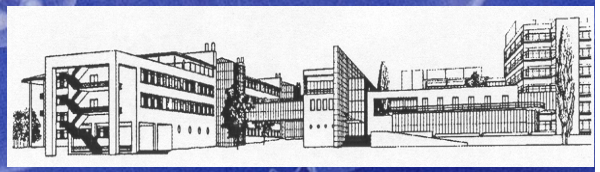
Mitochondrial dysfunction, common final pathway for aging and Alzmeimer‘s disease: Therapeutical aspects

3th International Conference and Exhibition on
Neurology & Theapeutics
September 08-10, 2014, Philadelphia (USA)

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Frankfurt am Main, Germany



The failure of Aß directed treatment strategies leaves all hopes for better antidementia drugs behind



The failures

1. Tarenflurbil, Semagacestat (LY-450139), Avagacestat (γ-secretase inhibitors)
2. Tramiprosate (inhibits Aβ aggregation)
3. Bapineuzumab and others (Aβ antibodies)
4. Aβ vaccination

Our hope

Drugs which protect mitochondria and/or improve their function

Mitochondrial dysfunction, the common player

PS1- Mutations

APP- Mutations

Trisomy 21

Altered APP processing

Oligomeric, intracellular Aß

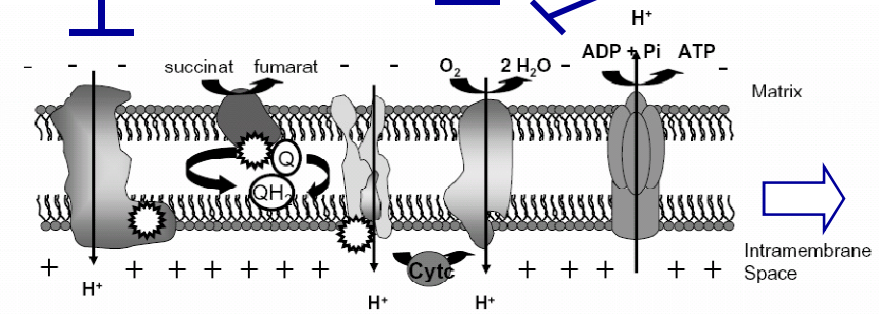
Fibrills/ Extracellular aggregation

Neuritic Plaques

Tau

?

Neurofibrillary tangles



- Reduced mitochondrial membrane potential
- reduced ATP levels
- elevated ROS production

diturbed calcium regulation

Mitochondrial and synaptic dysfunction

Enhanced Apoptosis

Aging

Leuner et al. (2007) Antioxid Redox signal; Müller et al. (2010) Mol. Neurobiol.

Complex I dysfunction leads to Aß generation in vivo

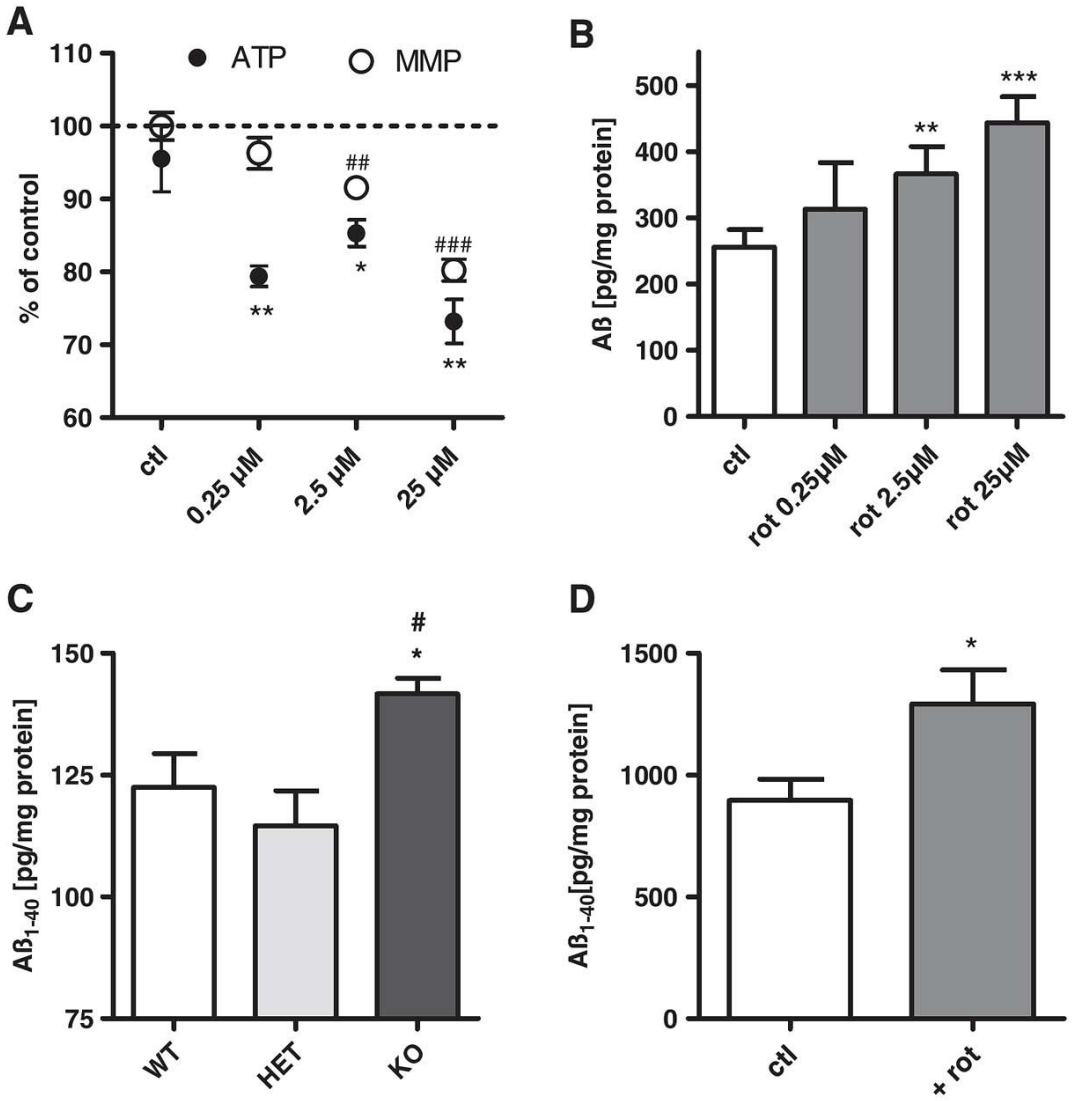
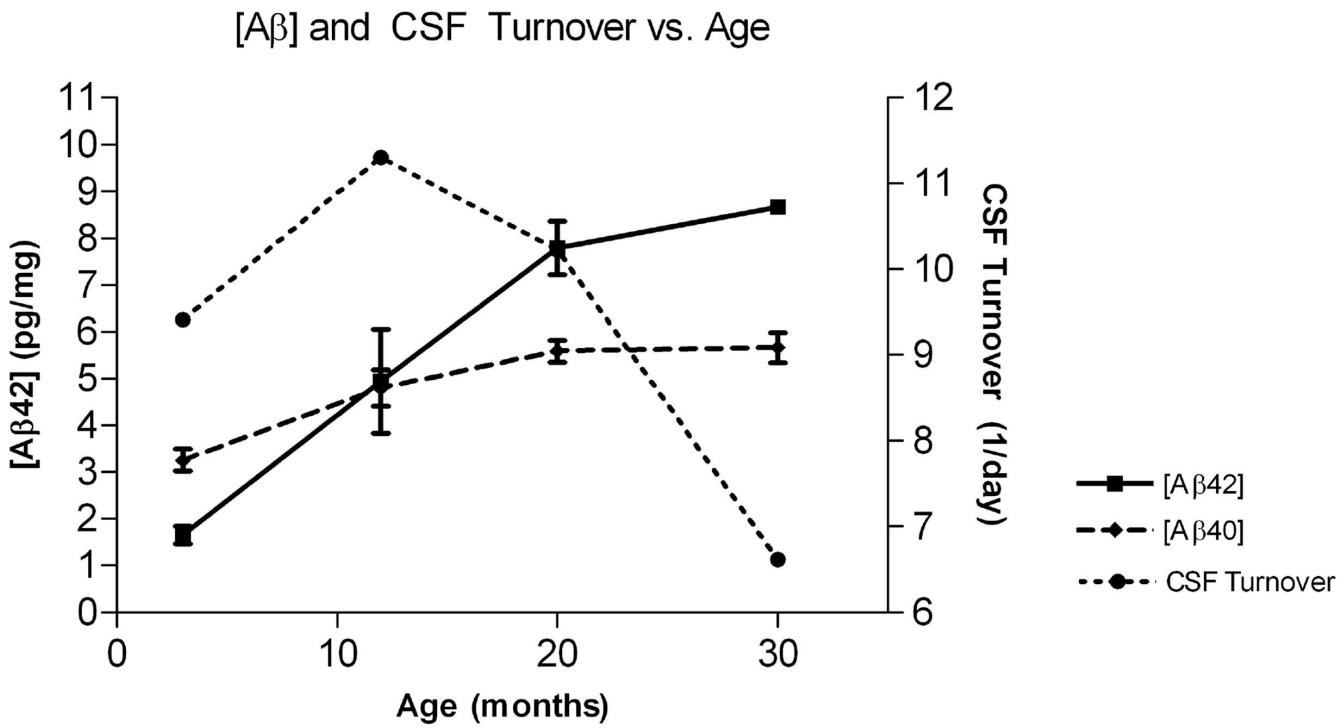


