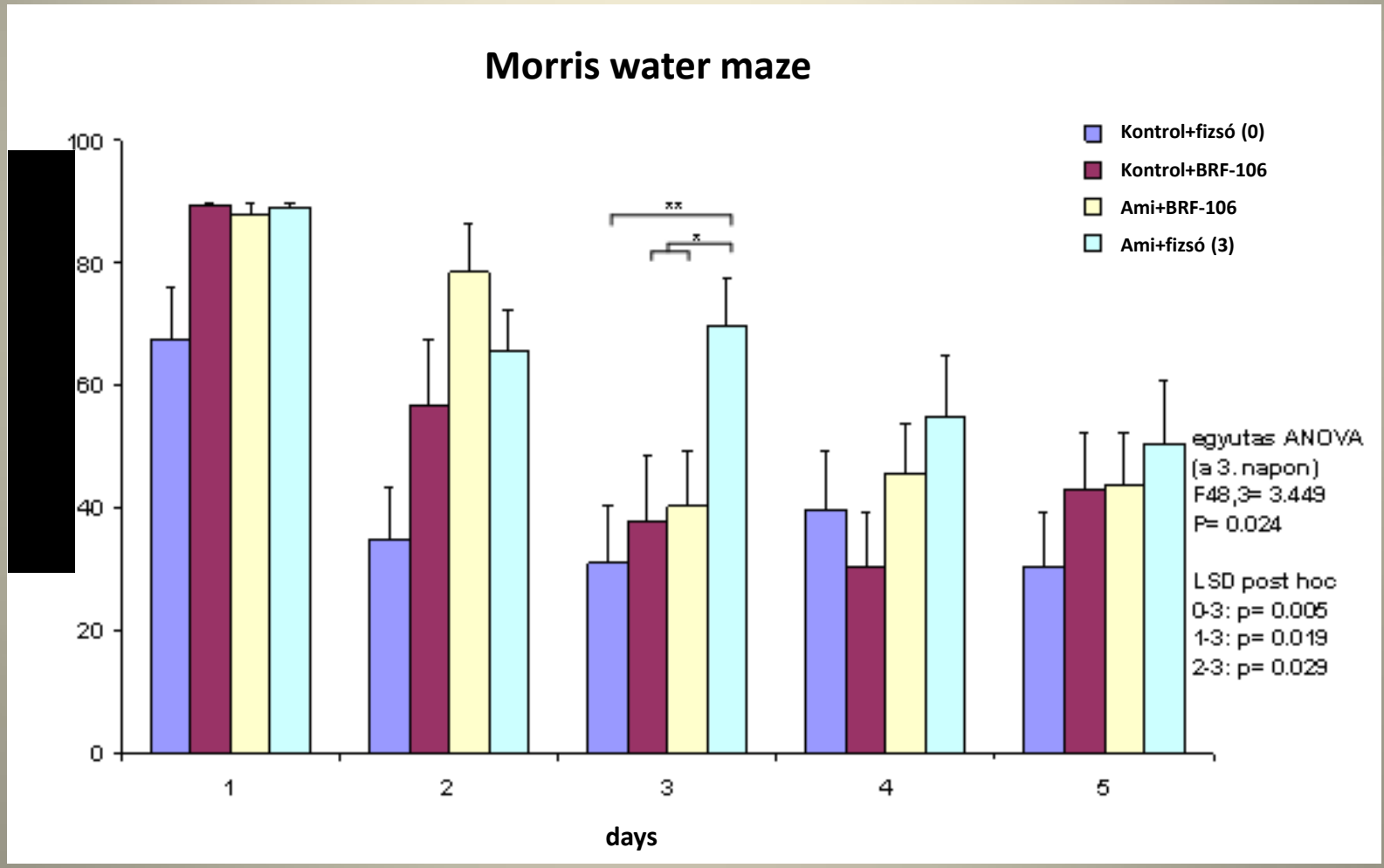
EFFECT OF BFR-101 ON NMDA-EVOKED FIRING RATE



