Reciprocal influence between APP expression and glucose metabolism in the hippocampus

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BELGIAN BRAIN CONGRESS
MICX – MONS October 8th, 2016

Introduction

Nowadays, there is evidence that brain glucose metabolism and Alzheimer's disease (AD) are linked. Patients suffering from type II diabetes present a higher risk to develop AD while in AD patients but also in preclinical stage (MCI) the brain glucose metabolism is reduced, leading to a general hypometabolism. Our hypothesis is that APP is involved in energy flux between the body and the brain. During ageing or in case of pathology such as Alzheimer's disease, Down Syndrome and insulin resistance, glucose availability can be reduced in the brain leading to a compensatory increase in the expression of APP. This compensatory increase could be the starting point of metabolic and neurotransmitter homeostasis disruption leading to cognitive deficits. The aim of this project is to better understand the link between APP expression and brain glucose metabolism and its impact on neuronal activity and synaptic connections.

Hypothesis and Mouse Model

Glucose metabolism ⟺ [APP]

1. APP is involved in glucose metabolism

[APP] ⇨ Glycolysis (GAPDH) / ATP production / Glutamate transport…

2. APP overexpression is correlated with glucose metabolism disruption in pathologies or ageing

Glucose hypometabolism ⇦ Down Syndrome ⇨ ⇧ [APP]

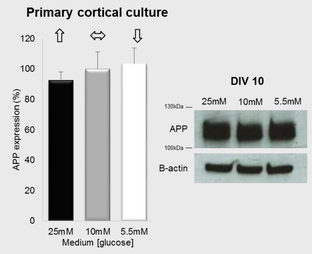
Glucose hypometabolism ⇦ Alzheimer's disease / Ageing / Brain insulin resistance ⇨ (or) ⇧ [APP]

Hyperglycemia, hypoglycemia and insulin resistance ⇦ Type 2 Diabetes ⇨ (or) ⇧ [APP]

3. Upregulation of APP mRNA when glucose is reduced is also found in the literature…

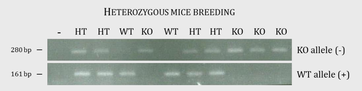
Rat primary cortical astroglial cells deprived of 95% of their glucose for 24h ⇨ ⇧ APP mRNA

4. …but our experiments did not show any overexpression of the APP protein when glucose supply is reduced in WT mice



5. So, until now, we mostly focused on the 3 APP expression levels available thanks to APP knockout mice

Genotype		Level of expression
WT	+/+	Normal expression
HT	+/-	Half expression
KO	-/-	No expression

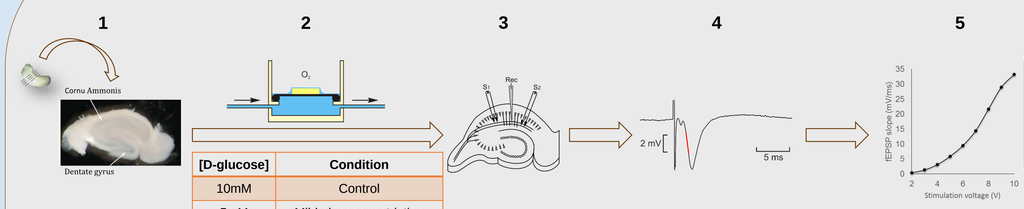


Advantages
- Allowing to study the physiological roles of APP and the importance of its expression level
- Excluding the role of Aβ oligomerization

Inconvenient
- No possible APP overexpression (without glucose metabolism or genetic modifications)

