Experimentally Induced Diabetes in Transgenic AD Mice Accelerates Brain Pathology

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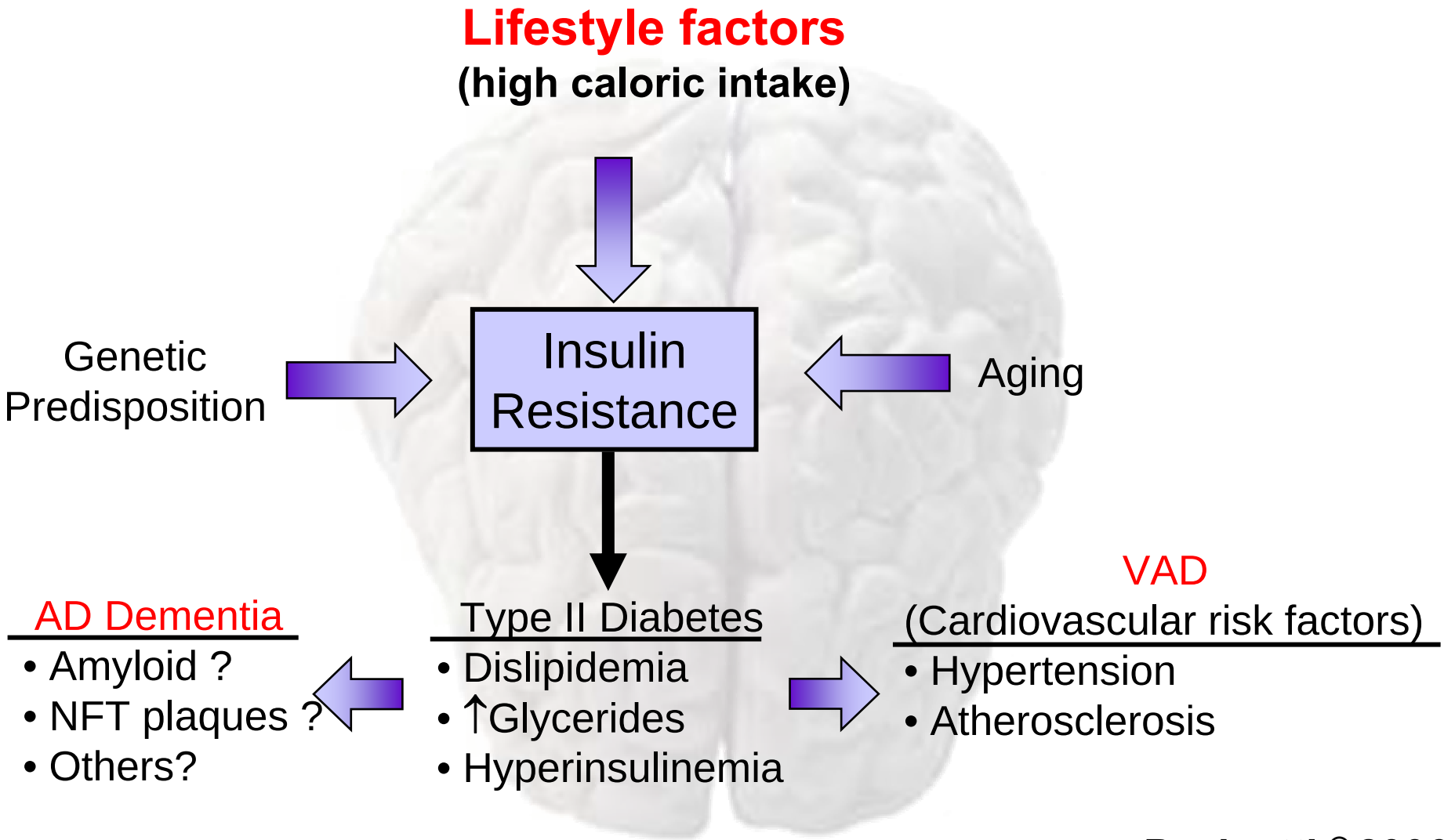
Type II Diabetes - Insulin Resistance A Risk Factor for Alzheimer's disease?

- Rotterdam Study- Type II diabetes (non insulin dependant diabetes; NDDM) doubles RR (1.9) of AD incidence even when cases with cerebrovascular disorders were excluded (Ott et al., 1999).