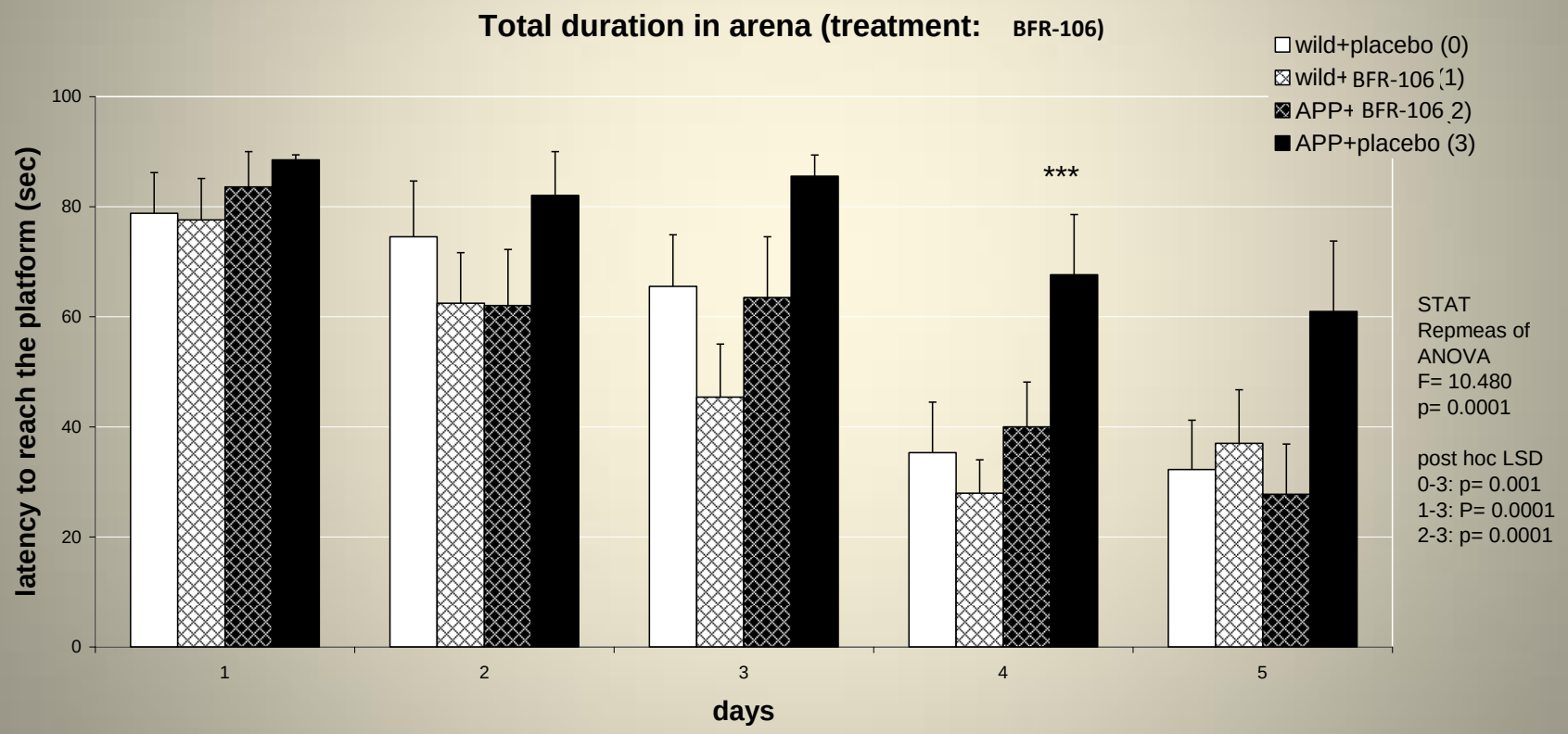
EFFECT OF ip-ADMINISTERED BFR-106 ON NMDA-EVOKED FIRING