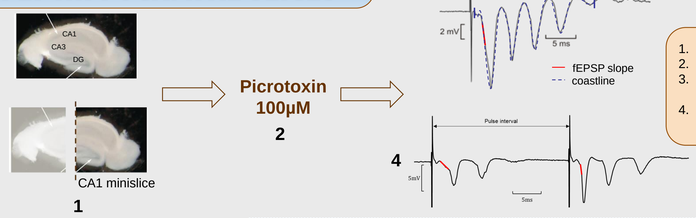
Electrophysiological Recordings

Synaptic activity in restricted glucose supply hippocampal slices



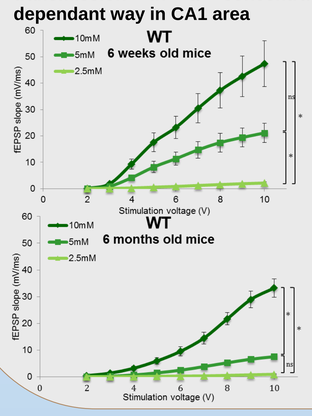
[D-glucose]	Condition
10mM	Control
5mM	Mild glucose restriction
2.5mM	Severe glucose restriction

1. Hippocampal slices preparation (APP +/+, +/- and -/- mice)
2. Resting period in an interface recording chamber (1h30). aCSF (32°C) contains 10mM, 5mM or 2.5mM of D-glucose
3. Electrodes positioning in CA1 area. Stimulation of the Schaffer collaterals and recording in the *stratum radiatum* of CA1
4. Evaluation of synaptic activity by fEPSP slope recording
5. Input-Output curve resulting from an increasing stimulation voltage difference from 2V to 10V in increments of 1V

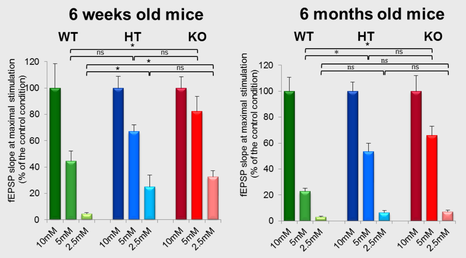


1. CA1 minislice preparation (APP +/+, +/- and -/- mice)
2. Resting period in presence of picrotoxin (100µM)
3. Epileptiform activity evaluation by recording the coastline (30ms)
4. Paired-pulse facilitation recording at 25, 50, 100 and 200ms of pulse interval

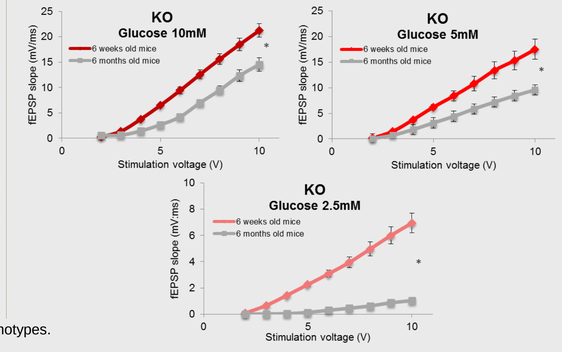
1. Glucose restriction reduces synaptic transmission in a concentration dependant way in CA1 area



2. The level of APP expression modulates the sensitivity to restriction in glucose supply in the hippocampus

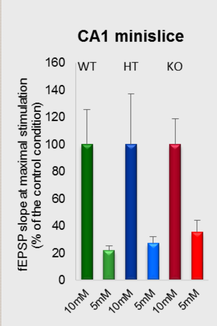


3. Ageing reduces the basal synaptic activity of the neuronal network and the tolerance to restriction in glucose supply in the hippocampus

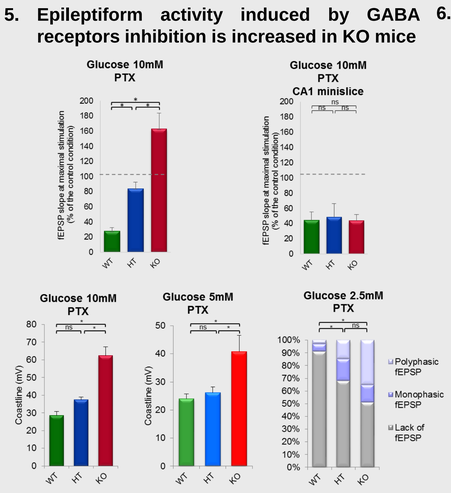


I-O curves distributions presented in 1 and 3 are similar in the other two genotypes.
HT mice always present an intermediate phenotype.