FIG. 7. Enhanced Aβ levels in a neuronal cell model and two animal models of complex I dysfunction. (A) SH-SY5Y cells were treated with rotenone (0.25, 2.5, and 25 μM) for 24 h and ATP and MMP as well as soluble $A\beta_{1\text{-}40}$ levels **(B)** were determined $n = 6 \pm$ SEM, unpaired *t*-test, **(A)** $^{*}p < 0.05$, $^{**}p < 0.01$, ATP ctl against rot, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ MMP against rot. **(B)** $^{**}p < 0.01$ ctl against rot 2.5 μM, $^{***}p < 0.001$ ctl against rot 25 μM. **(C)** APP transgenic animals received rotenone *via* i.p. application (10 mg/kg/body weight) or vehicle for 3 days and soluble $A\beta_{1\text{-}40}$ levels in brain homogenates were measured. $n = 6 \pm$ SEM per treatment group unpaired *t*-test $^{*}p < 0.05$. **(D)** In homozygous knockout (KO) *Ndufs4* mice, $A\beta_{1\text{-}40}$ levels are significantly increased compared to wild-type (wt) and heterozygous (HET) mice. Ndufs4 mice wt $n = 5 \pm$ SEM, HET $n = 5 \pm$ SEM, KO $n = 12 \pm$ SEM; unpaired *t*-test; $^{*}p < 0.05$ wt against KO, $^{\#}p < 0.05$ HET against KO.

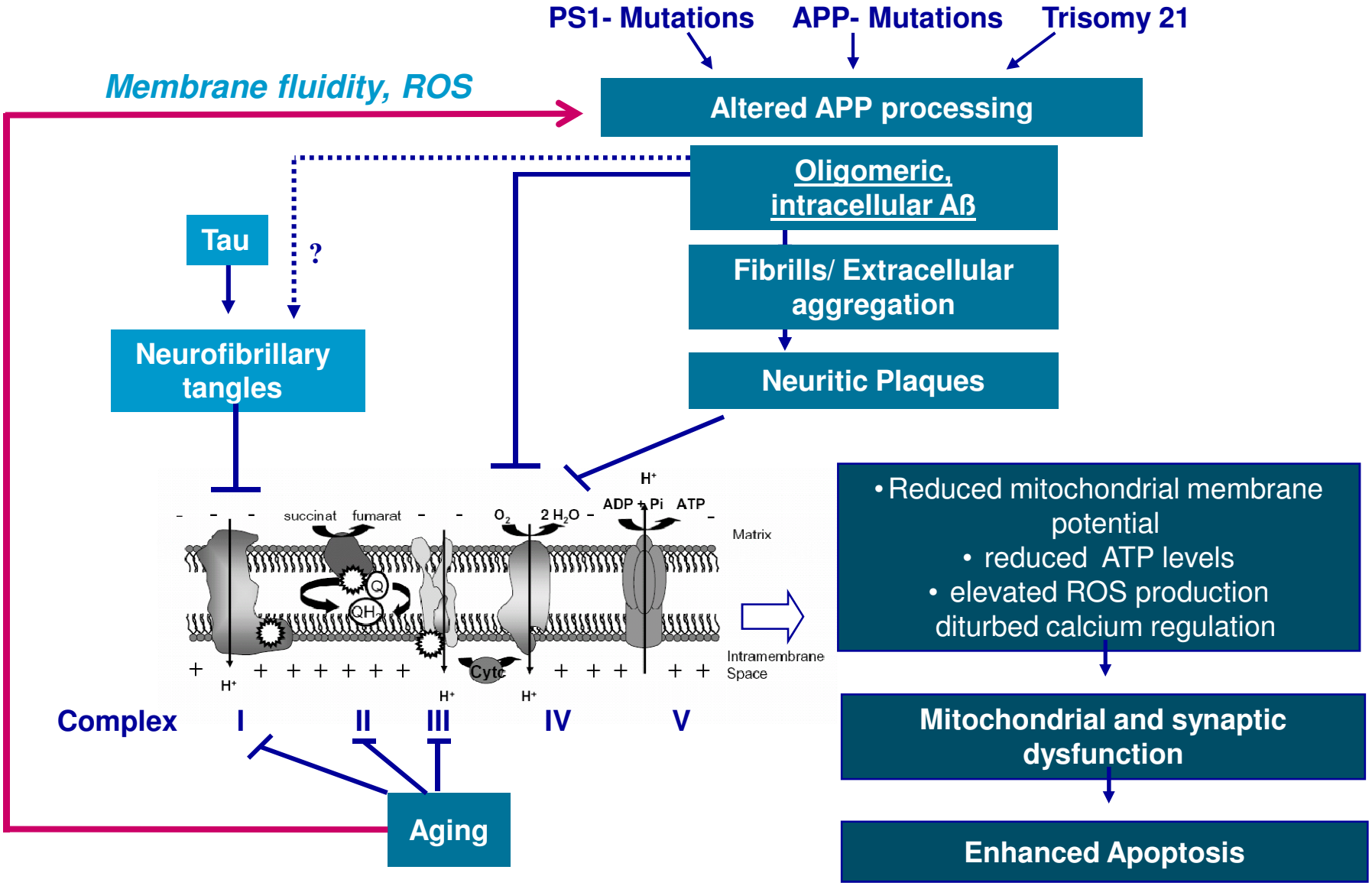
Aβ accumulation and CSF turnover



Graphs of the concentration of Aβ40 and Aβ42 in cerebral cortex plus hippocampus, and CSF turnover plotted against age. Aß40 (dashed line, diamonds) and Aß42 (solid line, squares) in pg/mg total protein concentrations are plotted on the left ordinate (3 months, n= 8; 12 months, n = 8; 20 months, n = 8; 30 months, n = 7). CSF turnover (dotted line, circles) is plotted on the right ordinate. Error bars for Aβ are SEM. CSF turnover is calculated from the means of CSF production and volume measurements. Note that Aß40 and Aß42 concentrations increase before there is a significant decrease in CSF turnover. Only later (after 12 months) in the lifespan of the F344/BN rat is there an inverse relationship between Aß accumulation and the CSF turnover rate.

Chiu, C. et al., Fluids and Barriers of the CNS 9 (3), 2012

Mitochondrial dysfunction, the common player



Leuner et al. Antioxid Redox signal (2007); Müller et al. Mol. Neurobiol. (2010)

SY5Y APPwt cells, a model for the very early interaction between aging and AD I

1. **Mitochondrial cascade hypothesis of sporadic AD (Swerdlow): Interaction of indivisually high baseline Aß production and individually high oxidative stress due to aging trigger the switch between „normal brain aging“ and the beginning of AD decades before clinical onset**

´Swerdlow et al., 2010, 2012

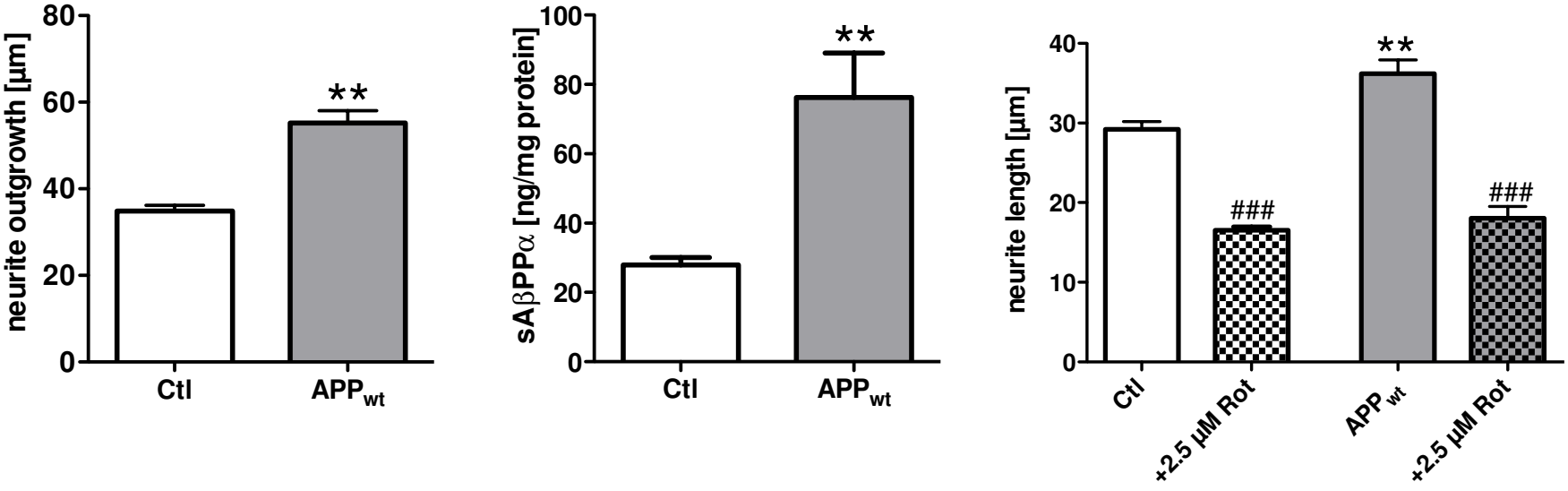
SY5Y APPwt cells, a model for the very early interaction between aging and AD II