- Insulin resistance, a major feature of Type II diabetes, is a significant risk factor pure AD (>2 fold RR). Association of diabetes and AD is strong among carriers of the ApoE4 (Peila 2002)

- Therapeutic evidence that certain insulin sensitizing drugs may beneficially influence AD:
  - biguanide (e.g. metformin)
  - glitazones (insulin sensitizing & PPAR-activating actions)

The Potential Role of Diabetes in Alzheimer's Disease



Diet Induced Diabetes in mouse models of Alzheimer's Disease type Neuropathology

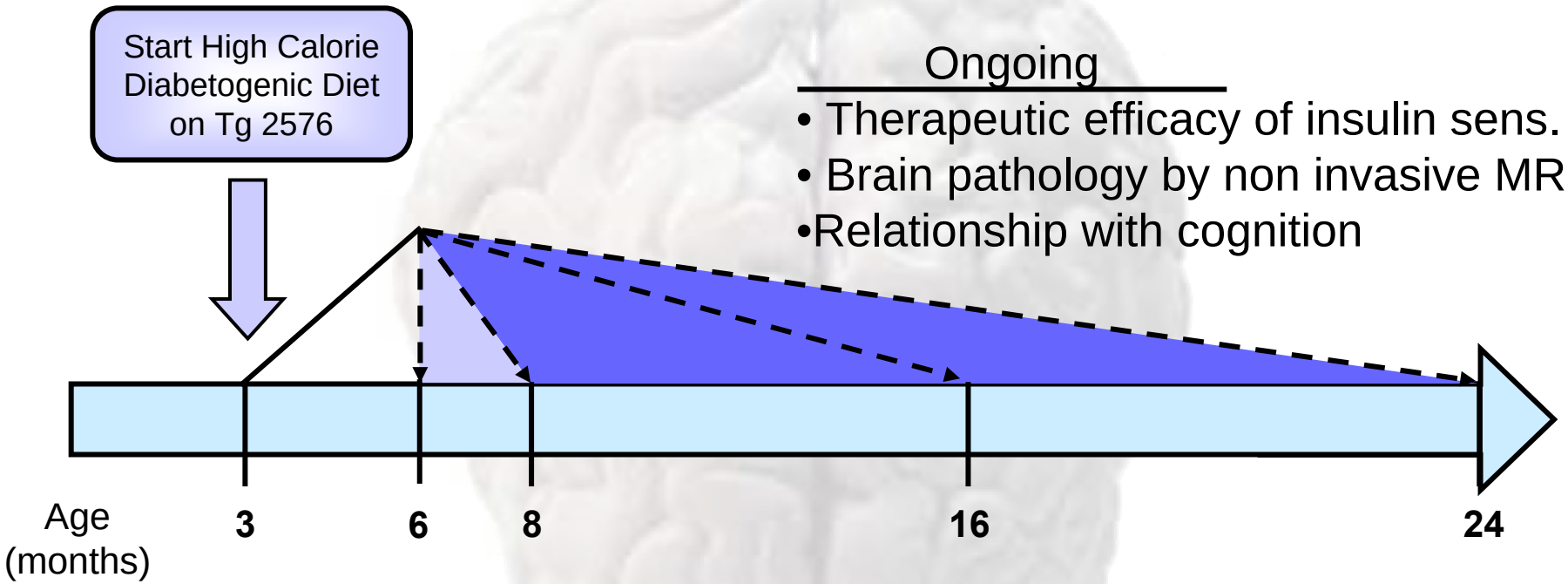
- Does insulin resistance promote AD type neuropathology through mechanisms that involve generation (e.g. γ-secretase) or impaired clearance (e.g. insulin degrading enzyme) of Aβ?

- The mechanism associated with insulin resistance mediated amyloidosis may involve abnormal regulation of insulin receptor (IR) functions in the brain.

- If insulin resistance promotes Aβ generation, are insulin sensitizing- anti-diabetic drugs beneficial to AD type amyloid neuropathology?