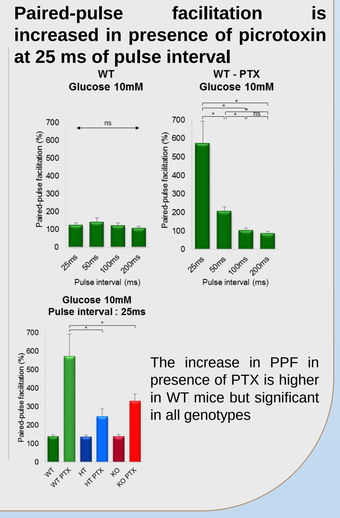
4. The sensitivity to glucose restriction is also observed in CA1 minislices



5. Epileptiform activity induced by GABA receptors inhibition is increased in KO mice



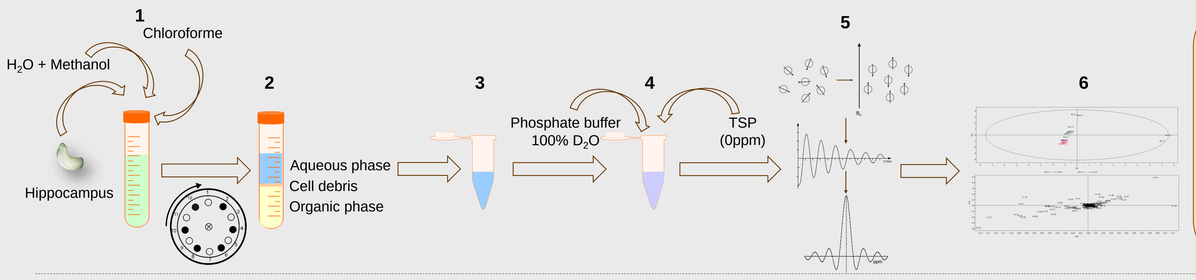
6. Paired-pulse facilitation is increased in presence of picrotoxin at 25 ms of pulse interval



The increase in PPF in presence of PTX is higher in WT mice but significant in all genotypes

