Neuroprotection by GDNF and a physical exercise therapy in the 3xTgAD mouse model

Susana Revilla Ortueta

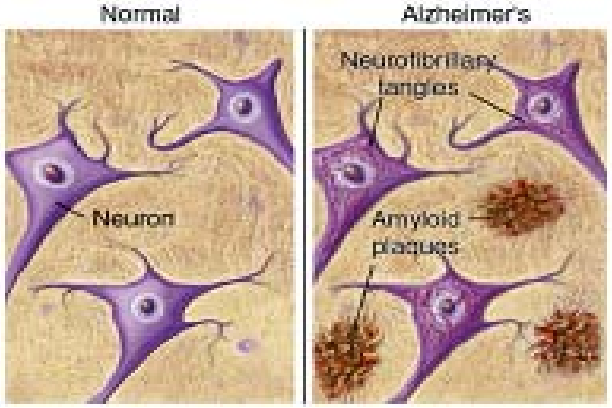
Alzheimer's Disease

Progressive neurodegenerative disorder

- **Clinical aspects:** Short term memory and attenction lost + cognitive impairment

- **Pathological hallmarks:** Extracellular plaques: ß-amiloid aggregation

  Neurofibrilar tangles: Hyperposphorilated Tau accumulation



- **Neuropathology:** Neuronal and synaptic lost

  Neurotransmission systems disfunction (BSPD)

Introduction

- Ethiology

  Genetics (Familiar Alzheimer 5 %)

  Risk factors (Sporadic Alzheimer 95 %)

Familiar (5 %)

APP protein processing

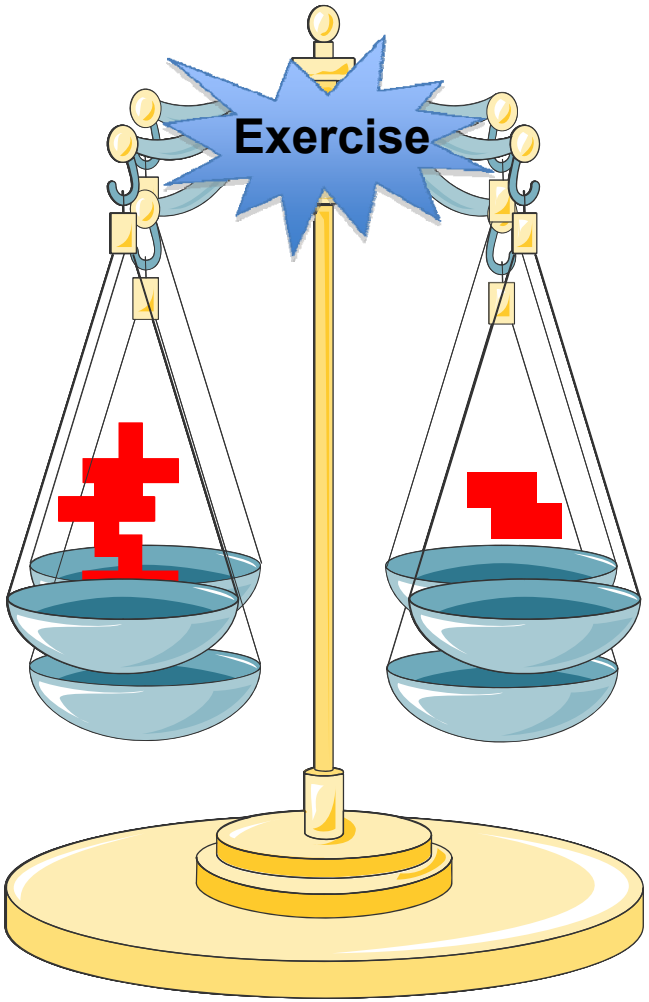
Gene	Locus	Inheritance
APP	21q21.2	Autosomal dominant
PSEN1	14q24.3	Autosomal dominant
PSEN2	1q31-q42	Autosomal dominant

Sporadic (95 %)

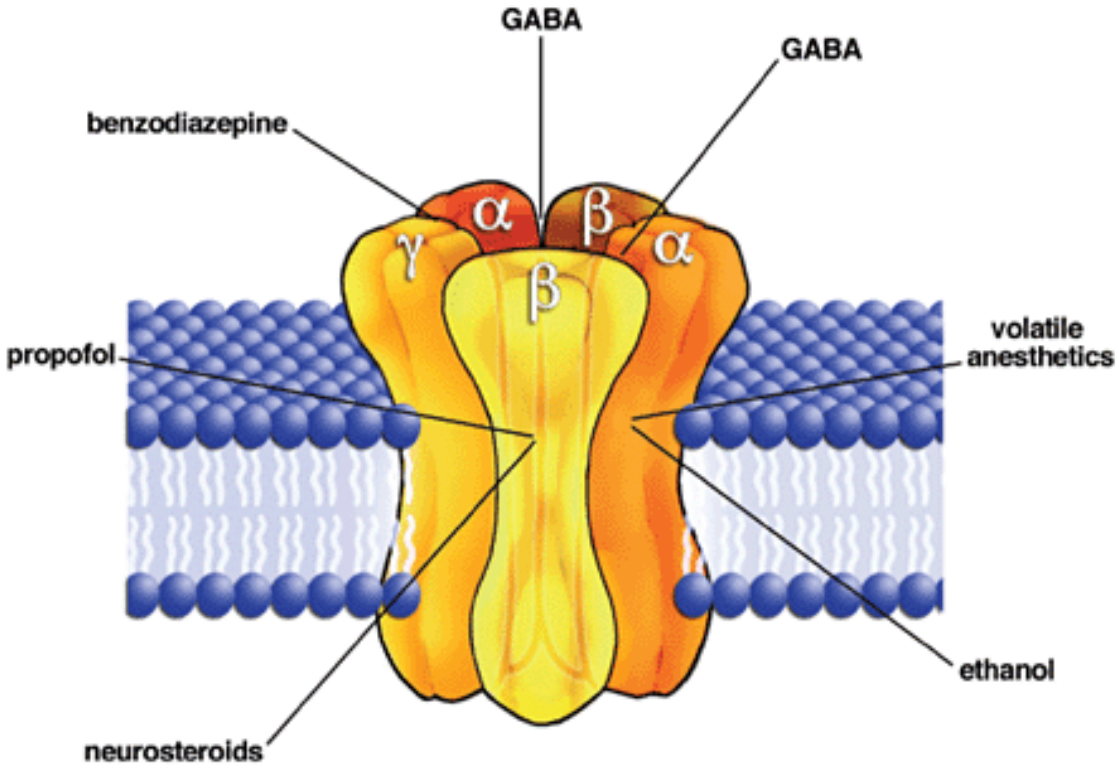
Cardio-vascular health

Susceptibility gene	Increased risk
APOE (4 -variant)	
VEGF	
MTHFR	
IL-1	
GSTP1	
TF and *HFE*	
Susceptibility gene	**Decreased risk**
APOE (2 -variant)	
Susceptibility gene	**Associated with age at onset**
GSTO1 and *GSTO2*	(conflicting results)

Neurotransmission systems affected in AD



Inhibitory Neurotransmission: GABA-A receptor



Neuropsychiatric symptoms in dementia

(depression, anxiety)

