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Reciprocal influence between APP expression and glucose metabolism in the hippocampus

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FREEDOM TO RESEARCH

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Working 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]

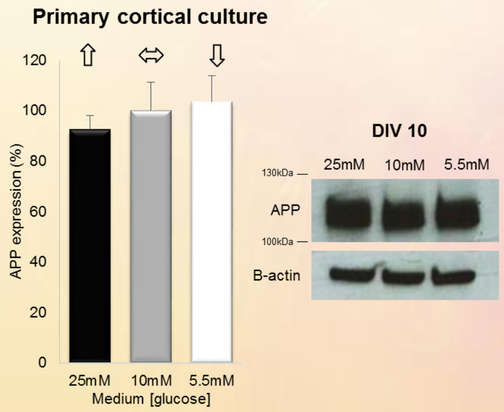
Glucose hypometabolism ⇨ Alzheimer's disease, Ageing, Brain insulin resistance ⇦ or ⇨ ⇧ [APP]

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…

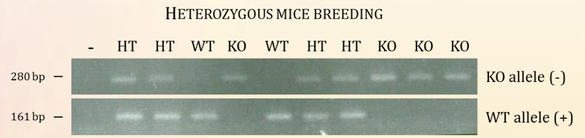
Rat primary cortical astroglial cells deprived of 95% of their glucose for 24h ⇨ ⇧ APP mRNA

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

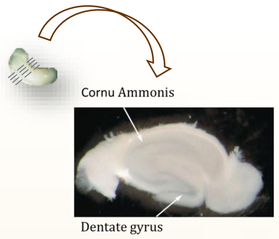
Inconvenient

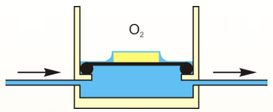
- No possible APP overexpression (without glucose metabolism or genetic modifications)

Electrophysiological recordings :

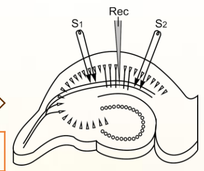
synaptic activity in restricted glucose supply hippocampal slices

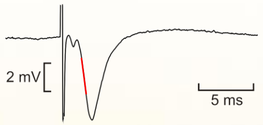
1 2 3 4 5

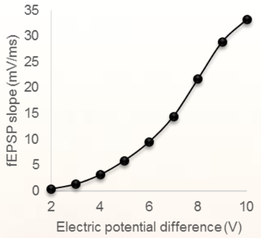




[D-glucose]	Condition
10mM	Control
5mM	Mild glucose restriction
2.5mM	Severe glucose restriction

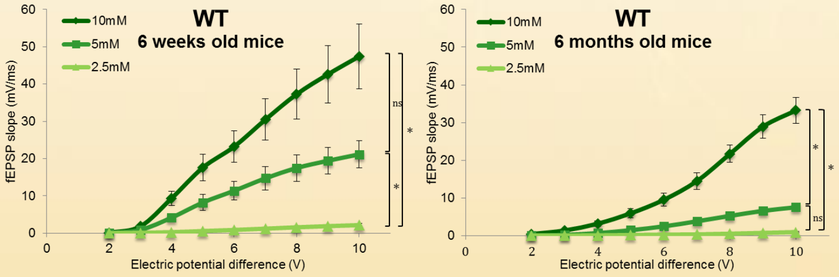




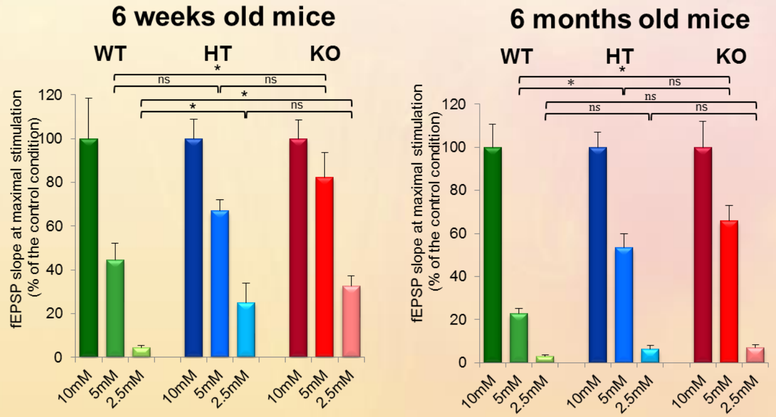


1. Hippocampal slices preparation (APP +/+, +/- and -/- mice)
2. Resting period in an interface recording chamber (1h30). aCSF (32°C) contains 10mM, 5mM or 2.5mM of D-glucose
3. Electrodes positioning in CA1 area. Stimulation of the Schaffer collaterals and recording in the *stratum radiatum* of CA1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