Potential Roles of Diet Induced Diabetes in Alzheimer's Disease Neuropathology

Scheme of Treatment



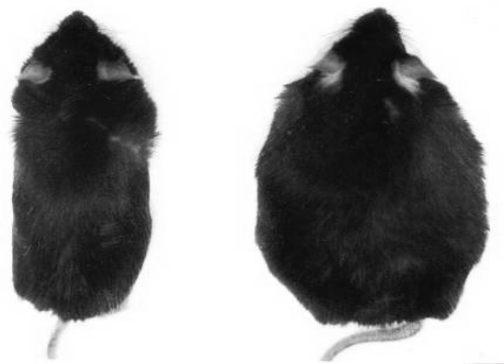
- Aβ generation (secretase acticities)
- A**β** clearance - IDE
- AD-type neuropathology

Potential Roles of Diet Induced Diabetes in Alzheimer's Disease Neuropathology

Dietary composition

	High Fat	CTL
Fat	**60%**	**20%**
Carbohydrate	**20%**	**60%**
Protein	**20%**	**20%**

Standard diet **HF diet**

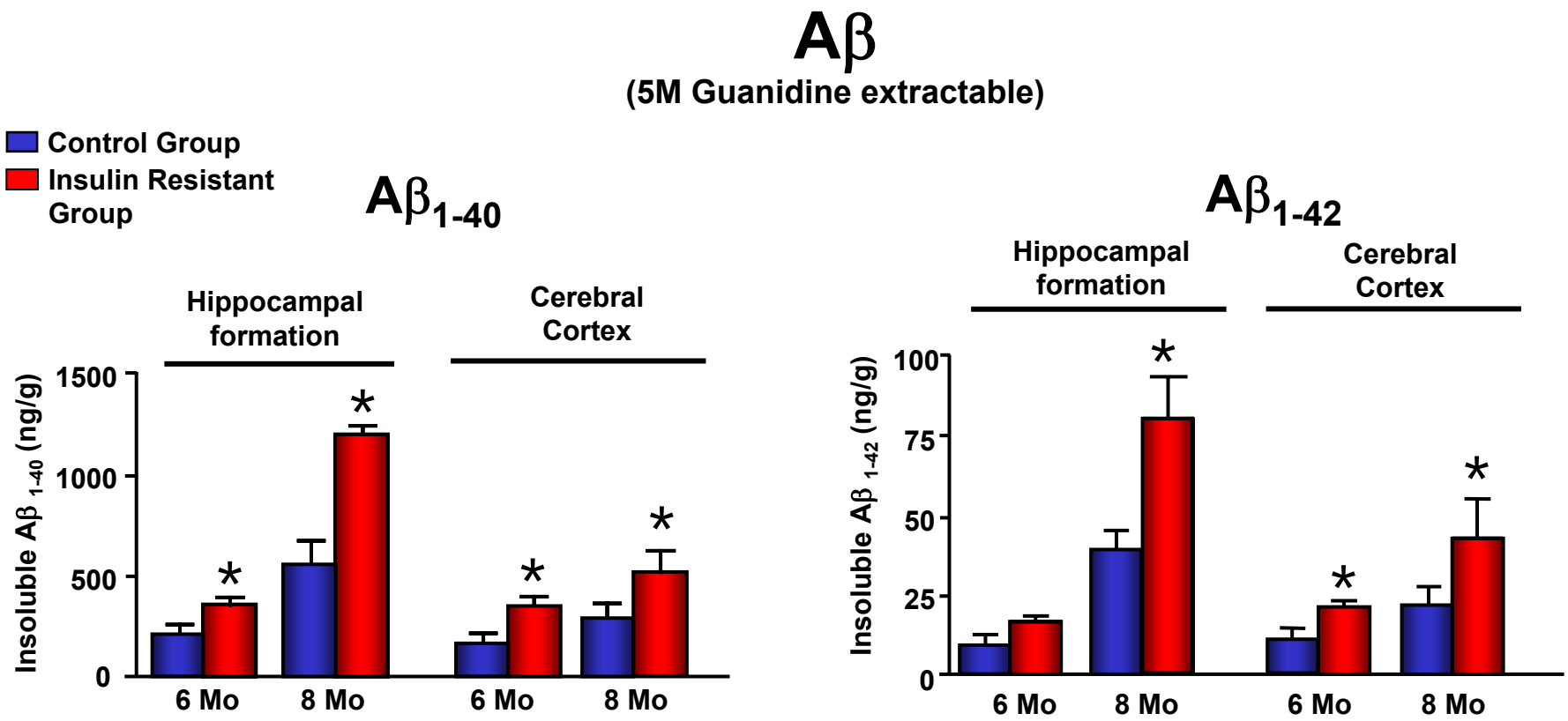


Tg2576 mice

Effects of diabetogenic high fat diet

- ↑ obesity
- ↑ fat pat deposition
- ↑ triglyceride
- ↑ insulin
- ↑ hyperglycemia
- = serum cholesterol content
- ↑ insulin resistance – Gluc Toler test