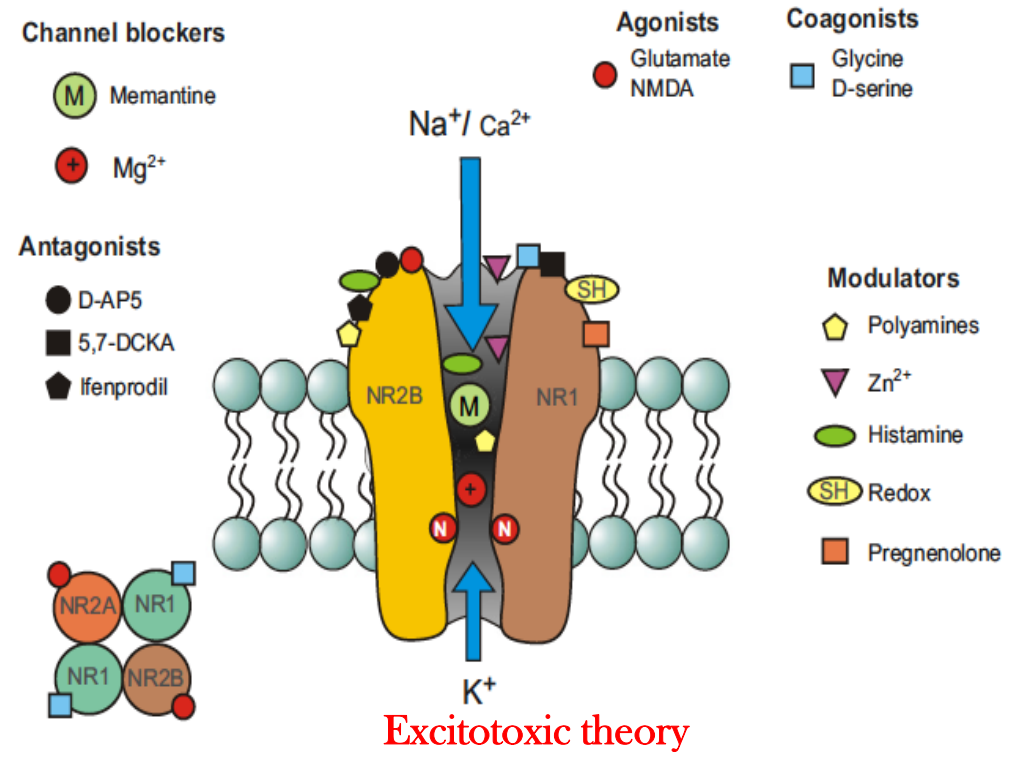
Pharmacology of

anxiolytic drugs (benzodiazepins),

sedatives and

convulsivant drugs

Neurotransmission systems affected in AD

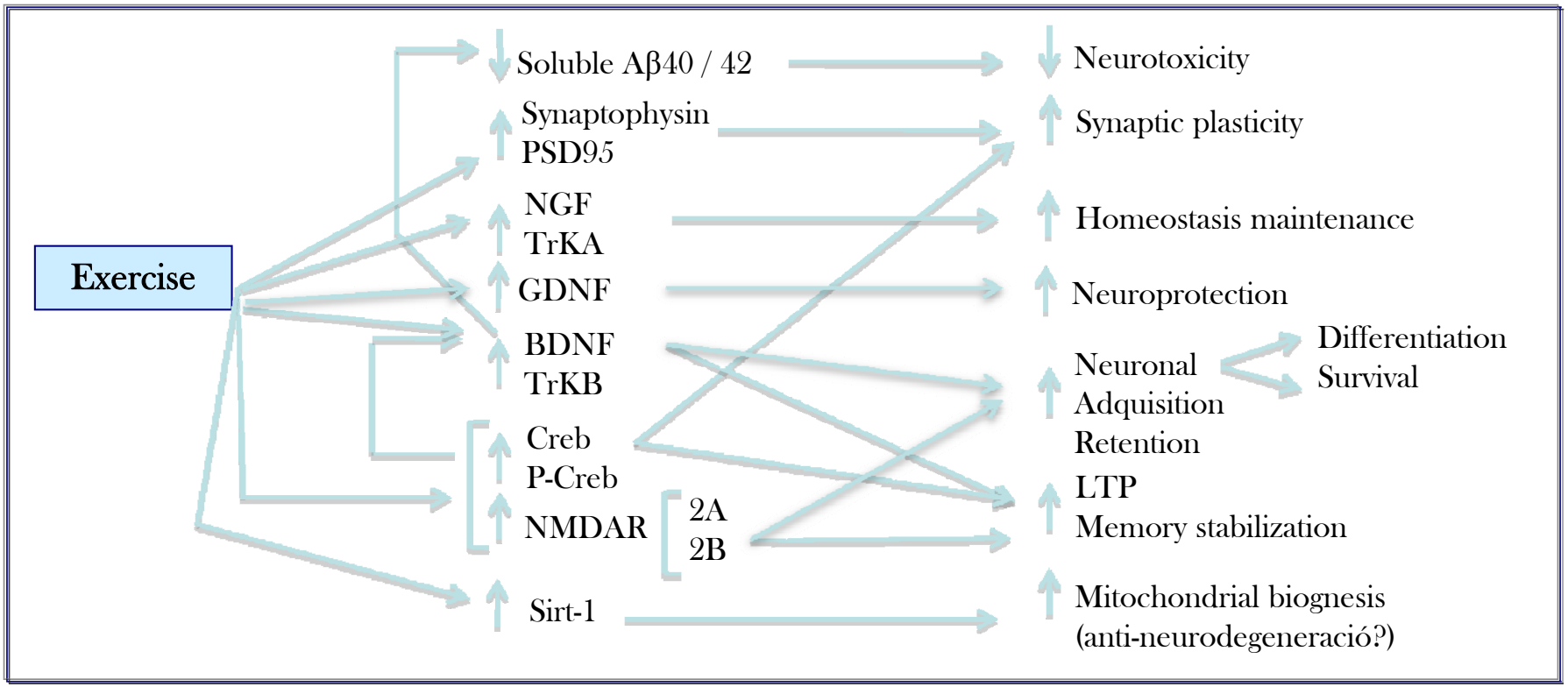


Excitotoxic theory

+ NMDAR → ↑ intraneuronal Ca^{2+} → + Neurotoxic compounds → Neuronal death

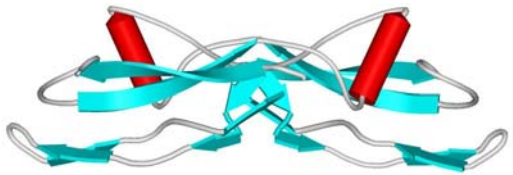
↓↓ Excitatory Aa

Exercise improves learning, synaptic plasticity and neuronal differentiation and survival



- Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. Molteni R. et al., European Journal of Neuroscienc 2002.
- Exercise can increase small heat shock proteins (shsp) and pre- and post-synaptic proteins in the hippocampus. Shuxin hua et al., Brain Research 2009.
- Moderate exercise changes synaptic and cytoskeletal proteins in motor regions of the rat brain. Ana F.B. et al., Brain research 2010.
- Treadmill exercise improves cognitive function and facilitates nerve growth factor signaling by activating mitogen-activated protein kinase/extracellular signalregulated kinase1/2 in the streptozotocin-induced diabetic rat hippocampus. C. H. Chae et al., Neuroscience 2009.
- Physical exercise protects against Alzheimer's disease in 3xTg-AD mice. García Y. et al., 2010 (in press). Gender-Specific Neuroimmunoendocrine Response to Treadmill Exercise in 3xTg-ADMice. Giménez-Llort et al., International Journal of Alzheimer's Disease 2010.

Neuroprotective effects of neurotrophic factors in neurodegenerative diseases



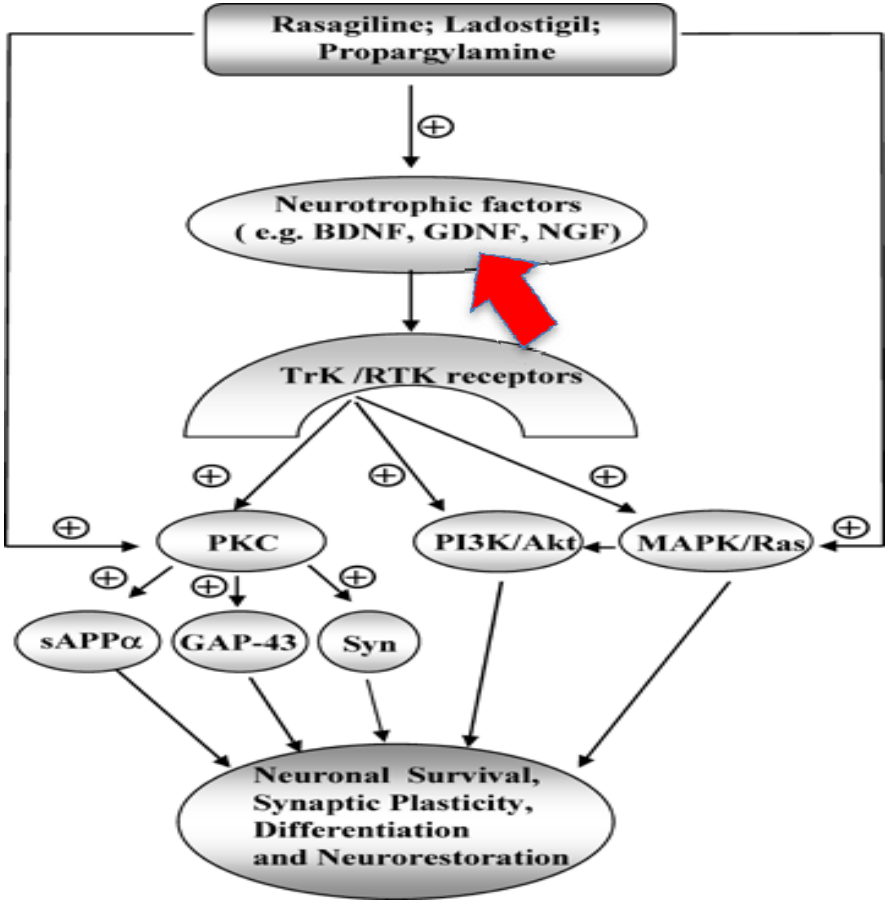
GDNF structure

Glial derived neurotrophic factor

Neuroprotection Neuroregeneration

Expression of GDNF transgene in astrocytes improves cognitive deficits in aged rats. (García-Matas et al., Neurobiol Aging, 2008)