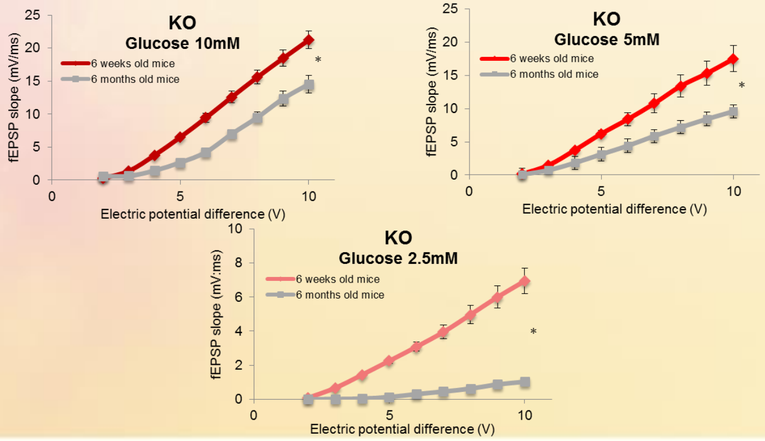
1. Glucose restriction reduces synaptic transmission in a concentration dependant way in CA1 area of hippocampal slices



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.

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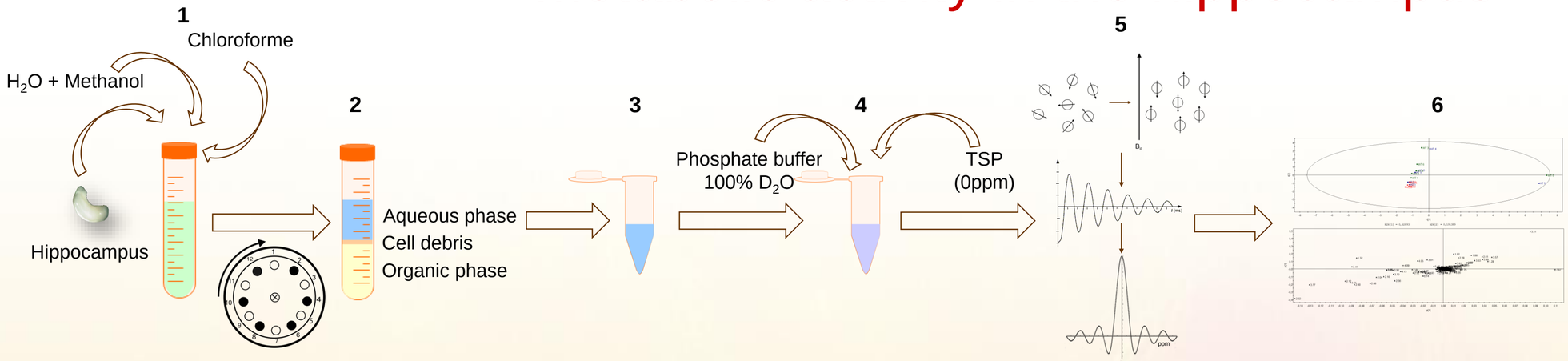
^{1H}NMR:
metabolic activity in the hippocampus

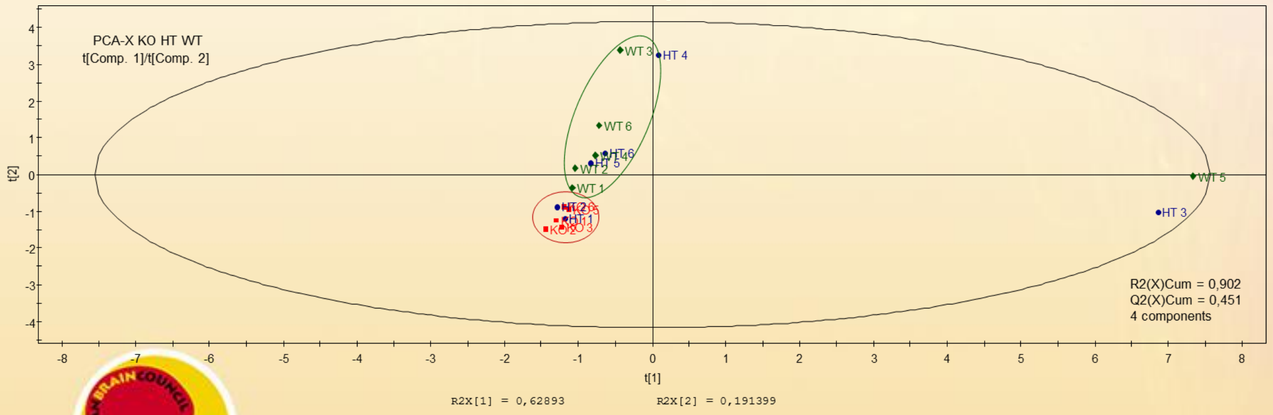


1. Hippocampi extraction, dissociation and metabolites extraction in methanol, H_2O and chloroform (4°C)
2. Centrifugation and phases separation
3. Evaporation of the aqueous phase (speedvac)
4. Metabolites resuspension in phosphate buffer 100% D_2O and a reference compound : TSP
5. Sample magnetization 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