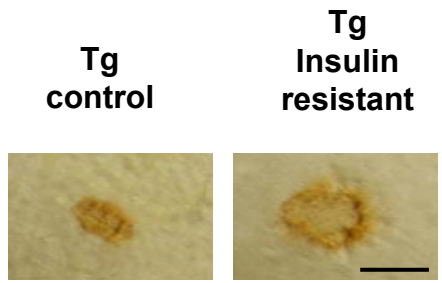
Diet Induced Diabetes Promotes Aβ Peptide Content in the Brain

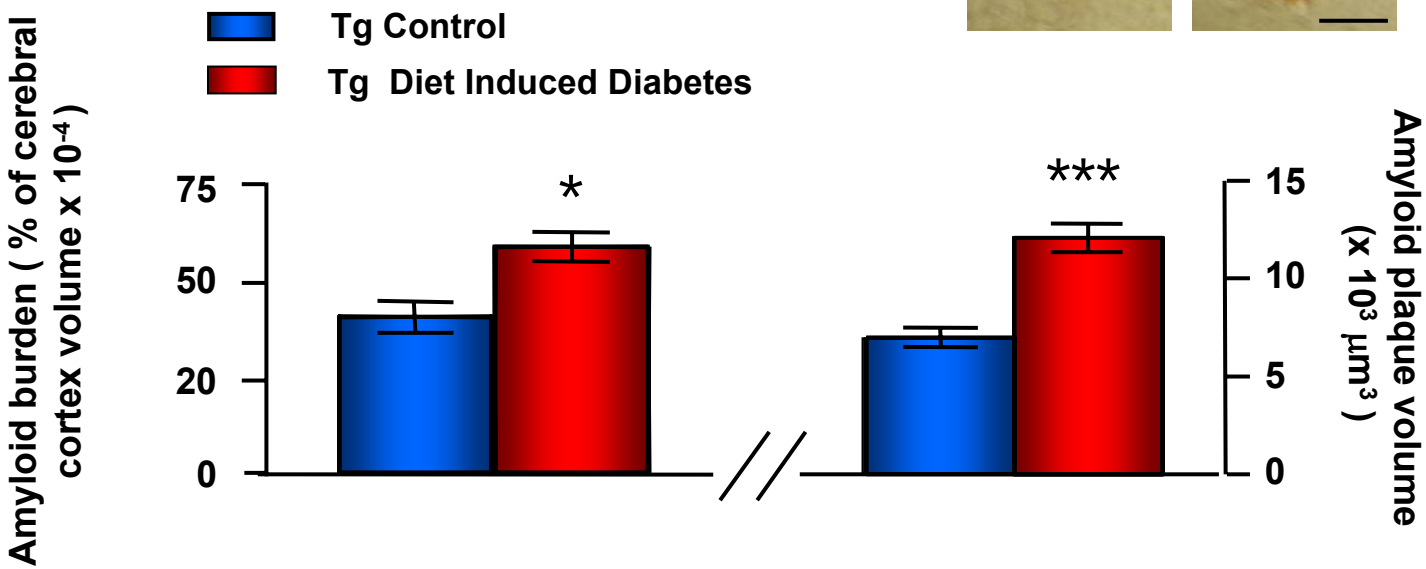


- Means ± SEM, n= 3-4 per group; *P <0.01 vs control group
- Diet induced insulin resistance lasted for 3-5 months respectively starting at 3 month of age

Diet Induced Diabetes Promotes AD-type β-amyloid Plaque Neuropathology in the Tg2576 Mouse Brain