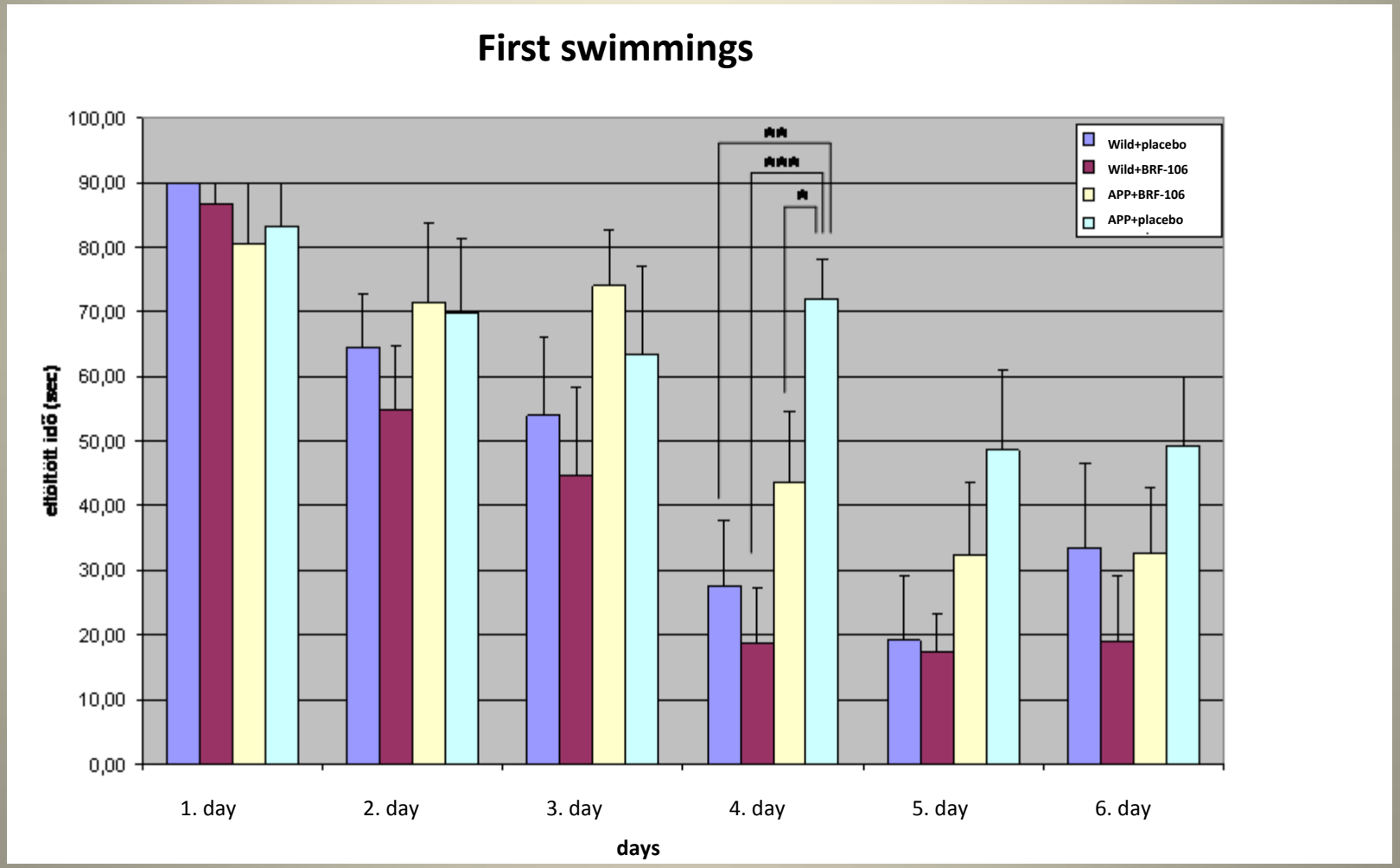
NEUROPROTECTIVE EFFECT OF BFR-106 IN OUR RAT MODEL OF AD (icv soluble Aβ 1-42)