^{1H}NMR

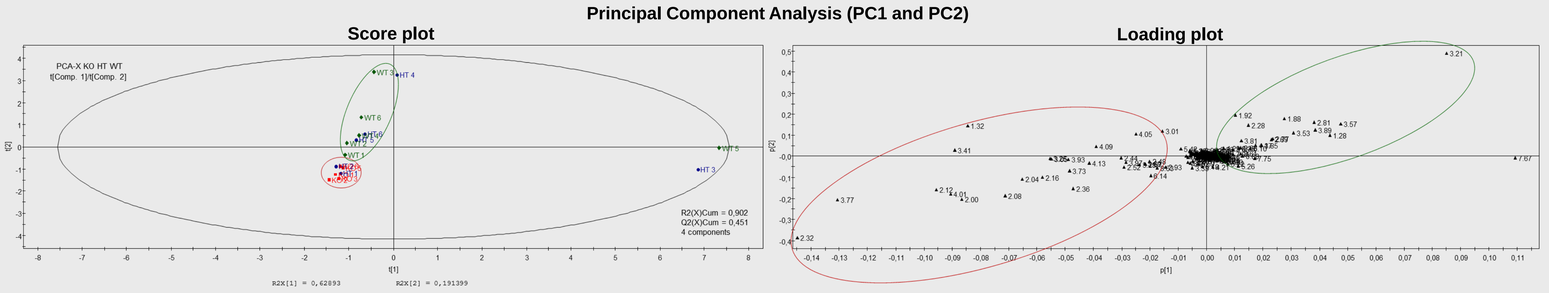
Metabolic activity in the hippocampus



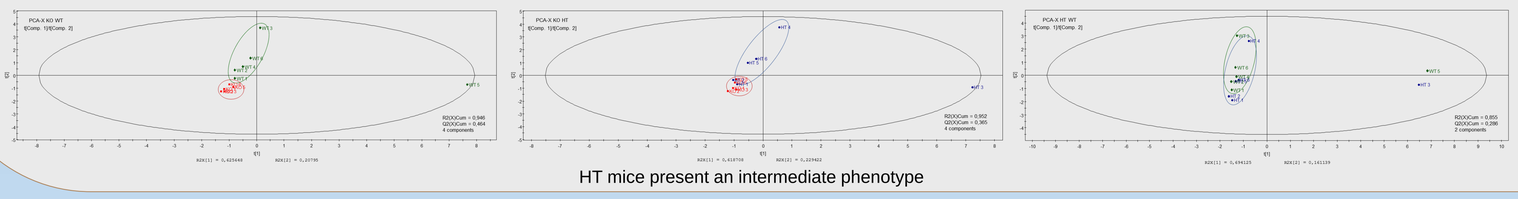
1. Hippocampi extraction, dissociation and metabolites extraction in methanol, H_2O and chloroform (4°C)
2. Centrifugation and phases separation
3. Evaporation of the aqueous phase (speedvac)
4. Metabolites resuspension in phosphate buffer 100% D_2O and a reference compound : TSP
5. Sample magnetization in a 500 mHz ^{1H}NMR spectrometer and spectra acquisition (Fourier Transform)
6. Spectra normalization (Mestre Renova) and ppm separation (loading and score plots) by Principal Component Analysis (PCA) in Simca. Corresponding metabolites are finally identified thanks to Chenomx and tables

1. The level of APP expression modifies the metabolic function in the hippocampus

Principal Component Analysis (PC1 and PC2)

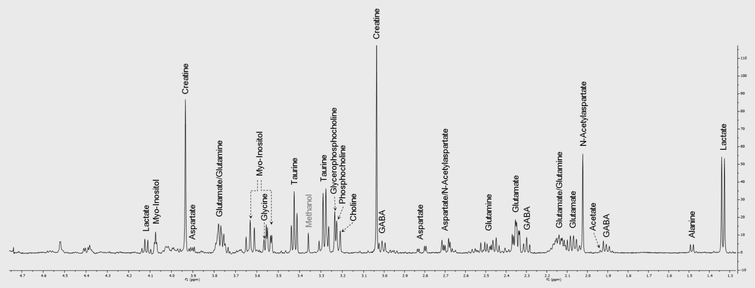


2. Metabolic modifications are more important between WT and KO mice while WT and HT mice present a similar hippocampus metabolism

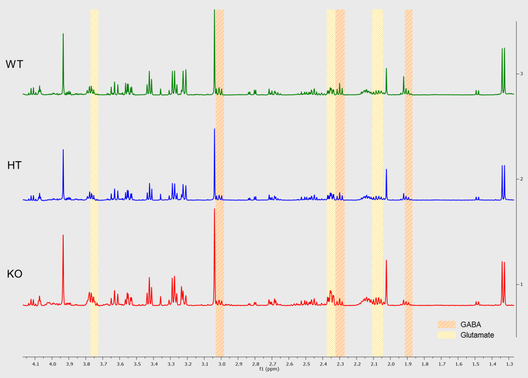


HT mice present an intermediate phenotype

3. Identification of aqueous metabolites detectable on a ^{1H}NMR spectrum obtained from the extraction of the hippocampi of an APP KO mouse



4. APP plays a role in neurotransmitters homeostasis in the hippocampus



The absence of APP increases the presence of glutamate but decreases the presence of GABA

Conclusion

The next step is to determine if the reduction in glucose supply causes an increase in the APP expression as described in the literature. If this hypothesis is validated, it could allow us to have a new level of APP expression: the overexpression one. This hypothesis is critical to determine if modifications found in *ex vivo* glucose restrictions can be related to molecular changes found in Alzheimer's disease and Down syndrome. Nevertheless, we can already conclude that **APP expression and glucose metabolism are indeed linked in the hippocampus** and that further investigations need to be conducted in the future to better understand this relationship.