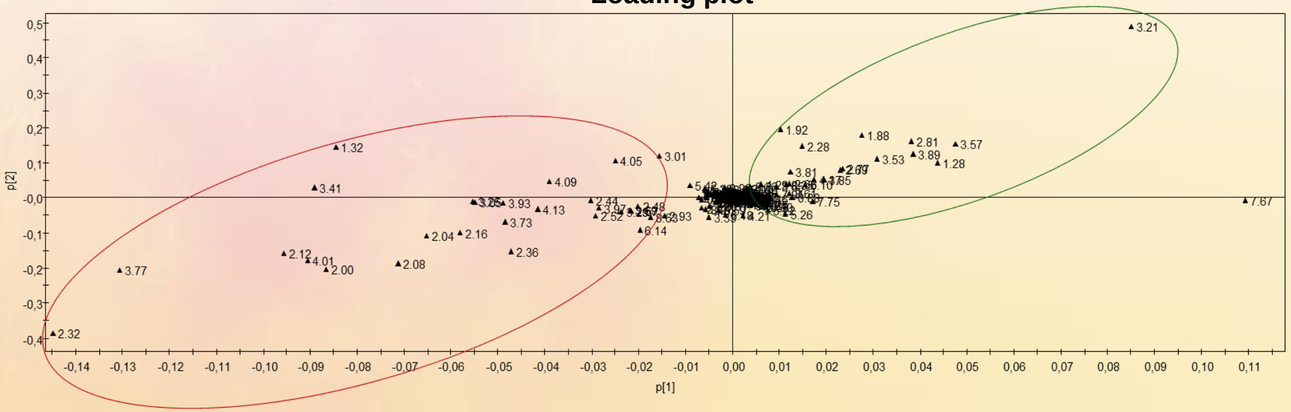
4. The level of APP expression modifies the metabolic function in the hippocampus

Principal Component Analysis (PC1 and PC2)