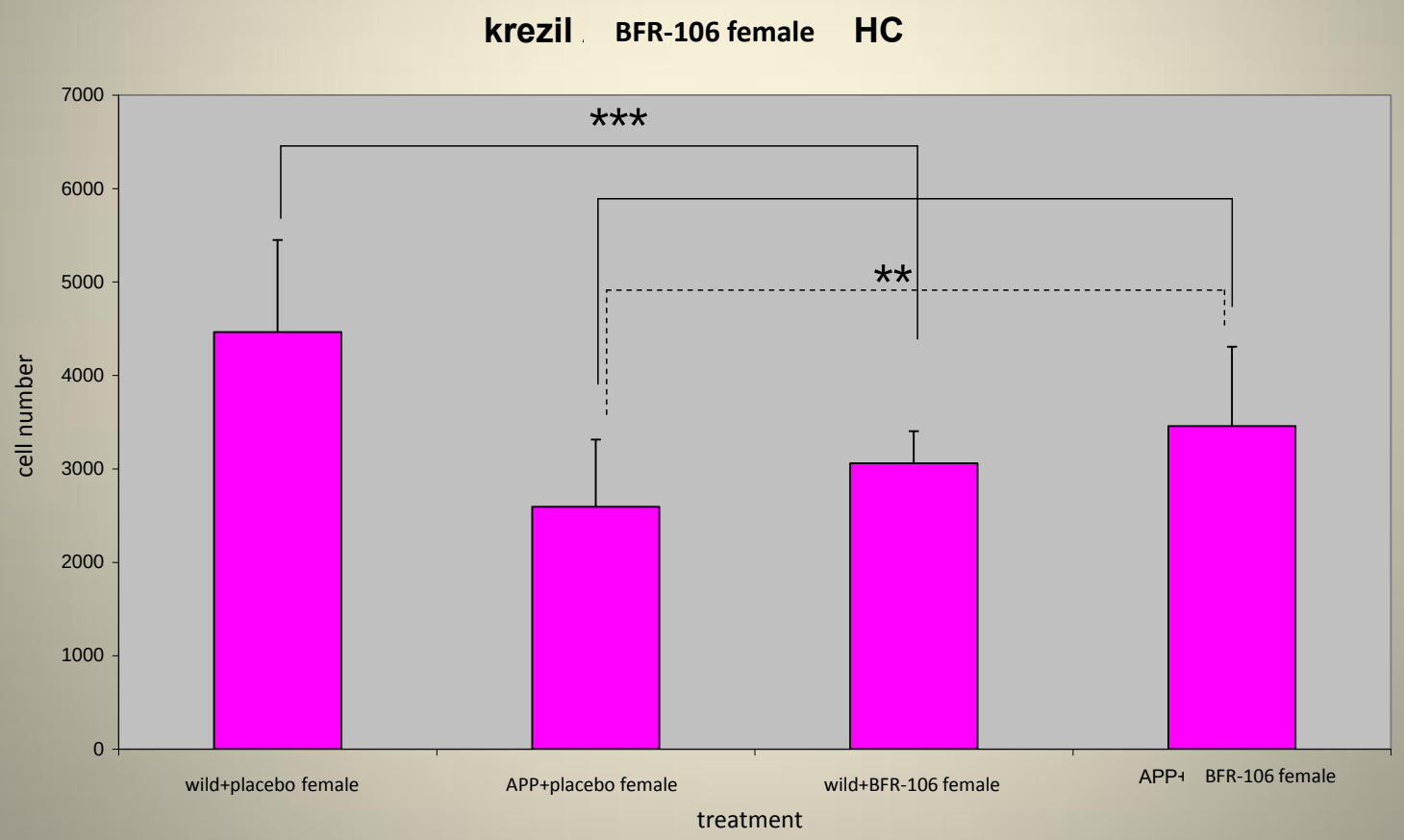
NEUROPROTECTIVE EFFECT OF BFR-106 IN APPxPS1 MICE (MWM)



NEUROPROTECTIVE EFFECT OF BFR-106 IN APPxPS1 MICE (MWM)