1. Doubling of Aß production leads to several mitochondrial defects (e.g. MMP, dynamics) but also to partial compensation e.g. enhanced glycolysis, and enhanced neuritogenesis by elevated sAPP production

2. Additional complex I dysfunction (induced by rotenone) interferes with the compensation mechanisms and causes very pronounced defects (largely enhanced Aß1-42, less sAPP, further reduction of neuritogenesis)

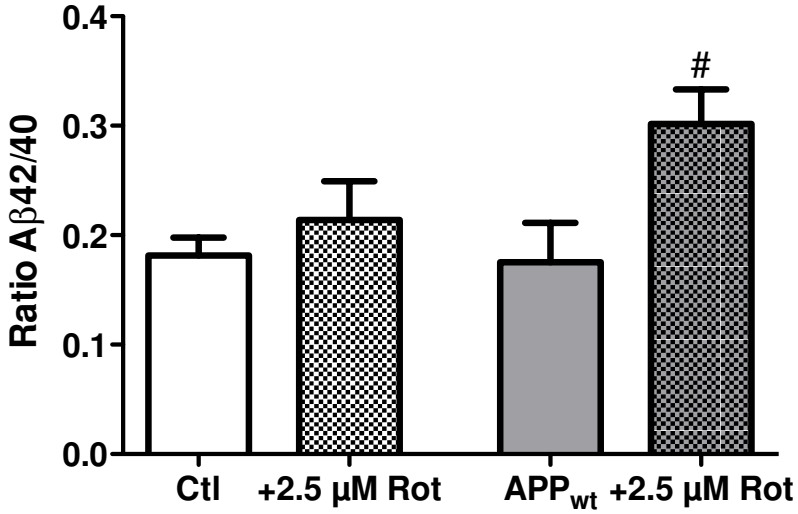
Stockburger et al. JAD in press

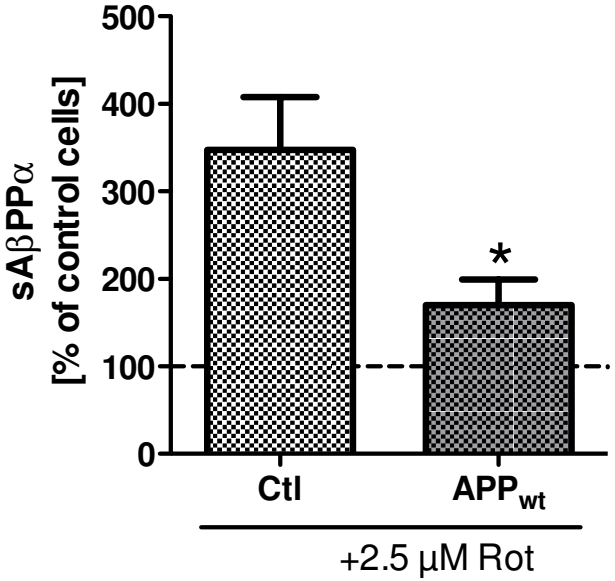
Enhanced sAPP production and increased neuritogenesis of SY5YAPPwt cells: Compensation gets lost following complexI dysfunction



Stockburger et al. JAD in press

ComplexI inhibition in SY5YAPPwt cells: Aß1-42 goes up and sAPP goes down





Stockburger et al. JAD in press

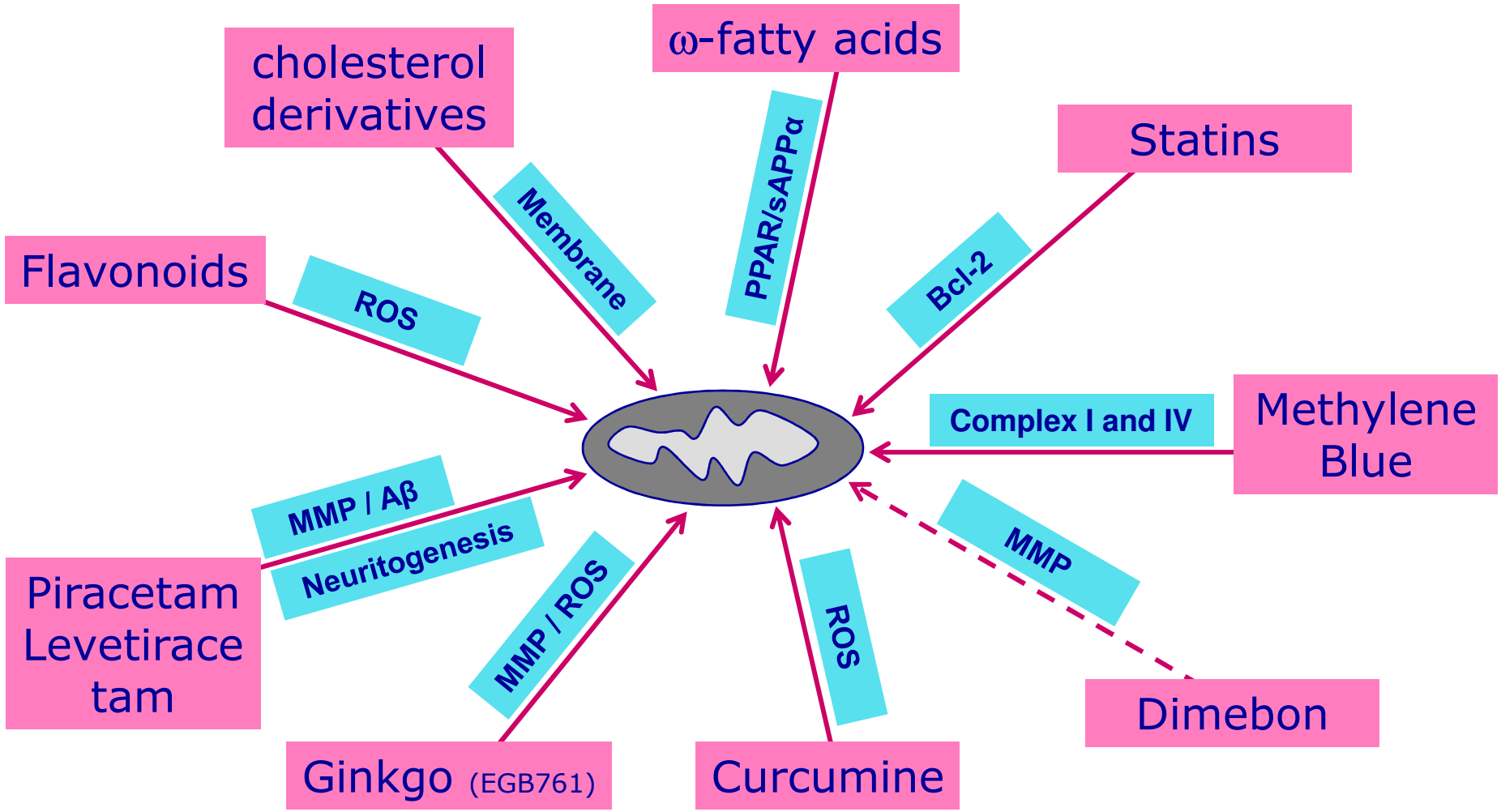
Conclusions I

1. Most sporadic (aging), histopathological (Aß, Tau), and genetic ($ApoE_4$) risk factors of AD synergistically lead to mitochondrial dysfunction

2. Mitochondrial dysfunction explains most of the functional deficits of AD brains (impairment of energy metabolism, synaptic dysfunction and deficits of information transfer, loss of synapses and synaptic conacts, loss of neurons by apoptosis

3. Mitochondrial improvement should be a relevant therapeutic approach

Mitochondrial protection, which is the best choice ?