Score plot

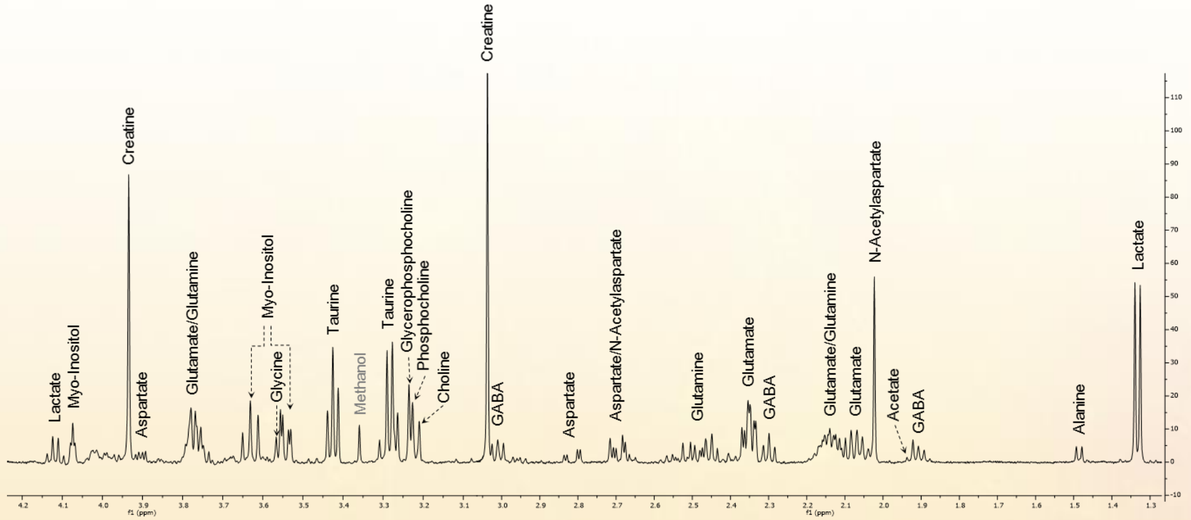


Loading plot

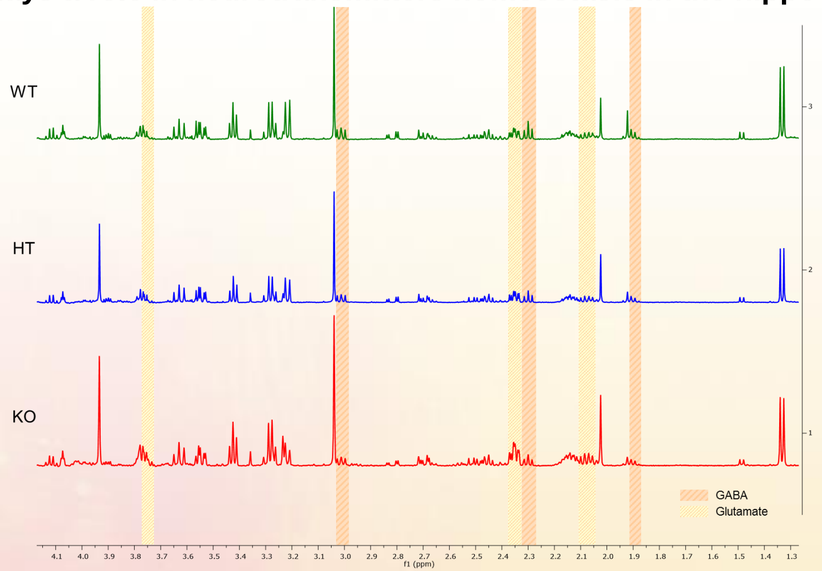


^{1H}NMR: metabolic activity in the hippocampus

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

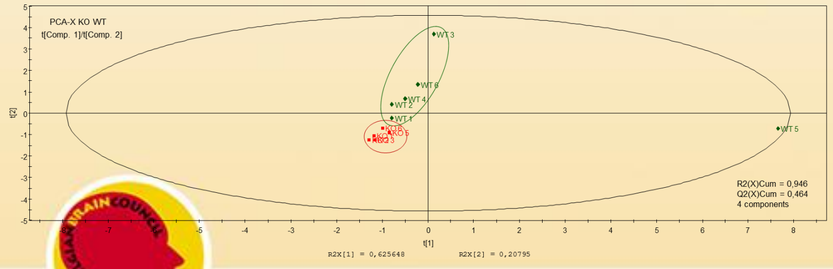


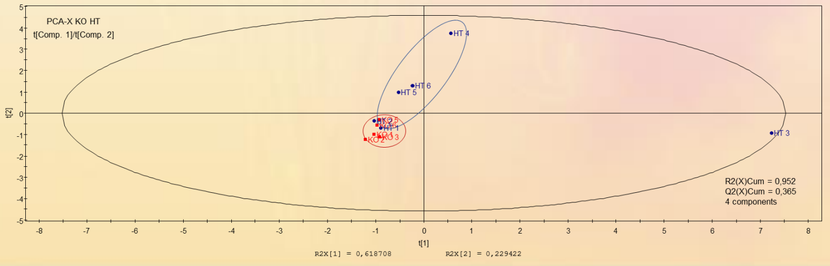
6. APP plays a role in neurotransmitters homeostasis in the hippocampus

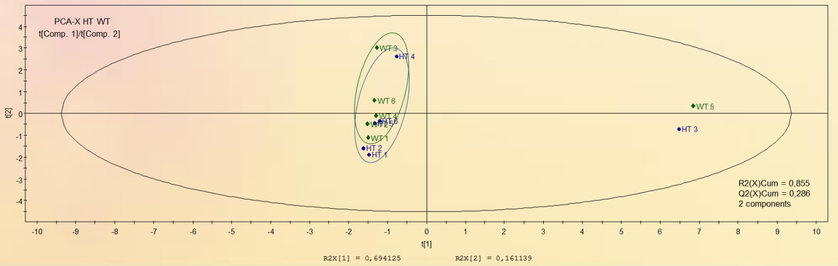


The absence of APP increases the abundance of glutamate but decreases the abundance of GABA

7. 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

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Questions left and conclusion

1. What kind of modifications could we observe in the hippocampus energy metabolism submitted to an *in vivo* hypoglycemia ?

- Continuous and controlled insulin administration to induce an *in vivo* hypoglycemia (50mg/dL)
- Hippocampi extraction
- Comparison of metabolite profiles modifications (^{1H}NMR) between sham condition and hypoglycemia for each genotypes

2. Will we be able to observe that APP is upregulated and/or overexpressed when glucose supply is restricted in one of our models ?

- APP protein quantification (Western-Blotting) from hippocampi submitted to an *in vivo* hypoglycemia
- APP mRNA quantification (RT qPCR) from our culture model and hippocampi submitted to an *in vivo* hypoglycemia

We can conclude that even if APP overexpression has not been observed in this project yet, **APP expression and glucose metabolism are linked in the hippocampus** and that further investigations need to be conducted to better understand this relationship