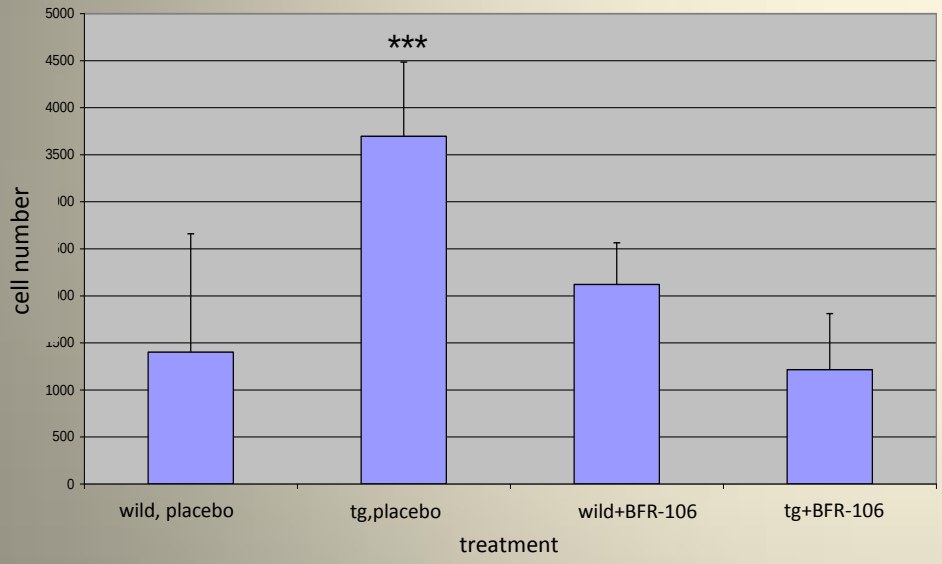


NUMBER OF VIABLE NEURONS AFTER BFR-106 TREATMENT, APPxPS1 MICE

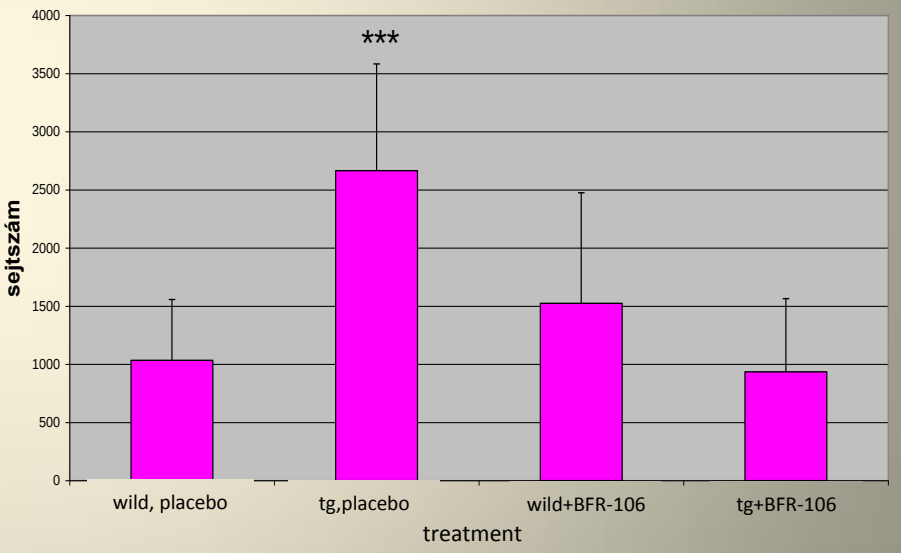


NEUROPROTECTIVE EFFECT OF BFR-106 IN APPxPS1 MICE