Leuner et al. Antioxid Redox signal (2007); Müller et al., Mol. Neurobiol. (2010; Eckert et al., Mol. Neurobiol. 2012

The three antidementia drugs: Pharmacological profiles

1) Ginkgo biloba extract EGb761®

   Antioxidant and radical properties, mitochondrial protection, PAF antagonism

- Abdel-Kader, R. et al.: Stabilization of mitochondrial function by Ginkgo biloba extract (EGb761). *Pharmacol Res (2007), 56(5), 493-502*.
- Muller,W.E.:*Effects of standardized Ginkgo biloba extract EGb761 on neuroplasticity. International Psychogeriatrics (2012), Suppl.1, S21-S24.*

2) Piracetam

   Improvement of membrane fluidity leads to improvement of neuronal and mitochondrial function

- Leuner, K. et al.: Improved mitochondrial function in brain aging and Alzheimer disease – the new mechanism of action of the old metabolic enhancer piracetam. *frontiers in Neuroscience (2010), published on 07 Sep. 2010.*

3) Dimebon

   Multiple receptor antagonism, Antagonism of PTP, mitochondrial improvement

- Eckert, S.H. et al.: Dimebon ameliorates amyloid-β induced impairments of mitochondrial form and function. Journal of Alzheimers Disease (2012), 31(3), 21-32
  Müller et al.: Mitochondrial quality control: Effects of Dimebon on mitophagy and apoptosis. Soc.Neurosciences (2013)

Dimebon, no effect in control cells, but significant restoration of mitochondrial dynamics in HEK_{sw} cells

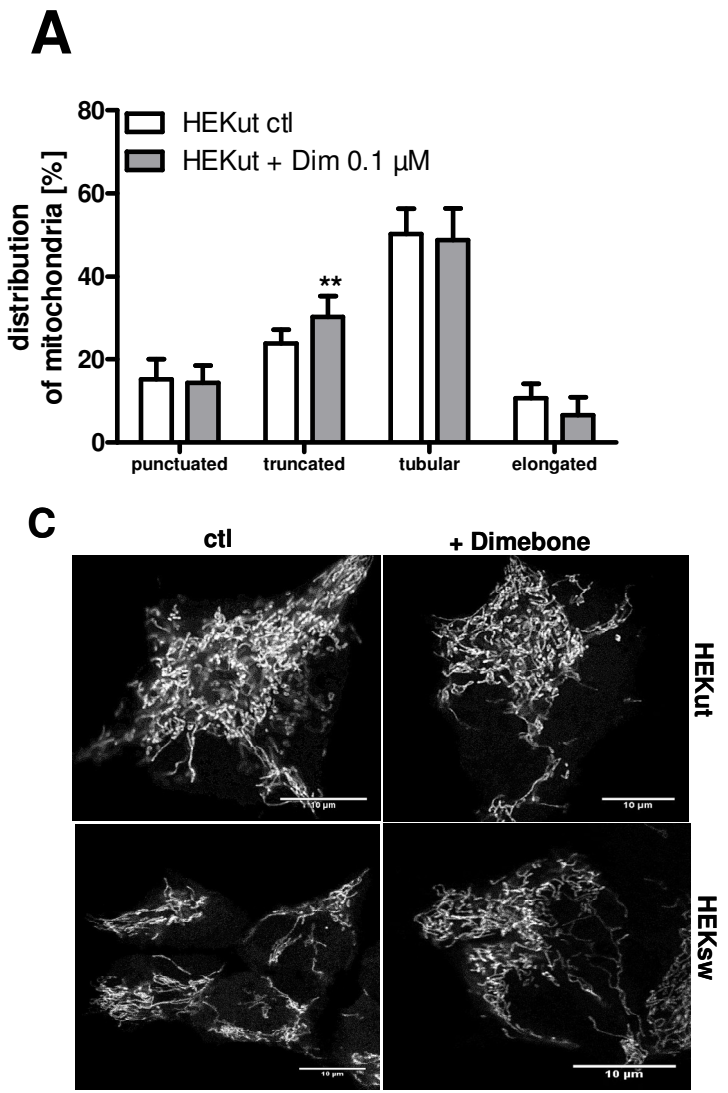
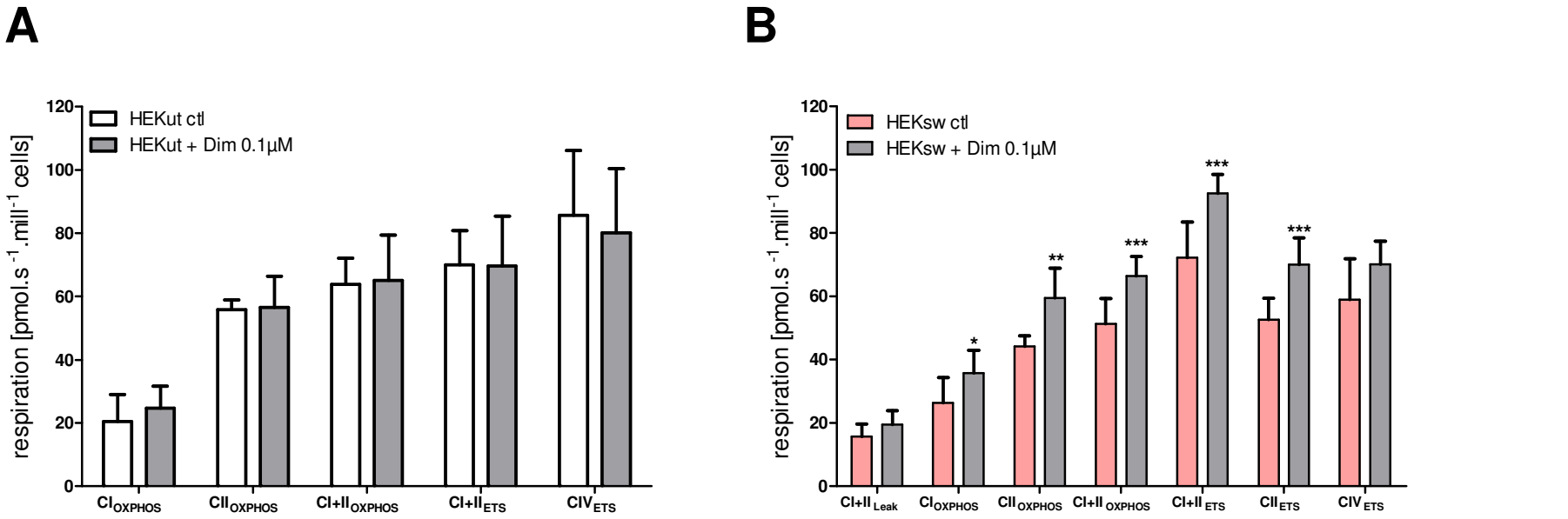
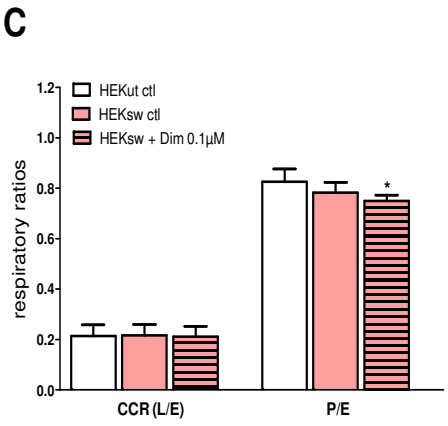


Fig. 2. In HEK_{sw} cells mitochondria are highly fragmented. Incubation with Dimebon for 6 h significantly shifts mitochondrial morphology in HEK_{sw} cells to tubular shape.
A HEK_{ut} cells were incubated with Dimebon for 6 h. Mitochondria were labelled with Mito Tracker CMXRos, fixed with PFA, and mitochondrial lengths were quantified with Image J. **B** HEK_{ut} cells were incubated with Dimebon for 6 h. Mitochondria were labelled with Mito Tracker CMXRos, fixed with PFA, and mitochondrial lengths were quantified with Image J. **C** Representative images of mitochondria in HEK cells in the presence and absence of Dimebon. Data represent the means ± SD with at least 100 measured mitochondria per experiment, n = 8-9, Two-way ANOVA with Bonferroni post tests, **p<0.01, ***p<0.001.