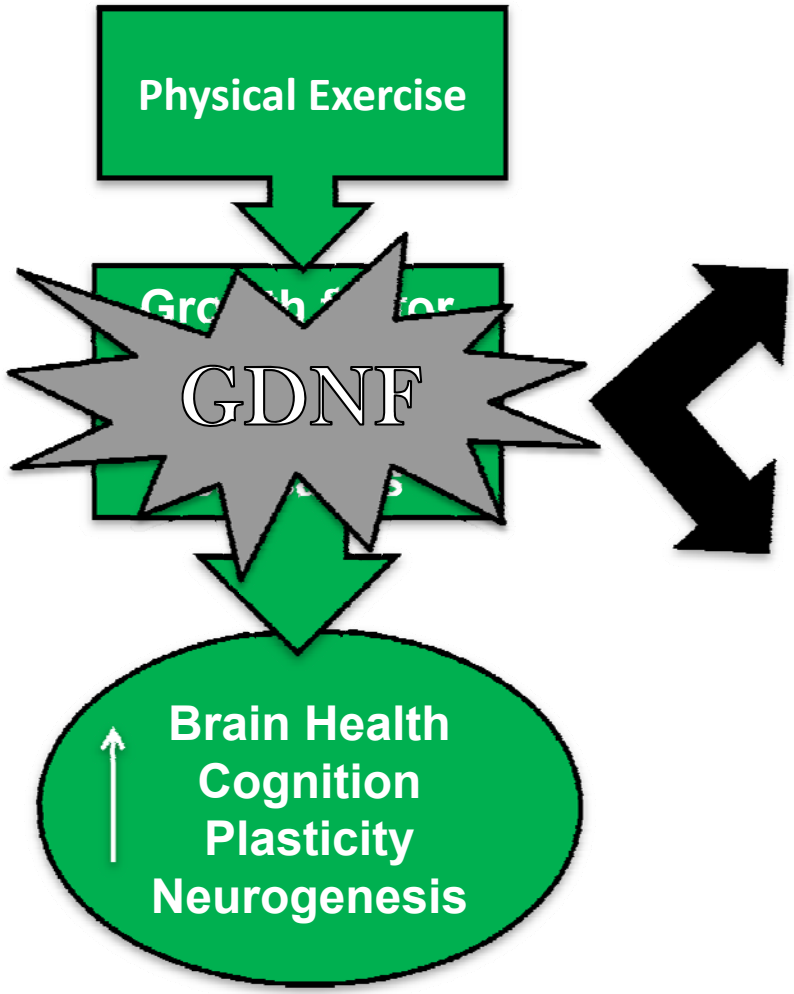
GDNF overexpression in CA1 HC astrocytes → Improvement spatial memory
Improvement neurotransmission
No motor changes



Weinreb et al., 2007

Introduction

Exercise improves brain health through growth factor cascades.



GDNF protein content in rat skeletal muscle is altered by increased physical activity in vivo and in vitro. (M. J. Mccullough et al., Neuroscience 2011).

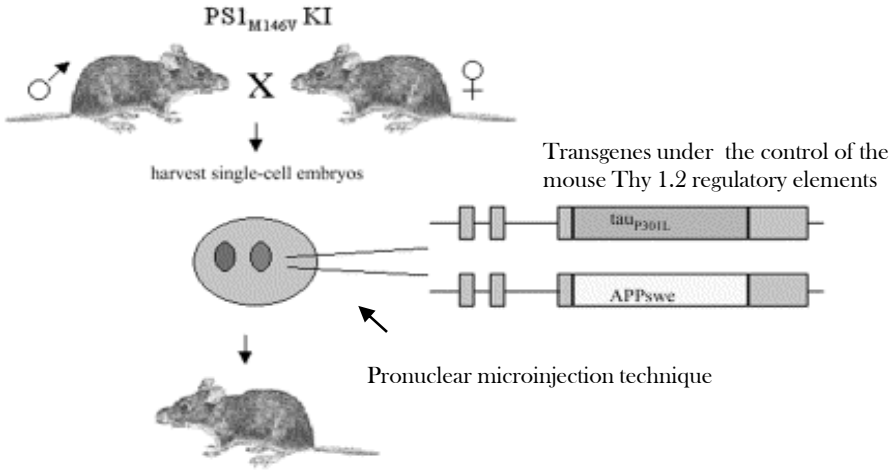
The neurotrophic effect of some AD agents are derived from the increased production of GDNF by astrocytes (memantine, Wu et al., 2009, Caumont et al., 2006; ladostigil, Weinreb et al., 2007).

Triple transgenic mouse: 3xTgAD

Oddo et al., Neuron 39:409-21, 2003
(Frank LaFerla, UCI, CA).

Spanish colony: Lydia Giménez-Llort, UAB.

Strategy used to develop 3xTgAD mice:



The 3xtgAD mice have a knock-in PSEN gene and are transgenic for APP and tau

Mutations: PS1 - M146V
APP - SWE
TAU - P301L

Recapitulation of salient features of AD:

- **Early:** intraneuronal amyloid β deposition & synaptic dysfunction
- **Later:** plaques & tangles

Objectives

1. To study neurochemical improvements induced by forced and voluntary exercise in 3xTgAD mice at moderate stages of AD.

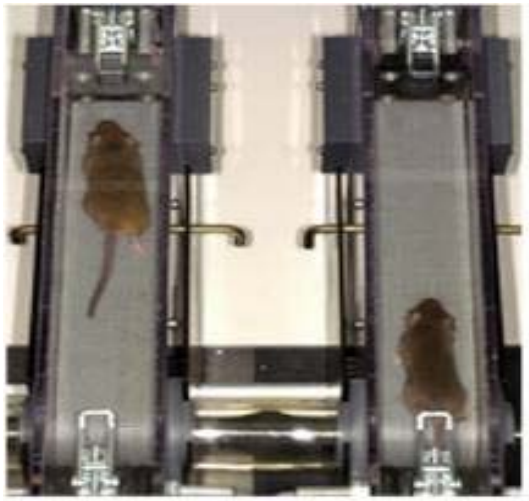
    - Functional characterization of GABAergic and glutamatergic neurotransmission systems.
    - Quantification ofAβlevels in the hippocampus.
    - Quantification of several protein expression levels which are affected in AD in the hippocampus.

2. To study the mechanism of action of GDNF in neuroprotection or neurodegeneration slowdown by gene therapy techniques in the 3xTgAD mouse model.

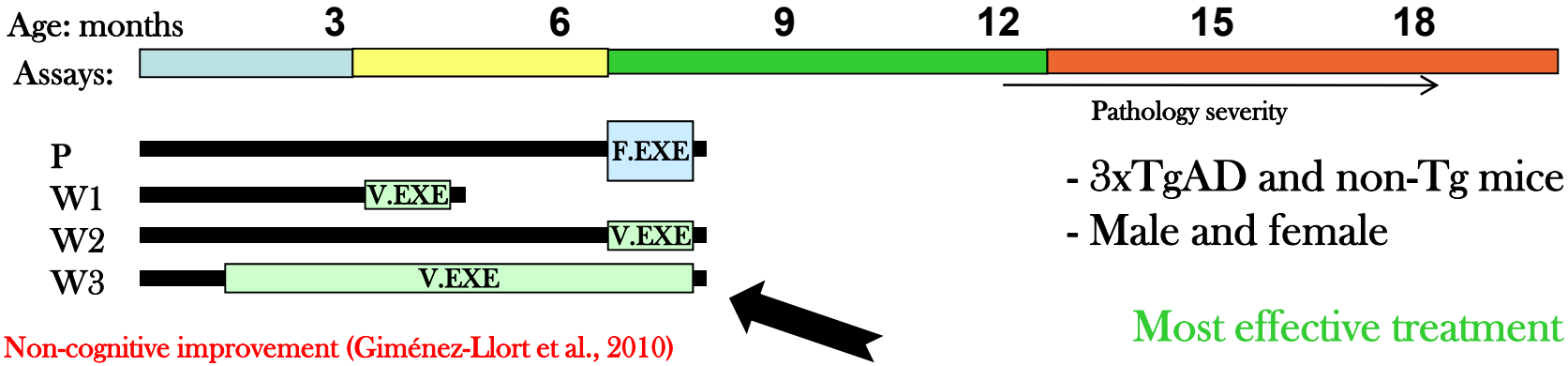
    - To assess the effects of GDNF overexpression after lentiviral transfection in hippocampus astrocytes (CA1 area) on the cognitive and neuropsychiatric status using GFP overexpression as control.