TAU-IMMUNOCHEMISTRY

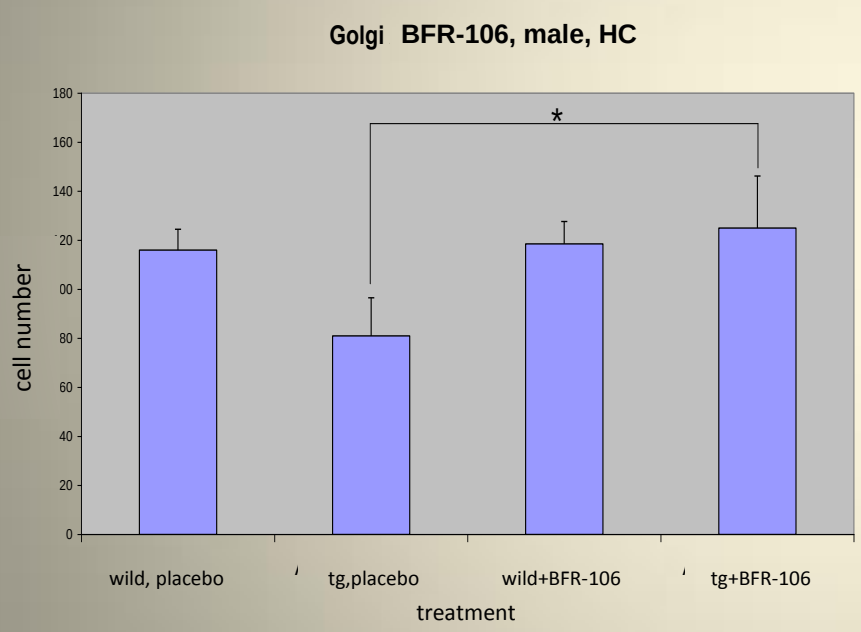
Tau BRF-106, male, HC

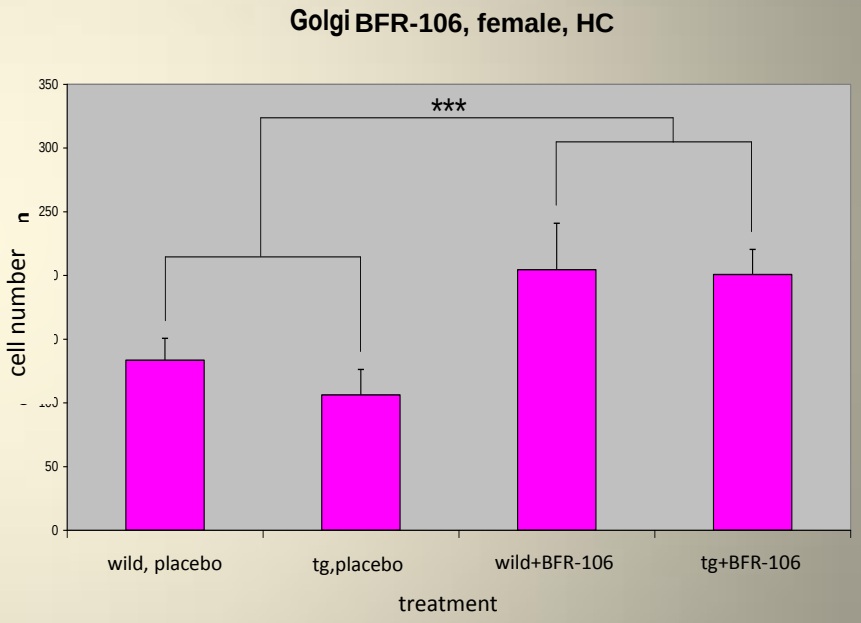


Tau BRF-106, female, HC