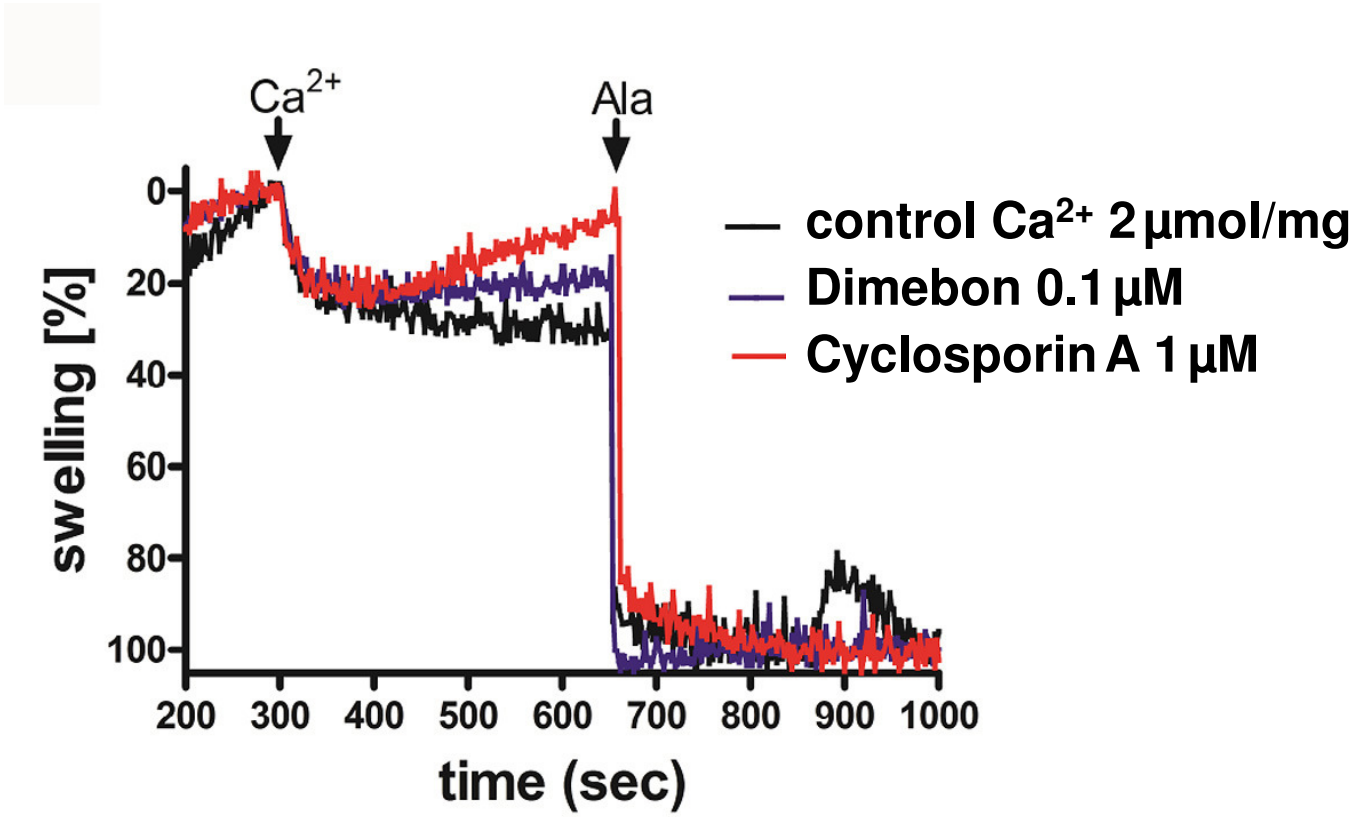
Dimebon increases Oxphos activity in HEK_{sw} but not in HEK_{ut} cells



A HEK_{ut} cells were incubated for 6 h with dimebon (0.1 µM). Respiration was measured using protocol one. **B** HEK_{sw} cells were incubated for 6 h with dimebon (0.1 µM). Respiration was measured using both protocols **A and B** Using the Oxygraph-2k, oxygen consumption in different mitochondrial stages was measured by injecting several substrates and inhibitors. $CI+II_{Leak}$, respiration on CI+II substrates to compensate for proton leak, is considered as state L; CI_{OXPHOS}, CI dependent oxidative phosphorylation; CII_{OXPHOS}, CII dependent oxidative phosphorylation; $CI+II_{OXPHOS}$, oxidative phosphorylation providing CI and CII substrates, is considered as state P; $CI+II_{ETS}$, non-coupled respiration with CI and CII substrates, is considered as maximum capacity of the ETS – state E; CII_{ETS}, non-coupled CII dependent respiration. ; CIV_{ETS}, non-coupled respiration with CIV substrates. Values represent the means ± SD from n = 6-9 experiments per protocol, Two-way ANOVA with Bonferroni post tests, *p<0.05, **p<0.01, ***p<0.001. **C** Ratios



Activity of the mPTP is reduced by cyclosporin A and dimebon (calcium induced swelling of mouse brain mitochondria)



Müller et al. Soc.Neurosciences (2012)

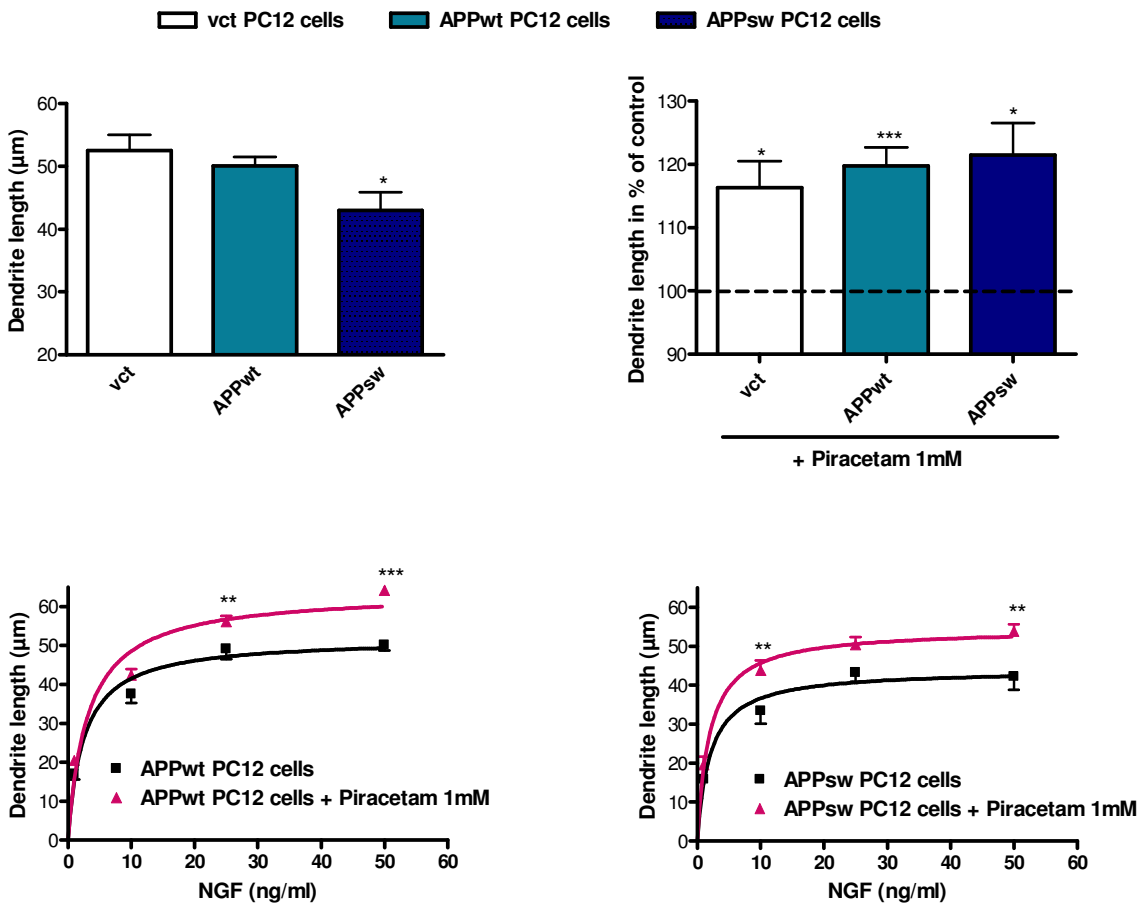
Piracetam – previous studies on mitochondrial protection

NMRI mice	• SOD, GR u. GPx↓ in aged animals • Mitochondrial membrane potential↑ in aged animals • Mitochondrial membrane potential↑ after H_2O_2, $A\beta_{1-42}$ or SNP insult
Tg AD mice	• Aβ↓ • ATP production↑ • Mitochondrial membrane potential↑
HEK APP_{wt}	• Aβ↓ • Aβ↓after SNP insult • Mitochondrial membrane potential↑ • Mitochondrial membrane potential↑ after SNP insult
PC12 cells	• ATP production↑ after serum deprivation and or SNP insult • Mitochondrial membrane potential↑ after serum deprivation and $A\beta_{1-42}$ or SNP insult • Neurite outgrowth↑ after $A\beta_{1-42}$ or SNP insult • Caspase 9 activity ↓ after SNP insult
PC12 $APP_{wt/sw}$ cells	• Neurite outgrowth↑