1. Physical exercise strategies

V. EXE



F. EXE

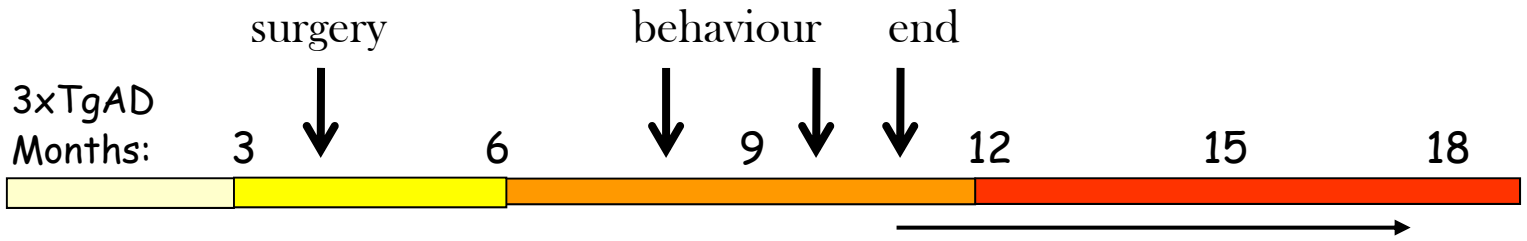


- 3xTgAD and non-Tg mice
- Male and female

Most effective treatment

P: Non-cognitive improvement (Giménez-Llort et al., 2010)
W: Cognitive and non-cognitive improvement (García-Mesa et al., 2011)

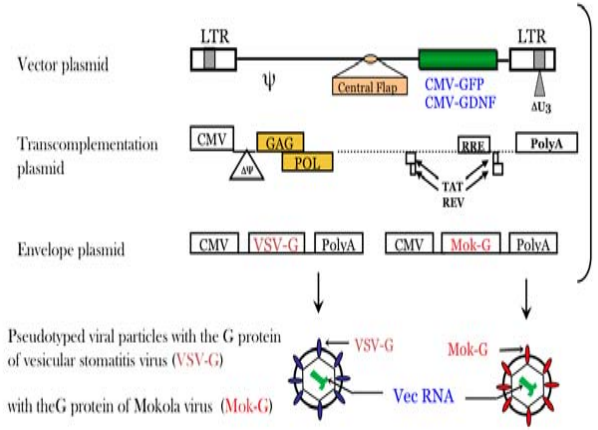
2. Astrocyte secreted GDNF against Alzheimer's disease



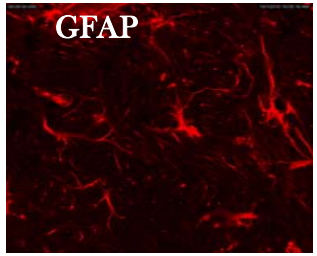
Hippocampus

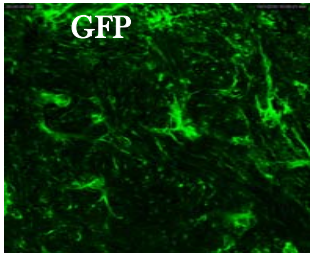
-2,00 AP
±1,2 ML
-2 DV

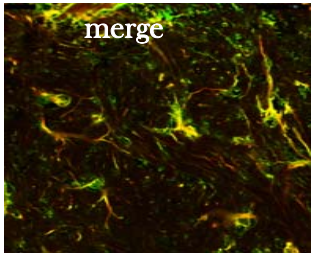
Corner test
Open field
Morris water maze

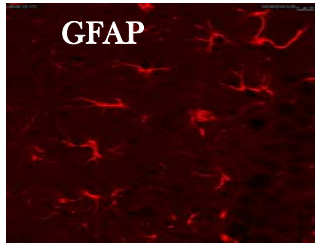


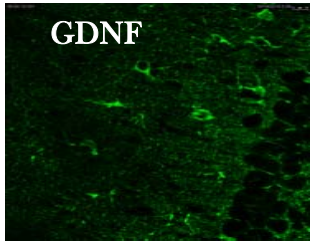
Provided by Dr. C. Sarkis (CNRS, París)