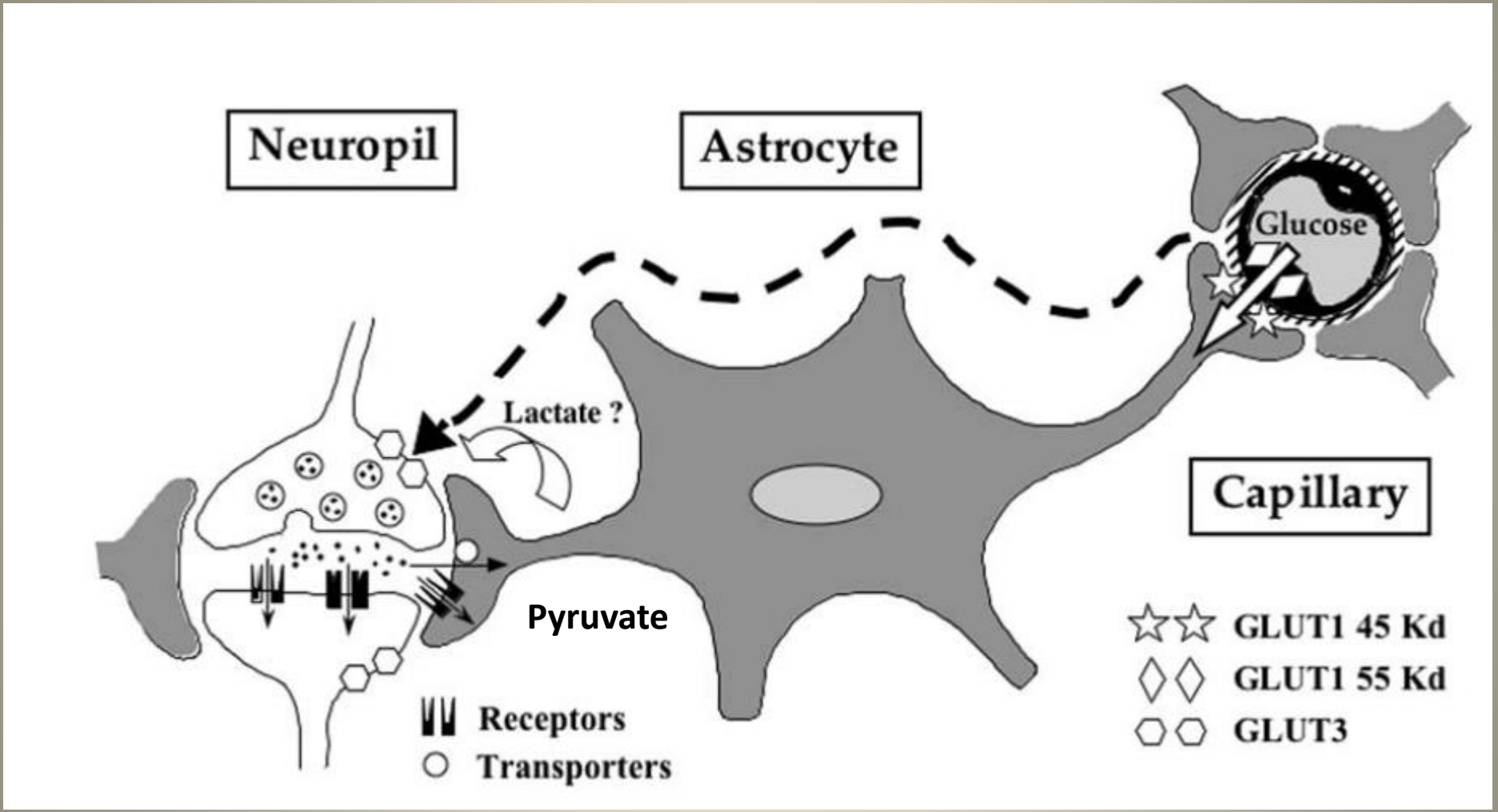


NUMBER OF DENDRITIC SPINES IN tg-MICE



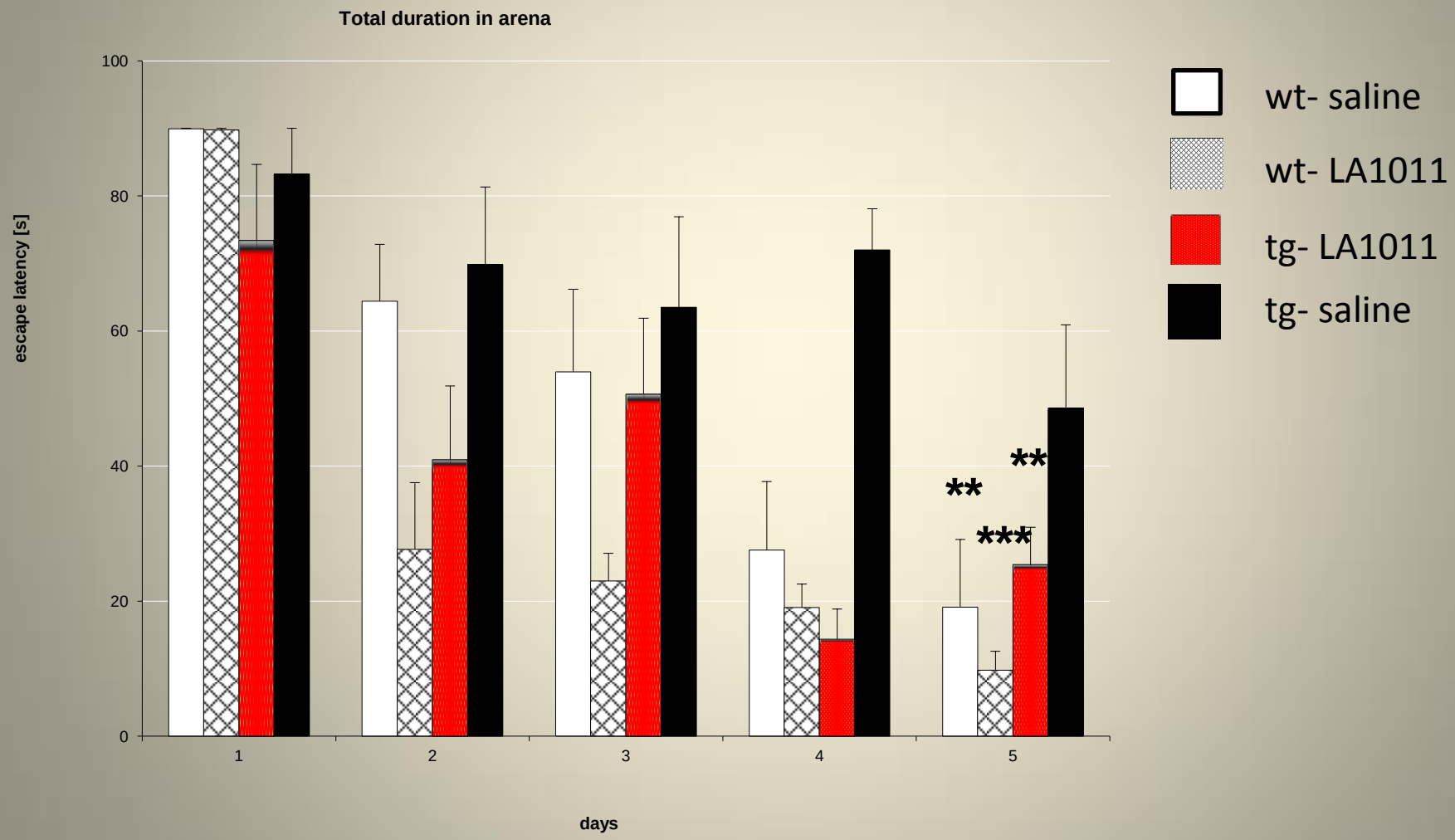


THE NEUROVASCULAR UNIT: HOW ASTROCYTES FEED HUNGRY NEURONS?