Stockburger et al. Biochem. Soc. Trans. 2013

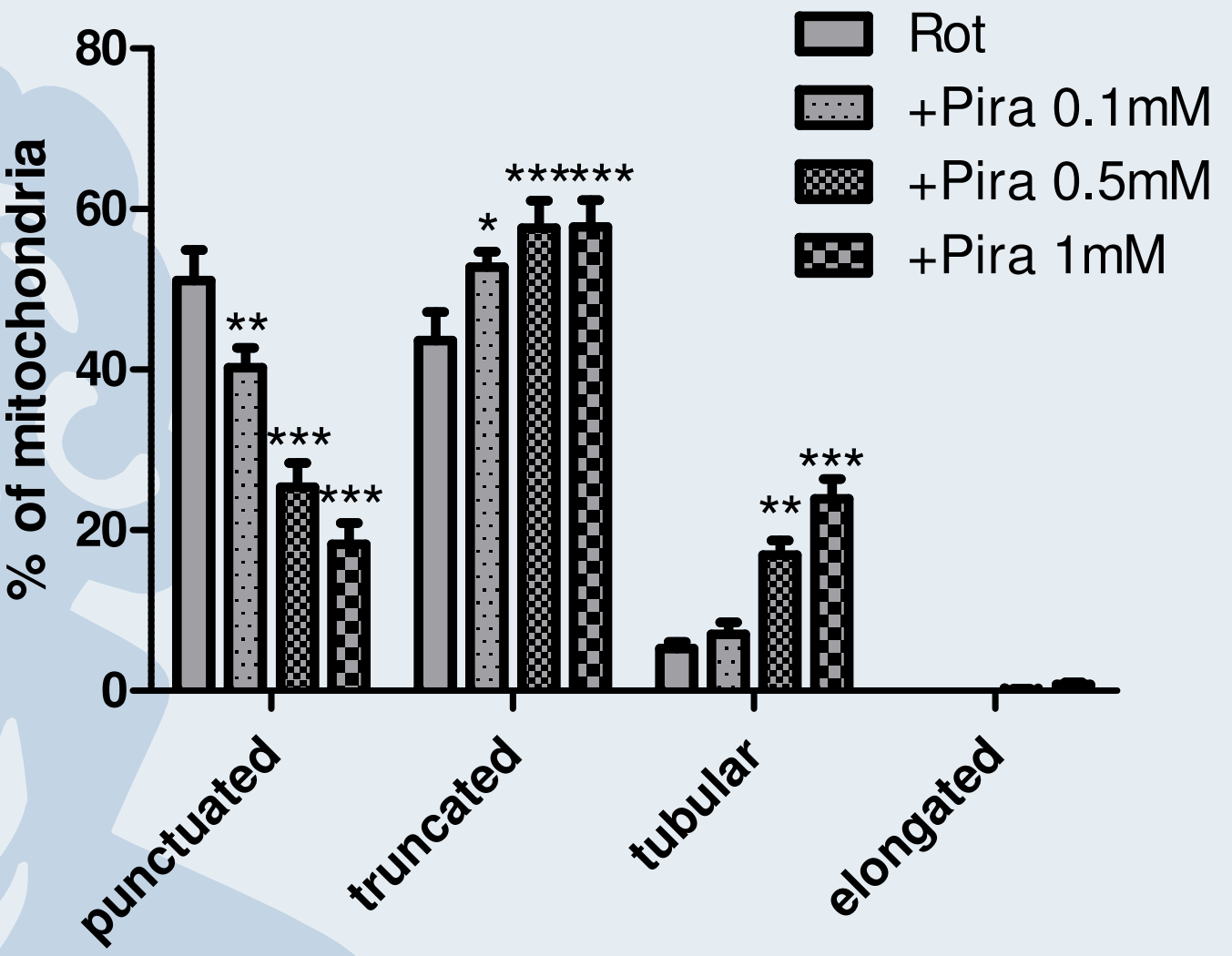
www.goethe-universitaet.de

Piracetam improves impaired neuritic outgrowth in PC12 cells expressing very low or intermediate (APPsw) levels of human Aβ



Kurz et al. (2010) Brit.J.Pharmacol. (2010)

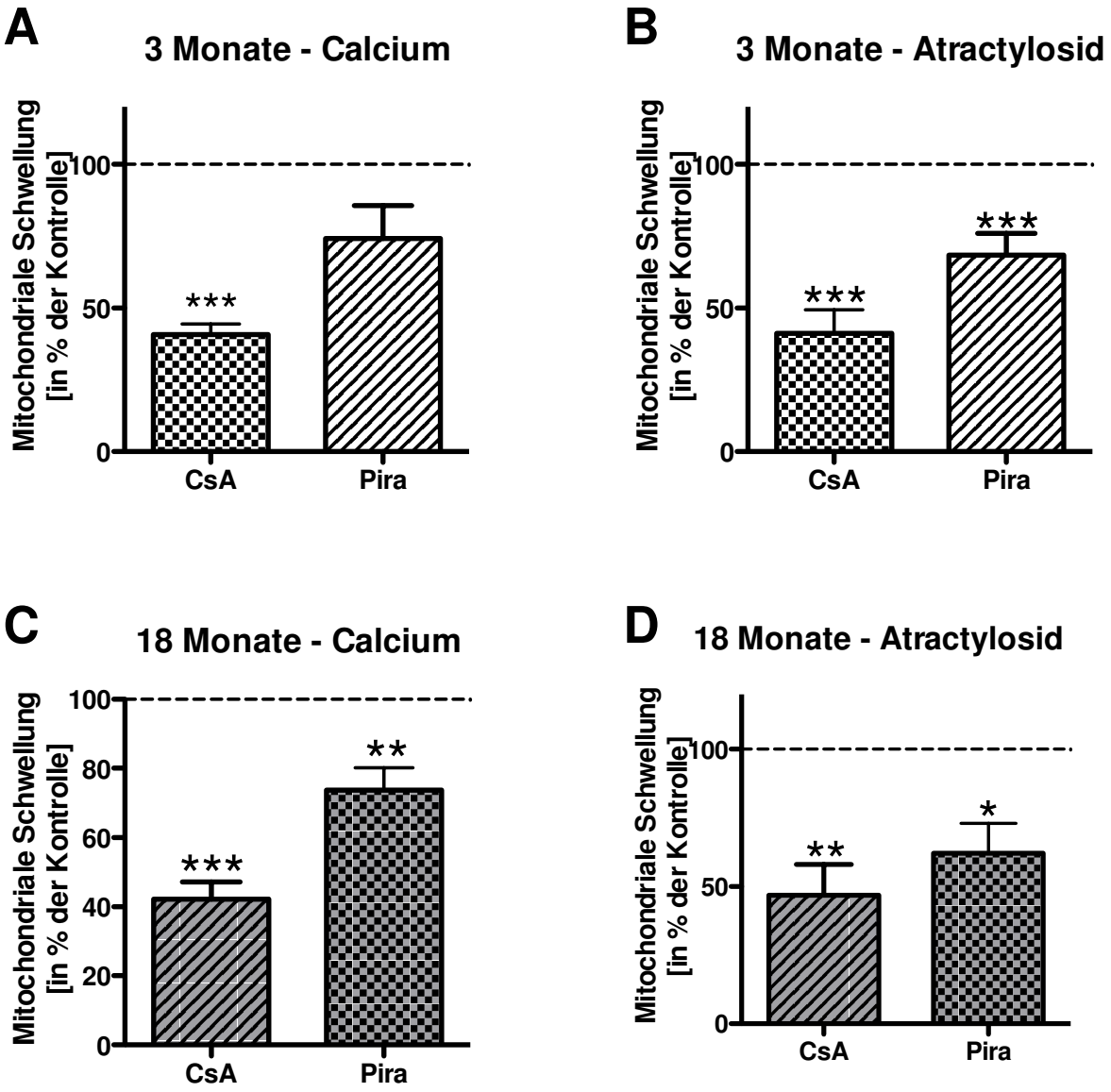
Piracetam restores miochondrial morphology in a concentration dependent manner



Stockburger et al. Biochem.Soc.Trans. (2013)

www.goethe-universitaet.de

Piracetam inhibits PTP following calcium or atractyloside activation



Stockburger et al., unpublished

Conclusions II

1. Mitochondrial protection by piracetam, Ginkgo extract, and dimebon not only improves mitochondrial function in models of brain aging and AD but also ameliorates major consequences of mitochondrial damage like enhanced Aß production and deficits of synaptic plasticity

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Safety and Tolerability of the γ-Secretase Inhibitor Avagacestat in a Phase 2 Study of Mild to Moderate Alzheimer Disease

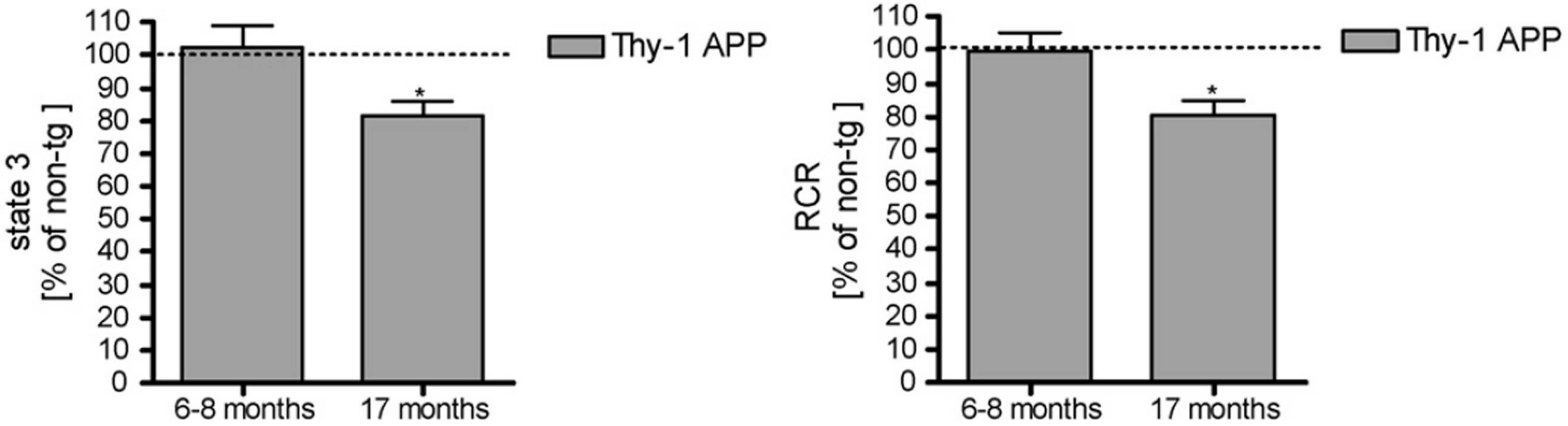
Vladimir Coric et al.