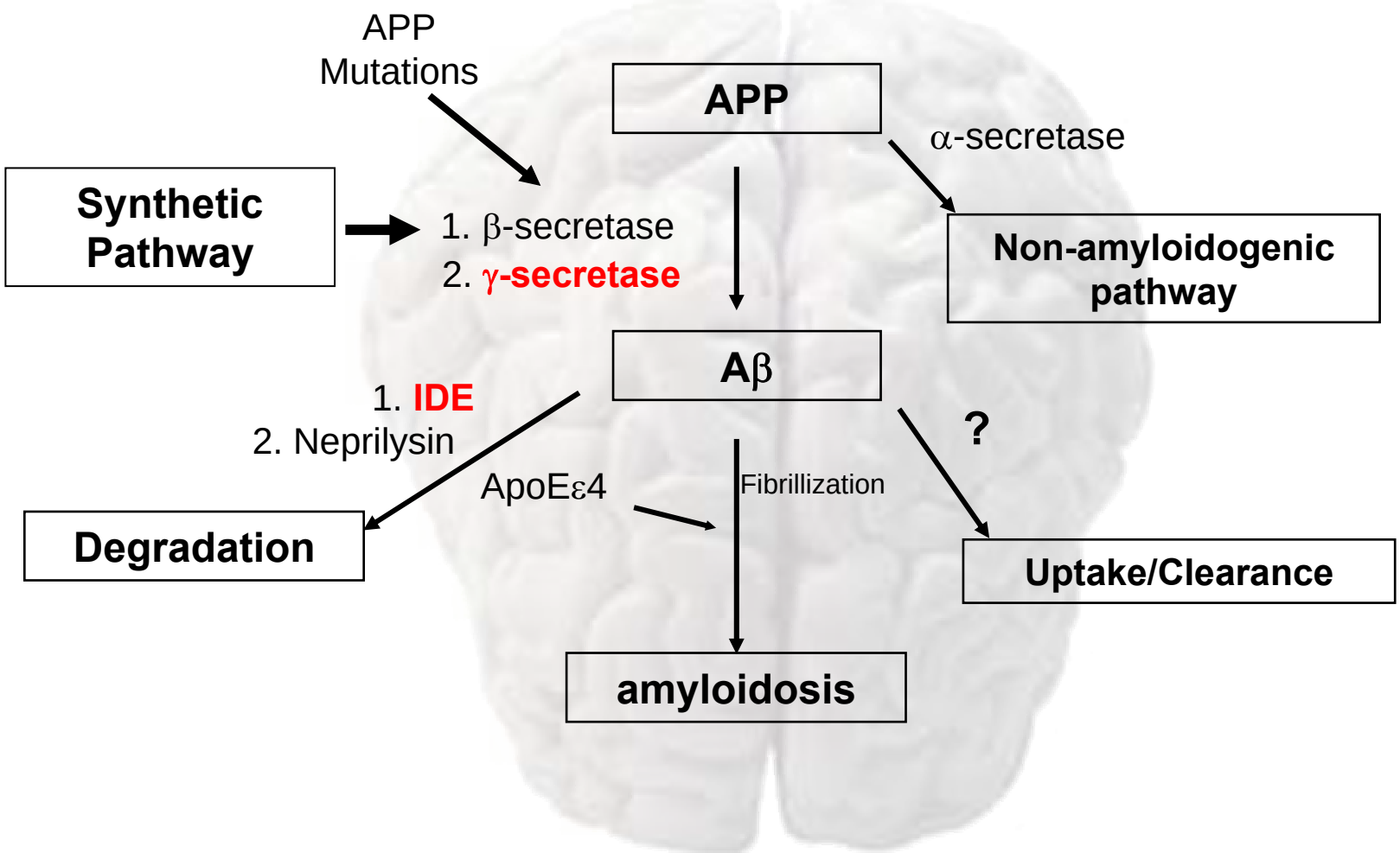
Stereological assessments of amyloid burden