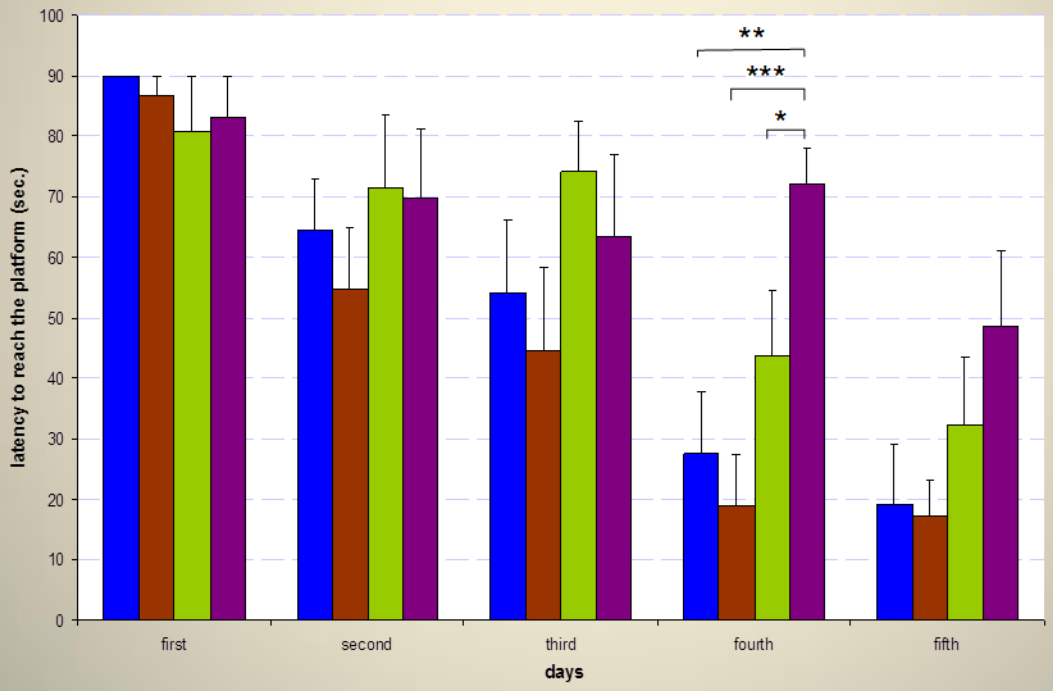
L. Pellerin: Mol. Neurobiol. 32: 59 (2005)

THE PROCOGNITIVE AND NEUROPROTECTIVE EFFECT OF LA1011 IN APPXPS1 TG MICE



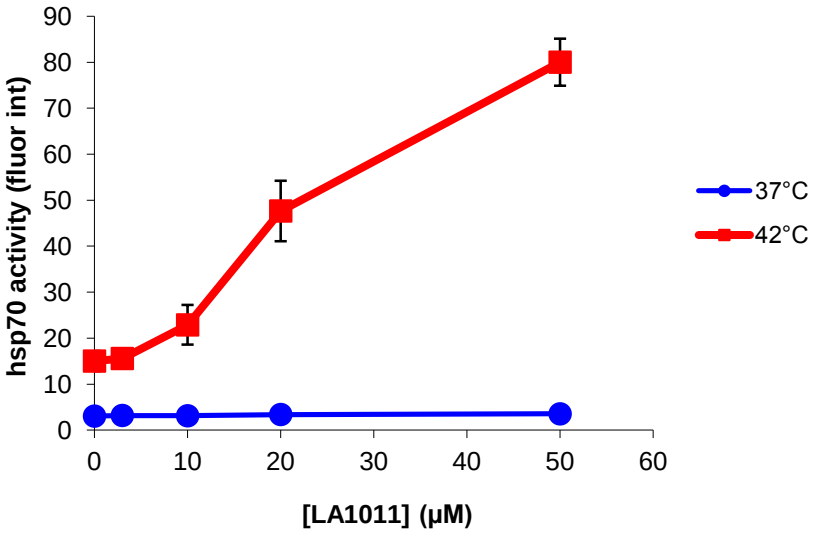
PROCOGNIVE AND NEUROPROTECTIVE EFFECT OF AN APP-ENDOCYTOSIS INHIBITOR BFE106

(Rat Morris water maze)



SELECTIVE HSP MODULATION (SHSY5Y model)

Co-inducer / booster



silencer