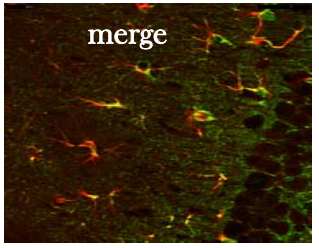






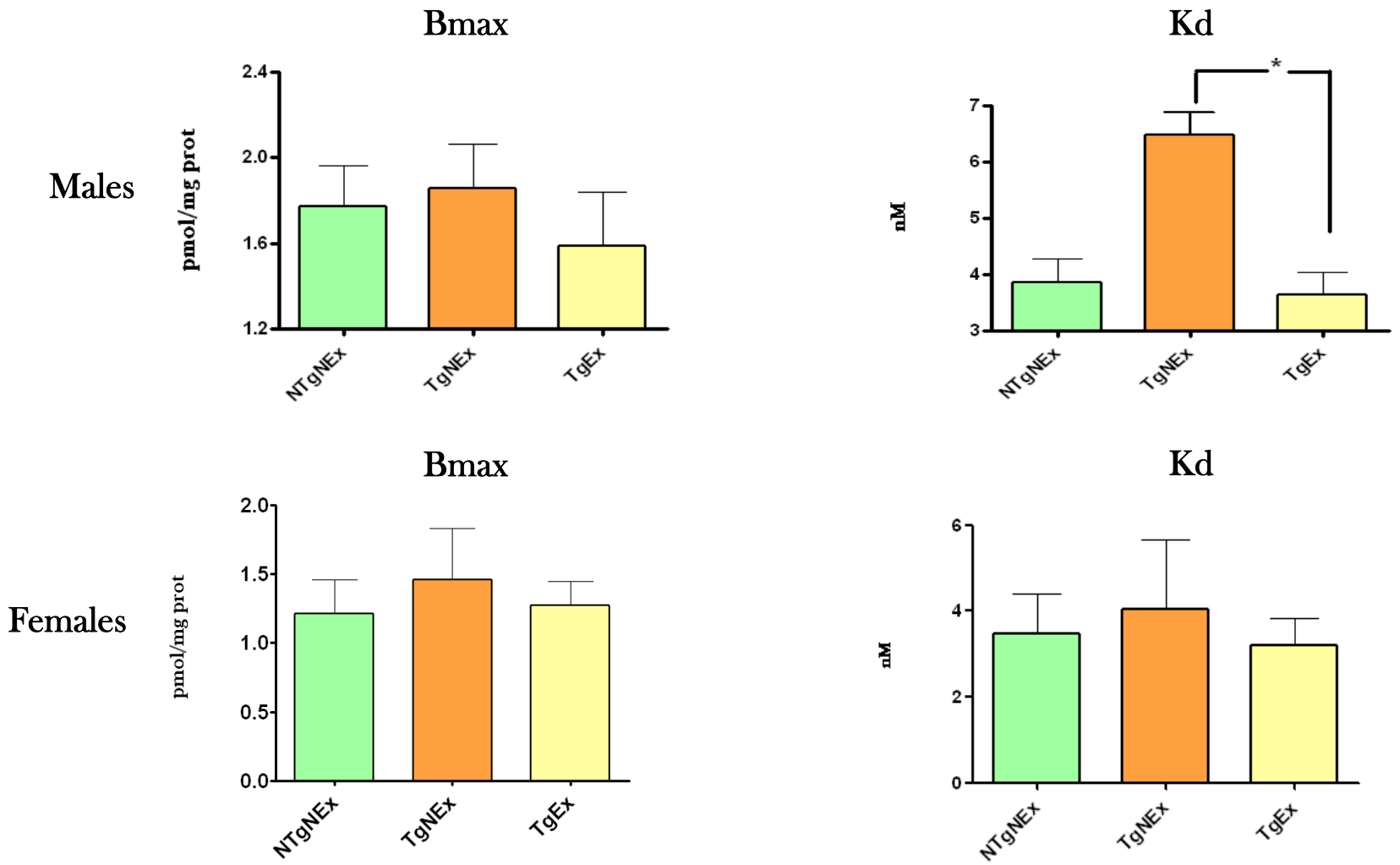






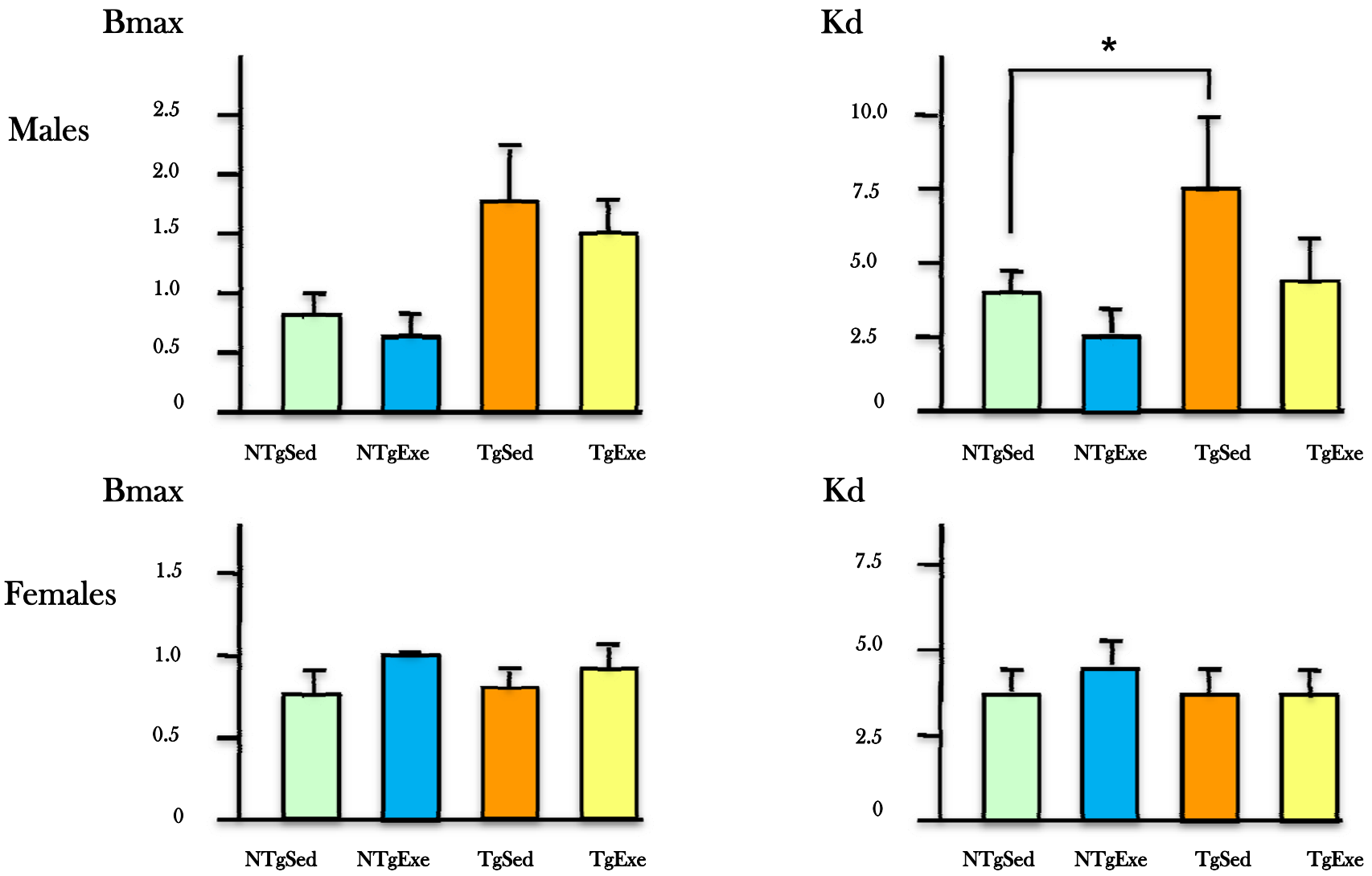
1. Effects of physical exercise on radioligand binding to their corresponding neurotransmitter receptors.

1.1. Forced exercise effects on GABAergic neurotransmision



One month of forced exercise therapy. Decrease of GABA-A receptor affinity (Kd) in 3xTgAD male mice that was recoverd by the effects of exercise. There were no changes in total binding sites density (Bmax). In females there were no changes in any settings. Results: mean ± SEM, n = 3, ANOVA: * $p < 0.05$ compared to TgNEx.

1.2. Voluntary exercise effects on GABAergic neurotransmision

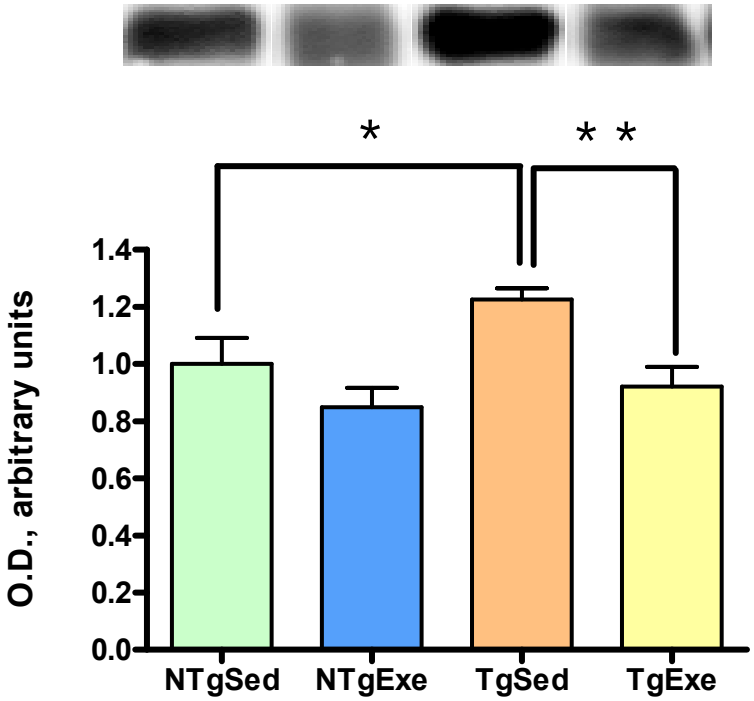


Six months of voluntary exercise therapy. 3xTgAD male mice showed a slight tendency to increase the total binding sites density (Bmax) and a significantly decrease of the GABA-A receptor affinity (Kd), both settings were restored after exercise. In females there were no changes in any settings. Results: mean ± SEM, n = 3-4, ANOVA: * $p < 0.05$ compared to NTgNEx

1.3. Effects of voluntary exercise on GABA α 5 subunit

7 months aged mice

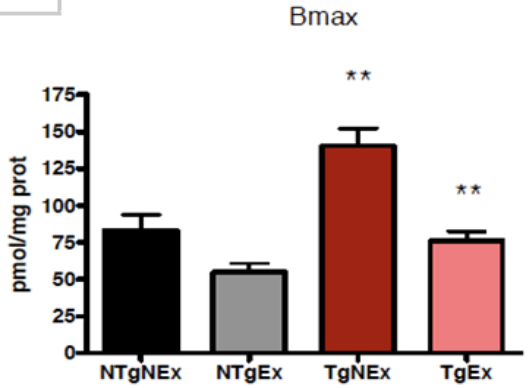
6 months of exercise

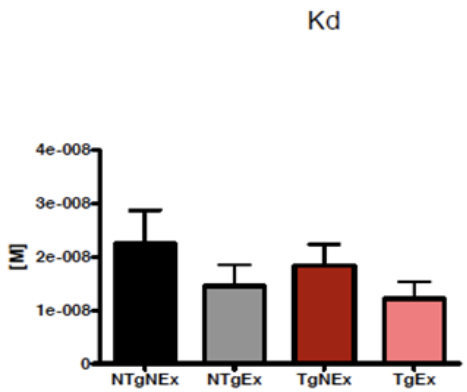


Levels of GABA- α 5 subunit was determined by Western Blot. Values are the mean ± SEM, n=5-9. Statistics: t-test and/or one-way Anova.

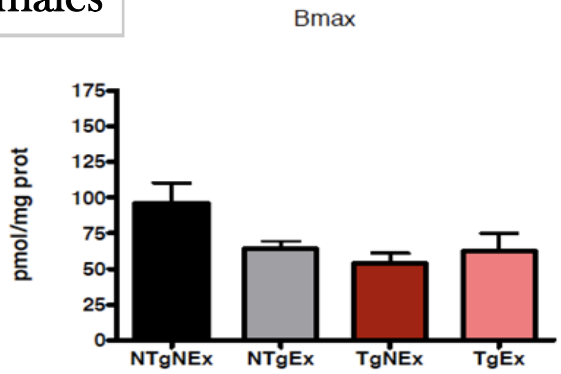
1.4. Voluntary exercise effects on NMDAR functionality