Arch. Neurol. 2012. 69(11): 1430-1440

Outcome after 24 weeks

- **Worsening of ADAScog by about 3 points**
- **Worsening of MMSE by 2 points**
- **Many severe side effects (GI,skin, cardiovascular etc.)**

Mitochondrial dysfunction (Oxphos activity) increases with aging in Thy1-APP mice



State 3 respiration and the respiratory control ratio are decreased in 17 months old but not in 6 months old Thy1-APP mice relative to control animals

Hauptmann et al., (2009) Neurobiology of Aging

The three antidementia drugs: Evidences of clinical efficacy

1) Ginkgo biloba extract EGb761®

   Positive effects on cognition in AD - patients in recent metaanalyses

- Wang, B-S. et al.: Effectiveness of Standardized *Ginkgo biloba* Extract on Cognitive Symptoms of Dementia with a Sixth-Month Treatment: A Bivariate Random Effect Meta-Analysis. *Pharmacopsychiatry (2010), 43: 86-91.*
- Weinmann, S. et al.: Effects of Ginkgo biloba in dementia: systematic review and meta-analysis. *BMC Geriatrics (2010),* ***10****: 14.*

2) Piracetam

   Positive effects on cognition over several groups of patients with geriatric memory disorders

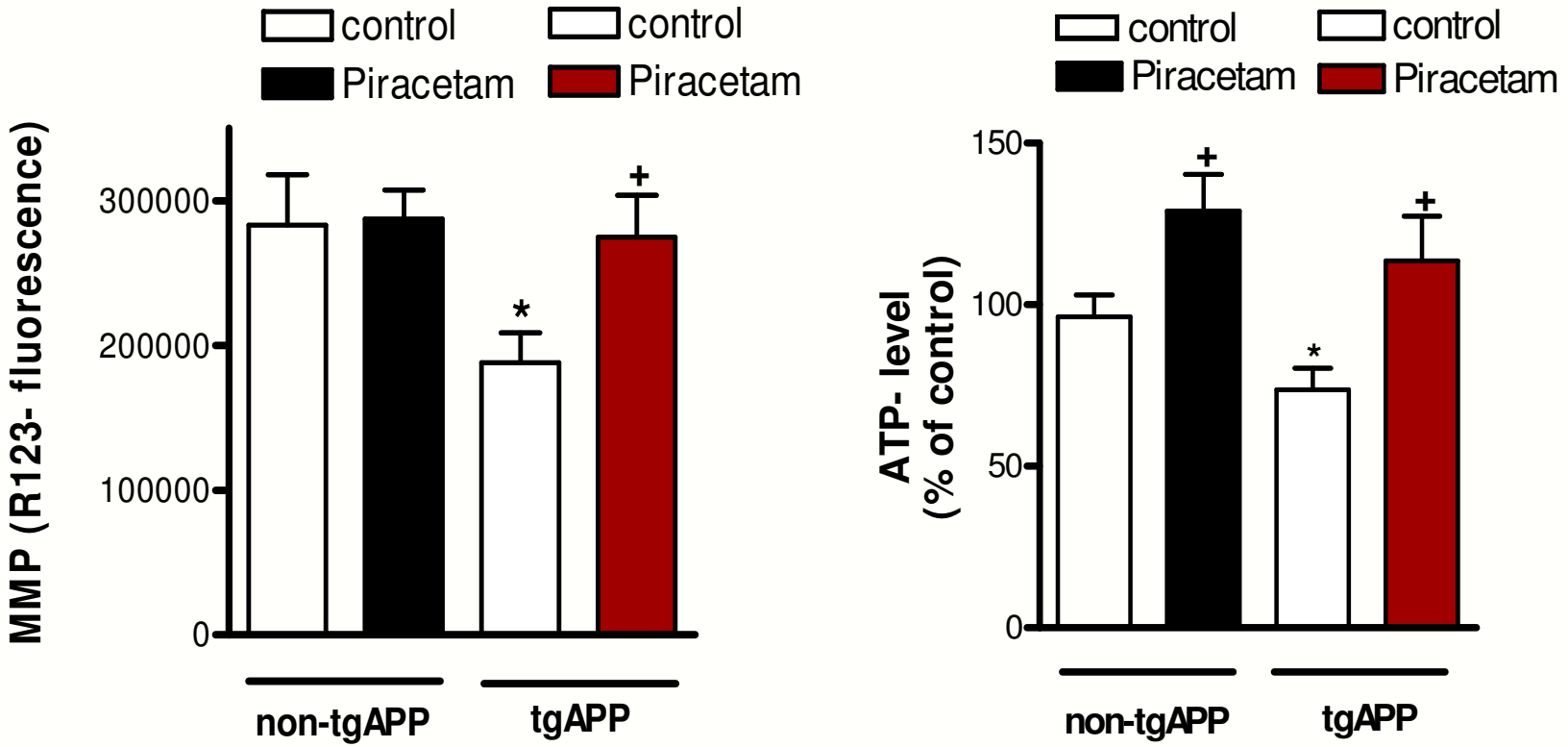
- Waegemans, T. et al.: Clinical efficacy of piracetam in cognitive impairment: a meta-analysis. *Dementia and Geriatric Cognitive Disorders (2002), 13(4), 217-224.*

3) Dimebon

   Positive effects on NPI over several studies

- Cano-Cuenca, N. et al.: Evidence for the Efficacy of Latrepirdine (Dimebon) Treatment for Improvement of Cognitive Function: A Meta-Analysis. *Journal of Alzheimer's Disease (2013)*

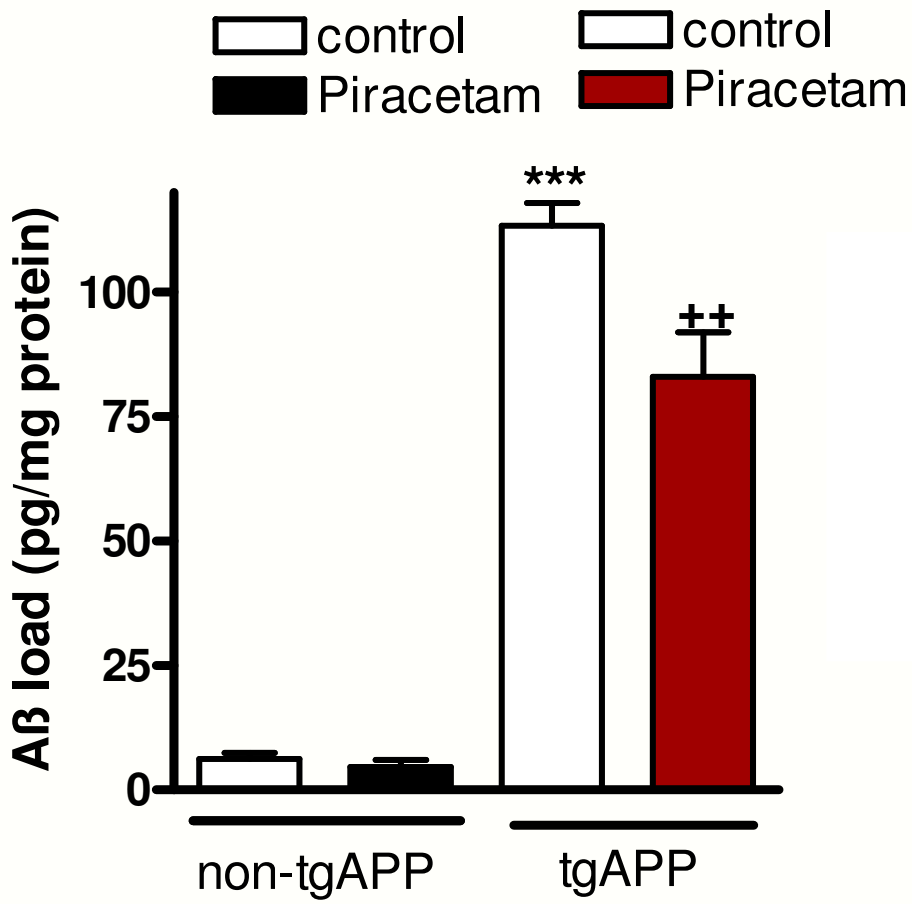
Treatment with piracetam (500 mg/kg, 14 days) normalised reduced mitochondrial membrane potential (MMP) and ATP levels in dissociated brain cells of APP transgenic mice



Kurz et al., *British Journal of Pharmacology 2010*

Treatment with piracetam reduces Aß load in APP mice

APP transgenic mice (brain)

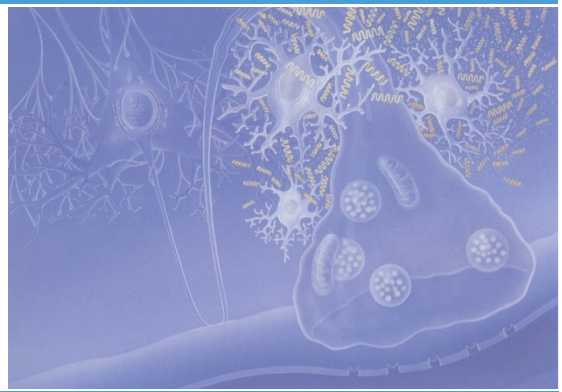


Mice transgenic for mutant human APP (swedish mutation) and non-transgenic littermates were treated for 14 days with piracetam (500mg/day orally).