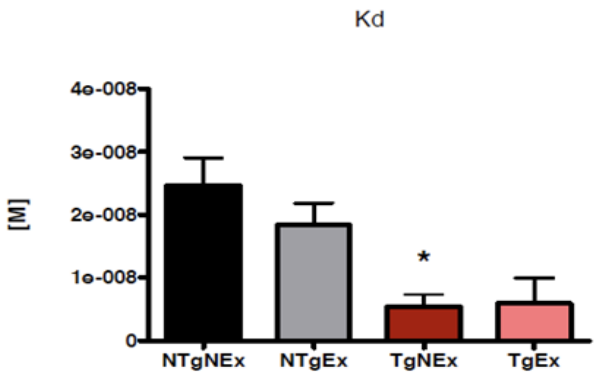
Males





Females





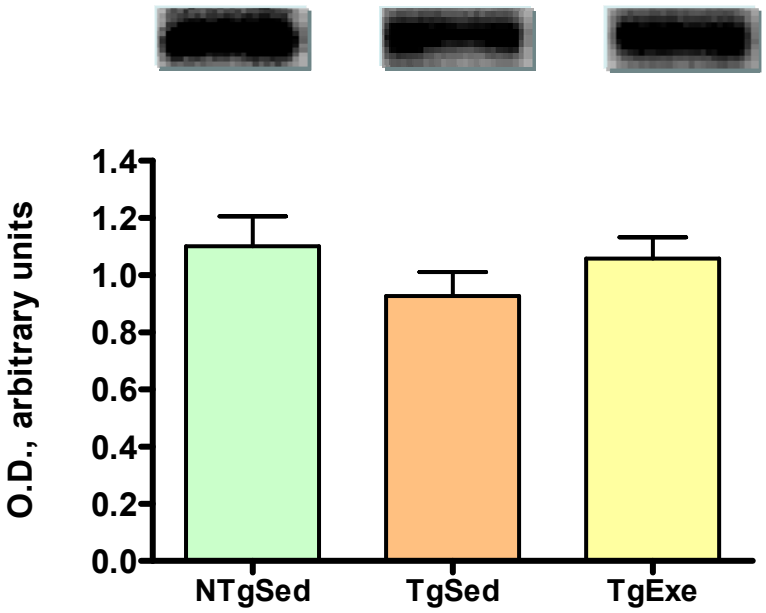
Exercise therapy of six months, from 1 to seven months of age. 3xTgAD male mice showed a significant increase of the receptors total density compared to NTg mice (** p <0.01) which was recovered after exercise (** p <0.01). NMDAR affinity did not suffer any change. In 3xTgAD females while receptor affinity increased, but without recovering after exrcise, the total density of receptors was not affected.

1.5. Effects of voluntary exercise on NMDAR

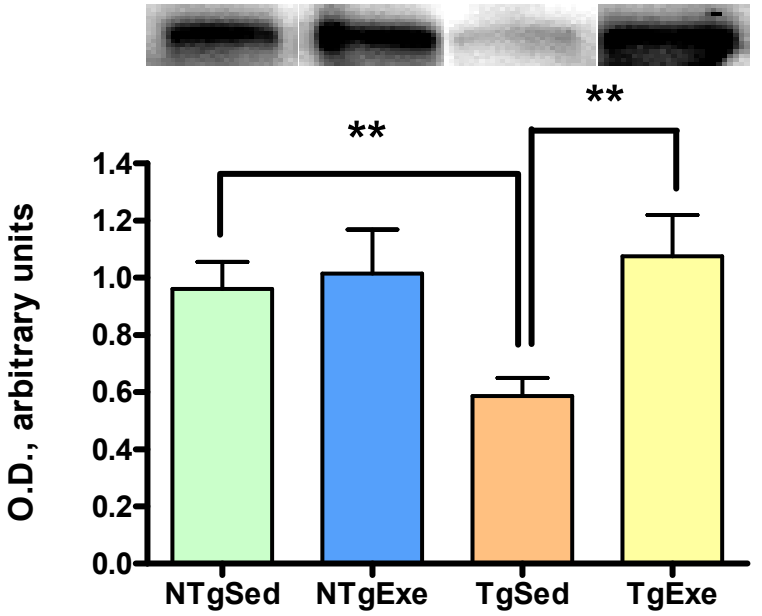
7 months aged mice

6 months of exercise

NMDAR 2A subunit



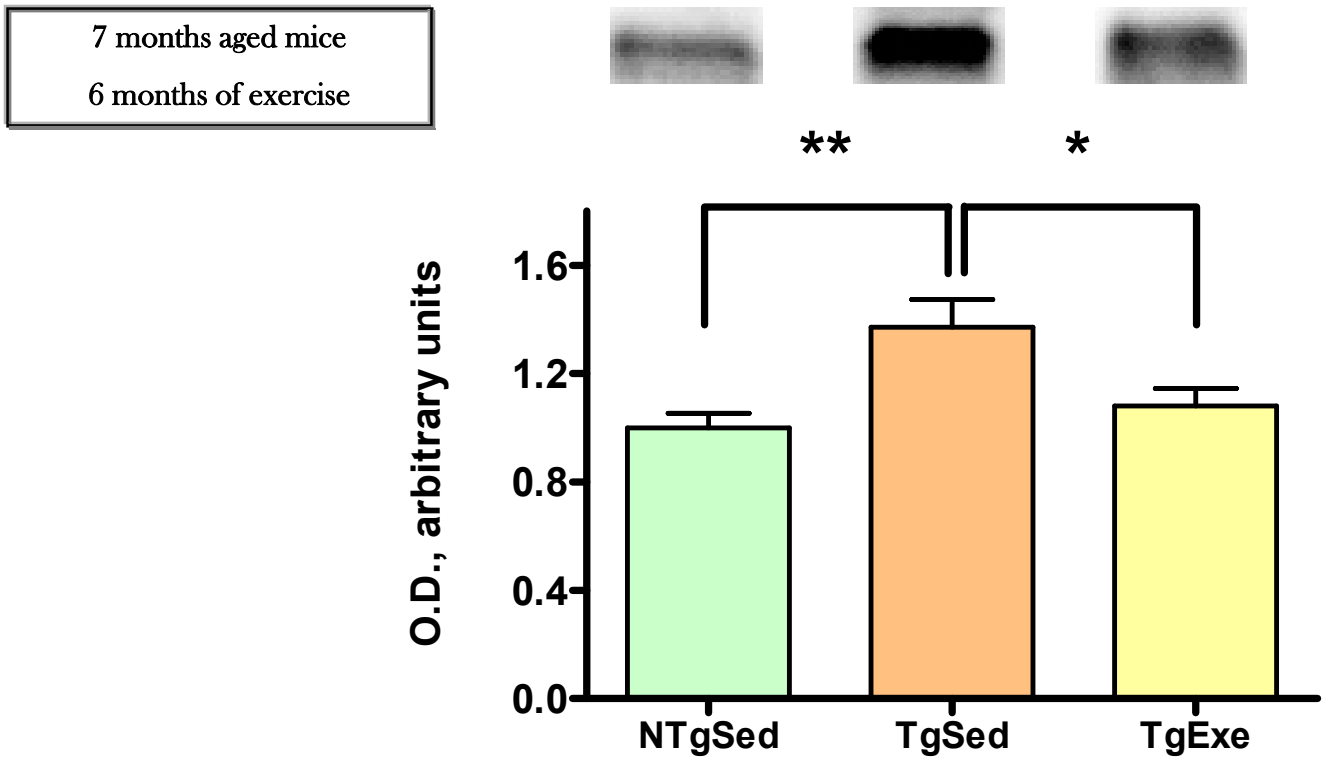
NMDAR 2B subunit



Levels of NMDAR 2A and 2B subunits were determined by Western Blot. Values are the mean ± SEM, n=5-9. Statistics: t-test and/or one-way Anova.

2. Effects of voluntary exercise on Aβ and other brain proteins expression levels in the hippocampus of 3xTgADmice.

2.1. Effects of voluntary exercise on amiloid β levels

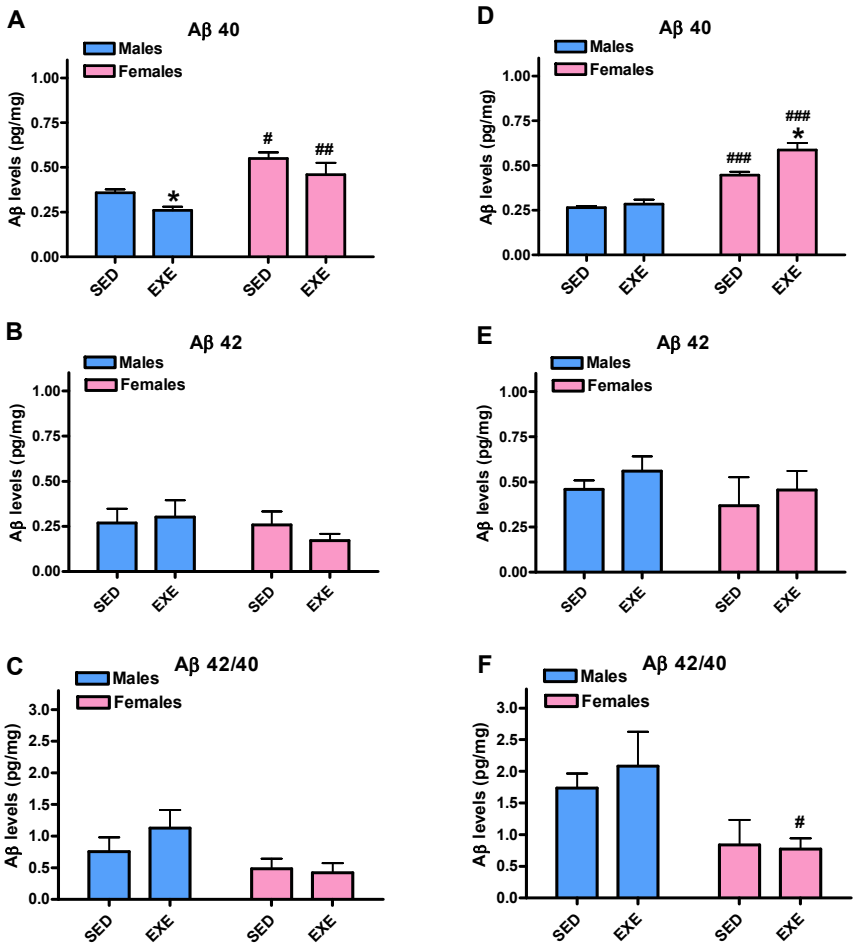


Levels of Aβ levels were determined by Western Blot. Values are the mean ± SEM, n=5-9. Statistics: t-test and/or one-way Anova.

2.2. Effects of voluntary exercise on soluble amiloid β levels

4 months aged mice

1 month of exercise



7 months aged mice

6 months of exercise

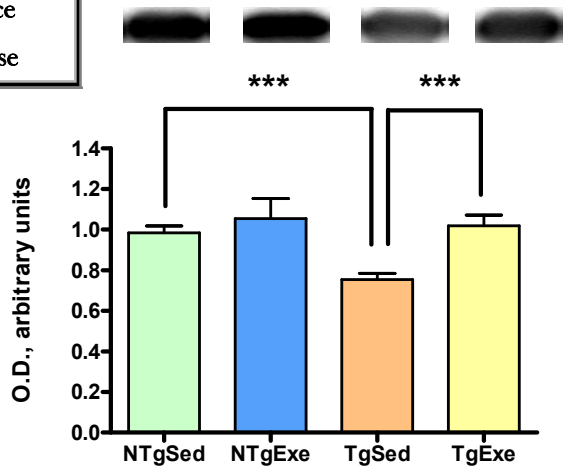
Levels of Aβ40 and Aβ42 were determined by sandwich ELISA. Statistics: two-way ANOVA followed by Bonferroni's *post hoc* test. Significant differences between groups: $^{*}P < 0.05$ compared to corresponding SED; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ compared to corresponding male group.

Results

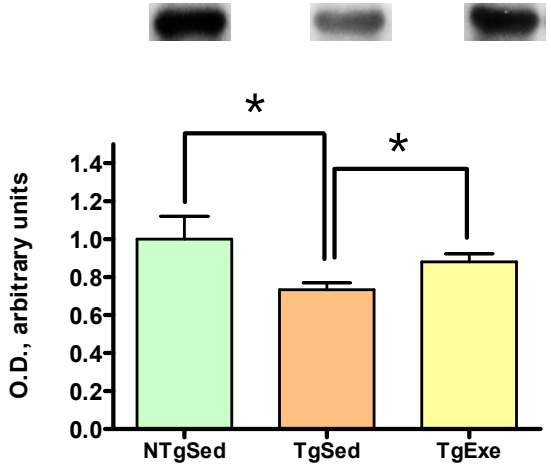
7 months aged mice

6 months of exercise

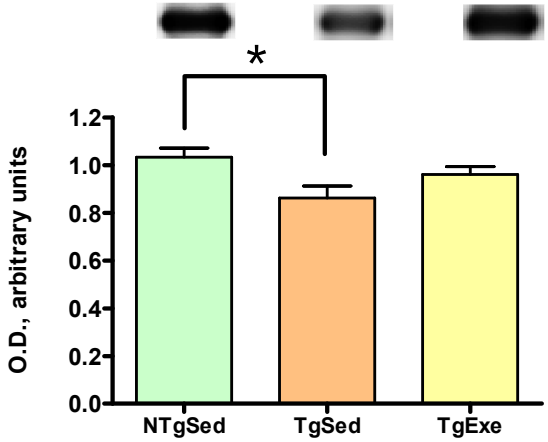
Pre-Synaptic marker : Synaptophysin



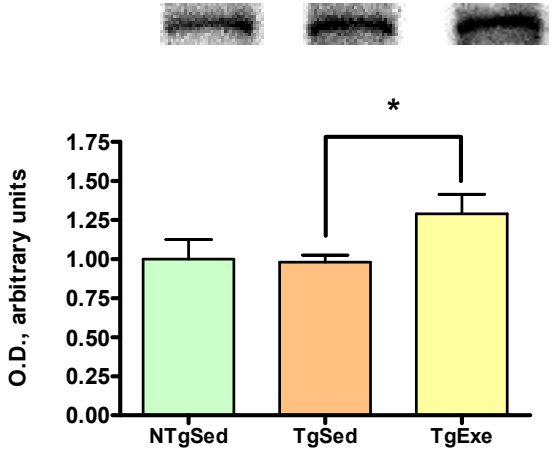
Synaptic plasticity : p-Creb



Post-Synaptic marker : PSD95



Neuroprotection against AD? : Sirt-1



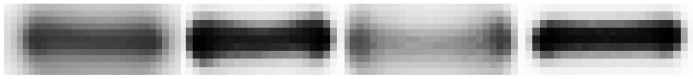
Levels of Synaptophisin, p-Creb, PSD95 and Sirt-1 proteins were determined by Western Blot. Values are the mean ± SEM, n=5-9. Statistics: t-test and/or one-way Anova.

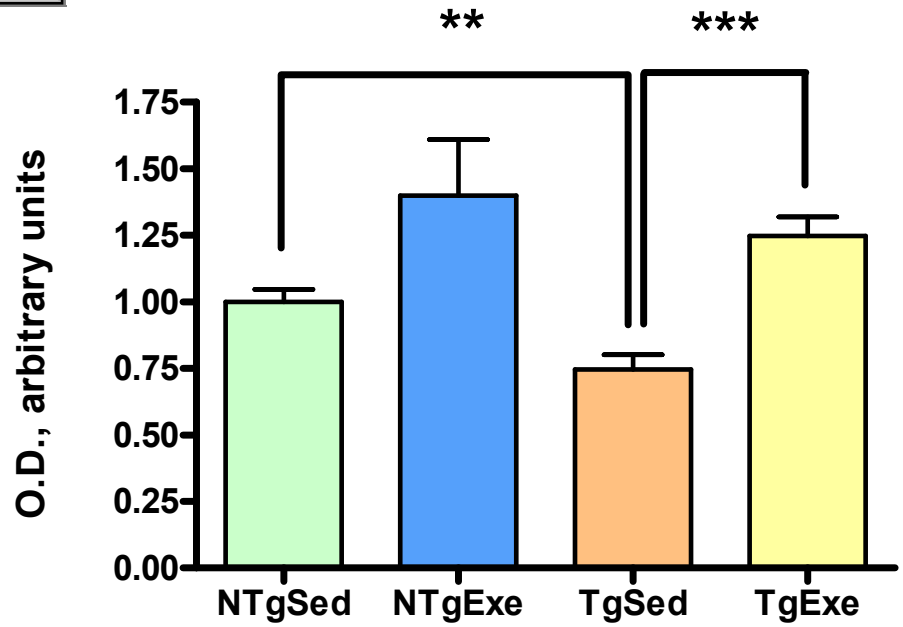
Results

Glial derived neurotrofic factor

7 months aged mice

6 months of exercise





Tissue analysis in progress (histology, RT-PCR)

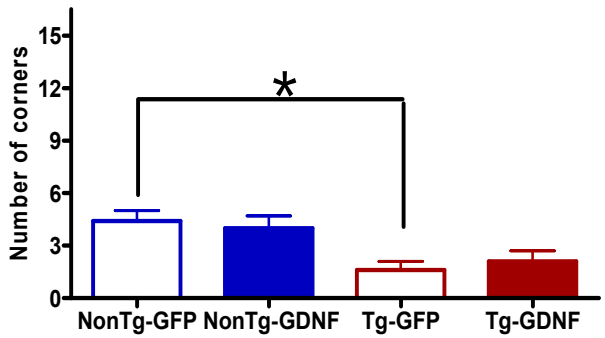
Levels of GDNF were determined by Western Blot. Values are the mean ± SEM, n=5-9. Statistics: t-test