Soluble $A\beta_{1\text{-}40}$ was determined in brain homogenates by Elisa

Kurz et al., *British Journal of Pharmacology 201*

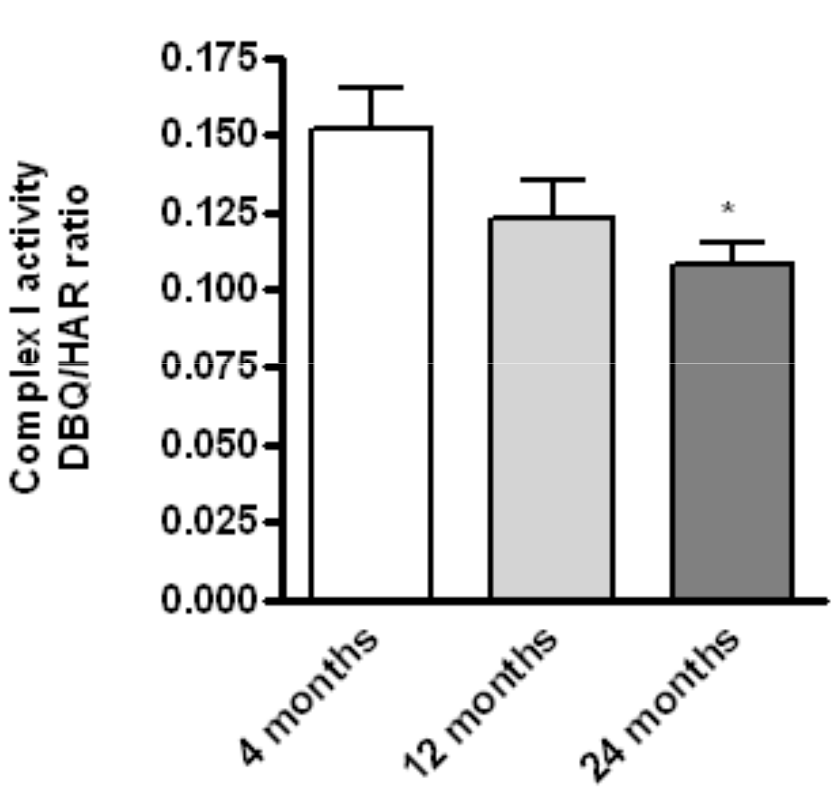
Mitochondrial dysfunction in Alzheimer‘s disease. The important mutual interaction with brain aging

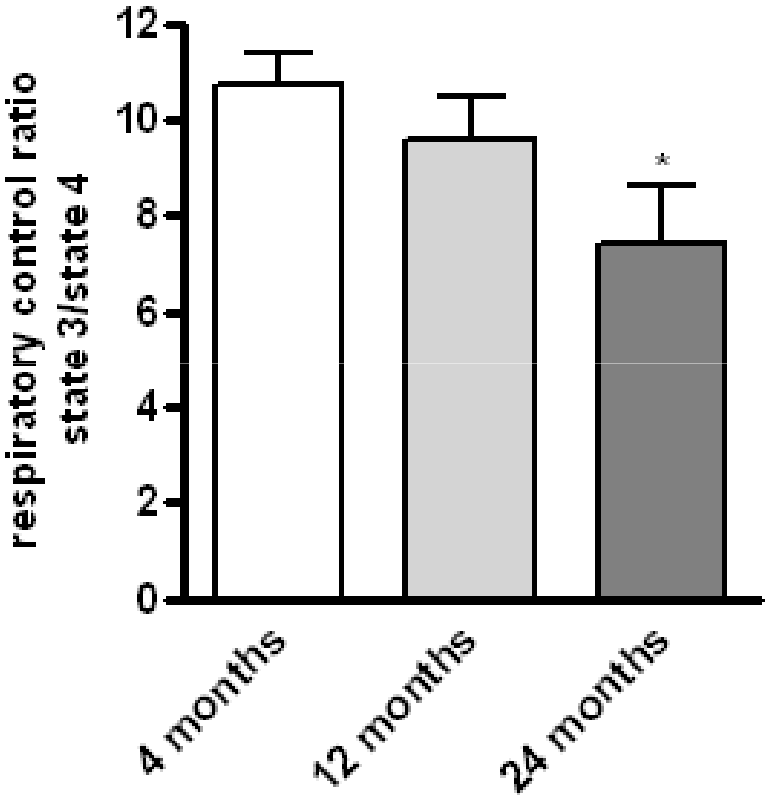


Our experimental model systems:

1. NMRI mice (3, 12, 22 months of age)
2. PC12 cells, SY5Y cells, or HEK cells transgenic for human APP or the swedish APP double mutation
3. SY5Y cells transgenic for human APP
4. Mice transgenic for the swedish APP double mutation (Thy1-APP)
5. Mice transgenic for the human Tau mutant P301L
6. Freshly isolated lymphocytes from non-demented aged controls, patients with minimal cognitive impairment (MCI), and Alzheimer patients (AD)

Mainly complex I function is impaired during aging

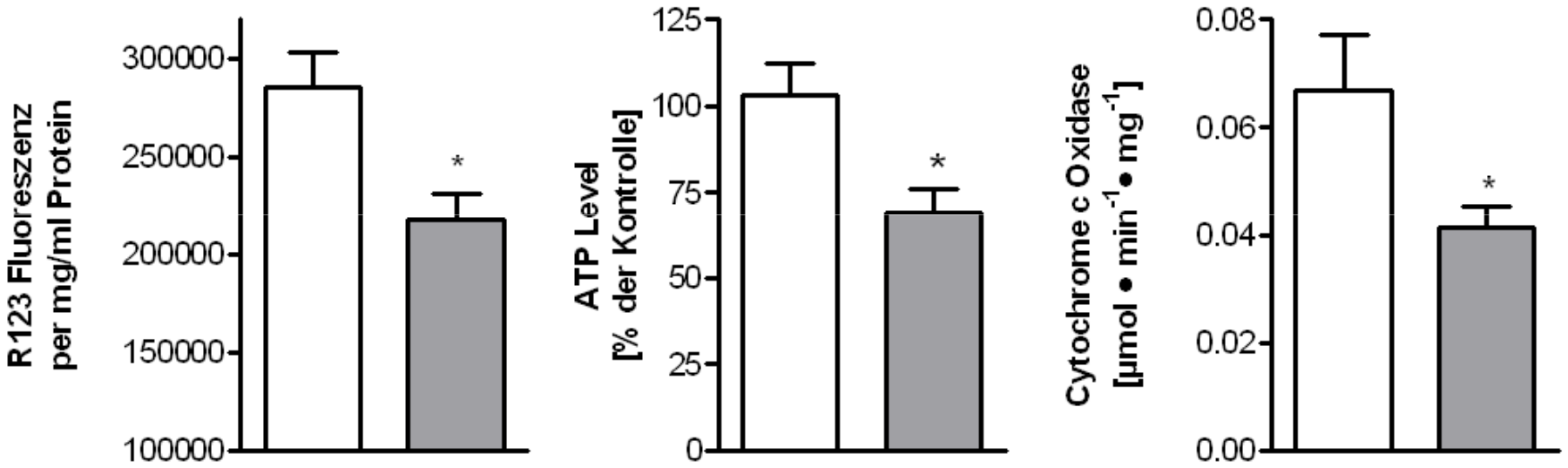




Complex I activity and mitochondrial function significantly reduced in aged NMRI mice.

Leuner, Hauptmann, Abdel-Kader, Scherping, Keil, Strosznajder, Eckert, Müller *Antioxid. Redox. Signal. 9:1659-75 (2007)*

Impaired mitochondrial function in Alzheimer cell and animal models



Pronounced mitochondrial dysfunction associated with reduced complex IV activity at an age of three months in AD animal model.

Hauptmann, Scherping, Dröse, Brandt, Schulz, Leuner, Jendrach, Eckert, Müller Neurobiol. Aging. 30:1574-86 (2009)