3. Effects of GDNF overexpression in hippocampal astrocytes on behavioural patterns.

Noncognitive behavioral patterns

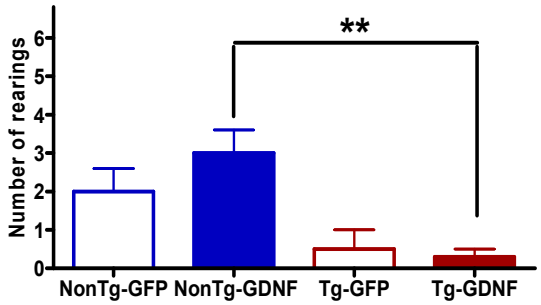
3.1. Corner test

Horizontal activity

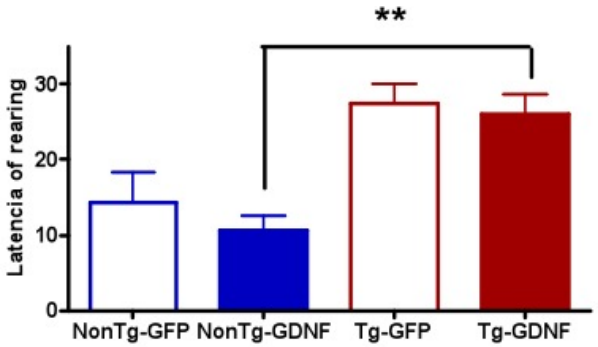


Anova, * p < 0,5 Treatment

Vertical activity

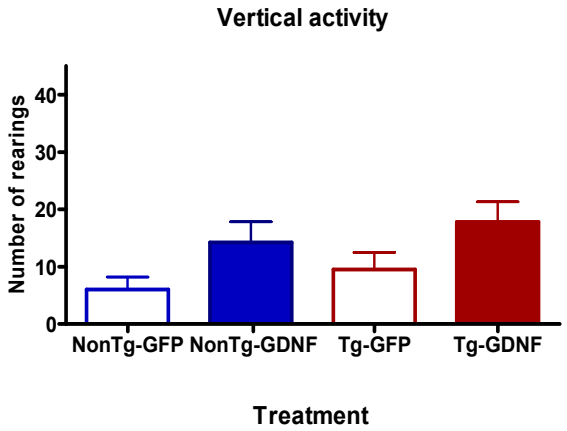


Anova, ** p < 0,01 Treatment



Anova, ** p < 0,01 Treatment

3.2. Open field



- ↑ neophobia and anxiety Tg vs NTg(no reversion by GDNF)
- Horizontal activity : < number of corners
- Vartical activity : < number of rearings

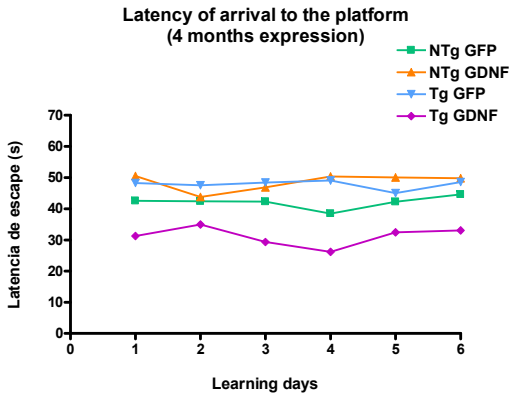
> latency of rearing

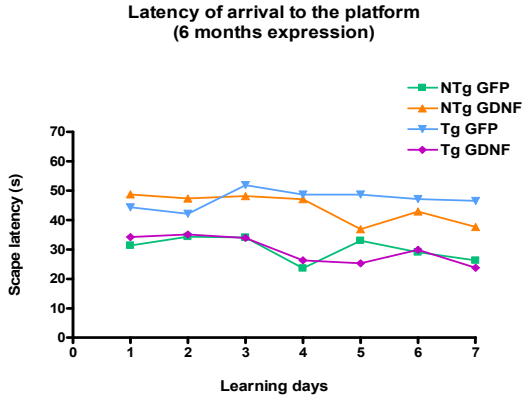
* Tendency to improve in NTg's

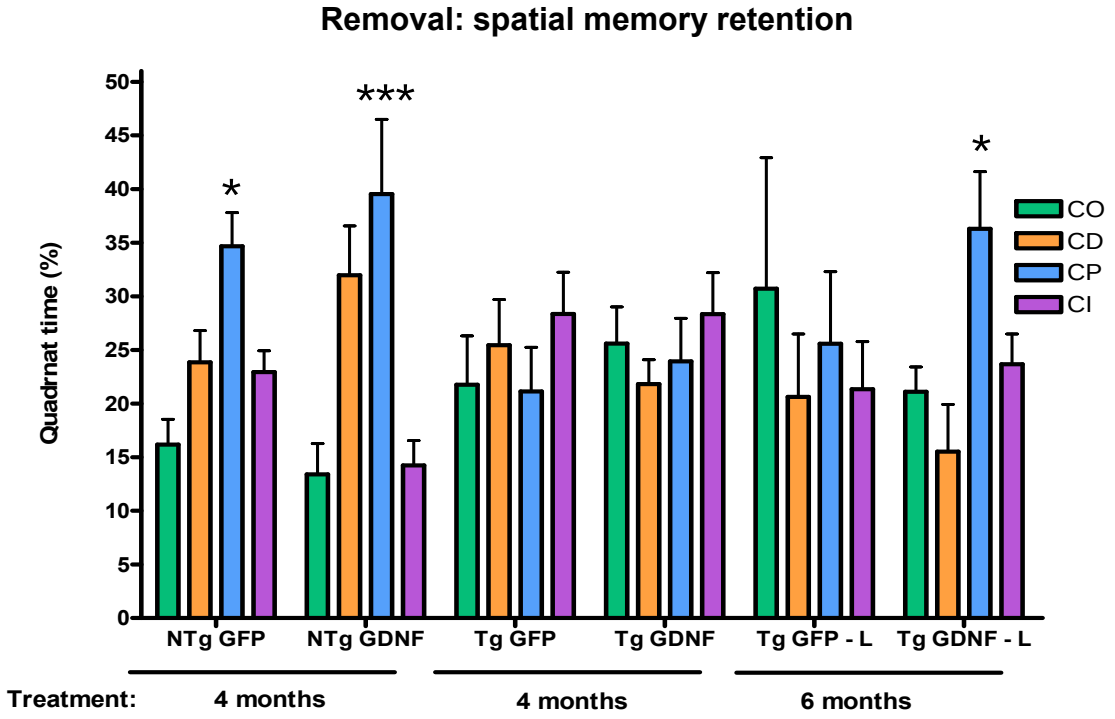
NTg's y Tg's

- ↑ Exploration after treatment with GDNF
- Vertical activity : > number of rearings

3.3. Morris water maze (MWM): cognitive behavioral patterns







Conclusions

1. Confirmation of the beneficial effects of exercise in moderate stages of AD.

↓

Brain functionalty

↓

Synaptic plasticity

- Compensatory mechanisms to mantain inhibitory circuit.
- A possible more advanced stage of excitotoxicity in females.
- The inhibitory signaling pathway releases enough GABA to maintain the balance between both inhibitory and excitatory neurotransmissions.

2. Voluntary exercise therapy decreased Aβ hippocampus levels.

- One month of exercise reduced soluble Aβ40 levels in 4-month-old male mice, whereas the small decreases in Aβ40 and Aβ42 in females were not statistically significant.
- Total amyloid levels, as determined by western blot, are decreased by physical exercise.

Conclusions

3. The benefits of physical exercise on synapse and general brain function demonstrated in the 3xTg-AD mouse model further support the value of this healthy life-style against neurodegeneration.

- Regarding to synaptic plasticity, learning, memory stabilization and long term potentiation the levels of most proteins implicated in these processes where recovered in 3xTgAD mice after the voluntary exercise therapy developed.

4. Confirmation of 3xTgAD phenotype as to the symptoms of cognitive impairment (learning and spatial memory retention) and non cognitive BSPD.

5. The overexpression of GDNF in hippocampal astrocytes improved spatial memory retention in 3xTgAD mice (reported in aged rats by Pertusa. M. et al., 2007).

- Memory and spatial learning need of the dorsal hippocampus.
- The CA1 region is essential for spatial discrimination tasks.

Conclusions

Institute of Biomedical Research of Barcelona (IIBB), CSIC-IDIBAPS, E-08036 Barcelona, Spain.

- Dra. Coral Sanfeliu Pujol
- Dra. Rosa Cristòfol Martínez

Yoelvis García

Jofre Serret

Rubén Corpas

Albert Parull

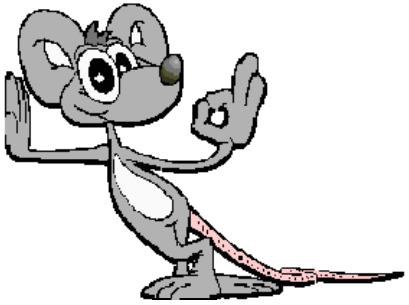
Patricia Molina

- Dra. Cristina Suñol Esquirol

Institute of Neuroscience and Medical Psychology Unit, Department of Psychiatry and Forensic Medicine, Autonomous University of Barcelona, E-08193 Bellaterra, Barcelona, Spain.

- Dra.Lydia Giménez-Llort

Financiated by La Marató de TV3 062931-0 and ISCIII RD06/0013/1004.



Thanks!