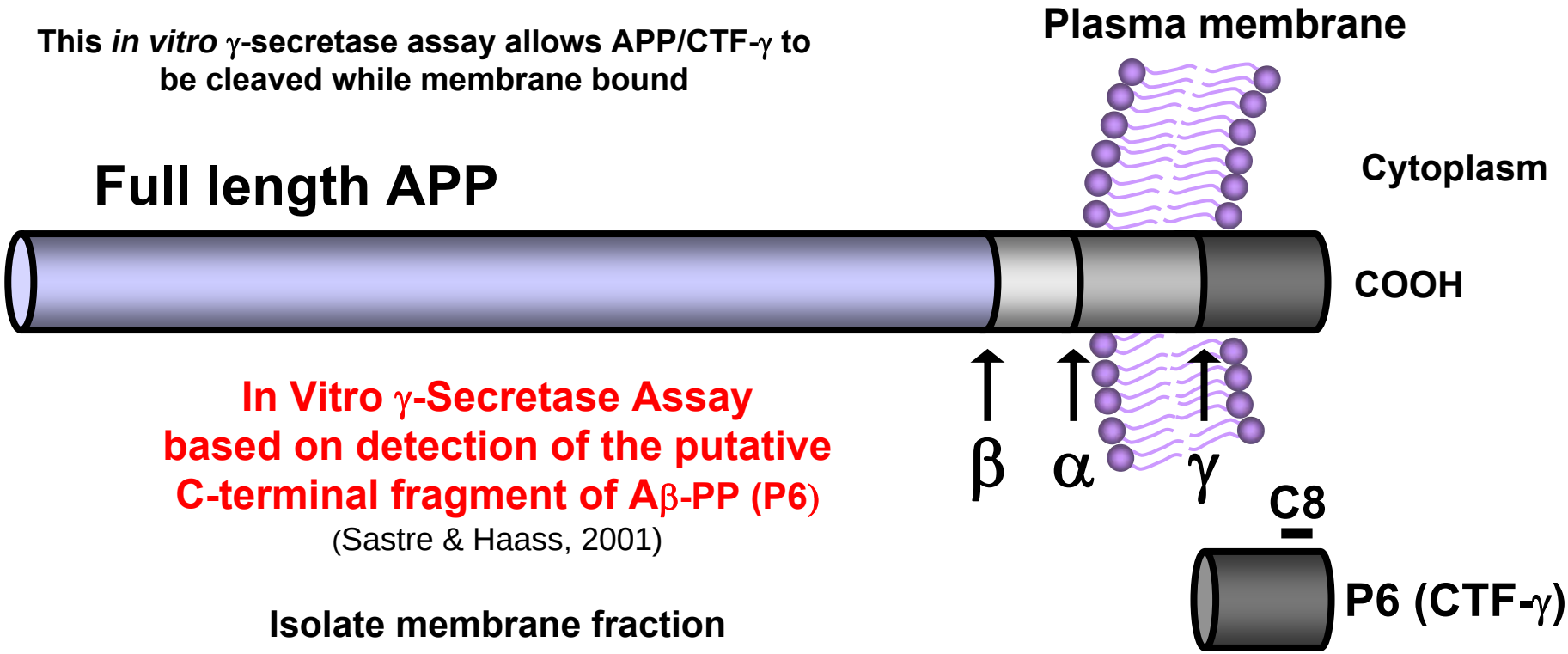
N=4-6, P=0.01 ANOVA

Potential Mechanisms Through which Diet Induced Diabetes May Influence AD β-amyloidois in the Brain



Quantification of C-Terminal Fragment (CTF)-γ of APP as Index of γ-Secretase Activity

This *in vitro* γ-secretase assay allows APP/CTF-γ to be cleaved while membrane bound



In Vitro γ-Secretase Assay based on detection of the putative C-terminal fragment of Aβ-PP (P6)

(Sastre & Haass, 2001)

Isolate membrane fraction (100,000 × g)

Control

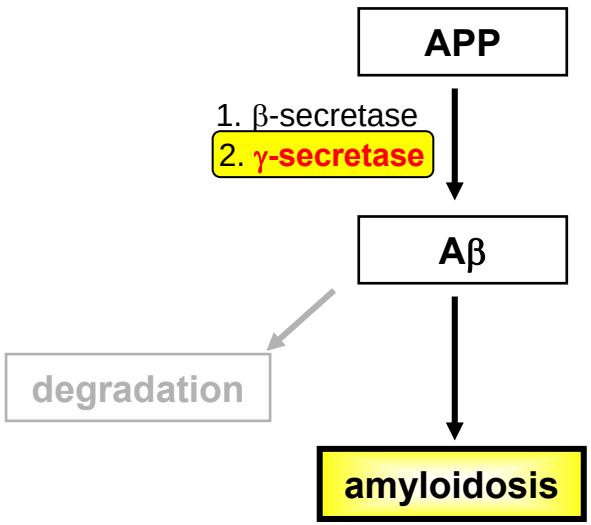
Incubate at 37°C for 2 h

Un-reacted control (4 °C)

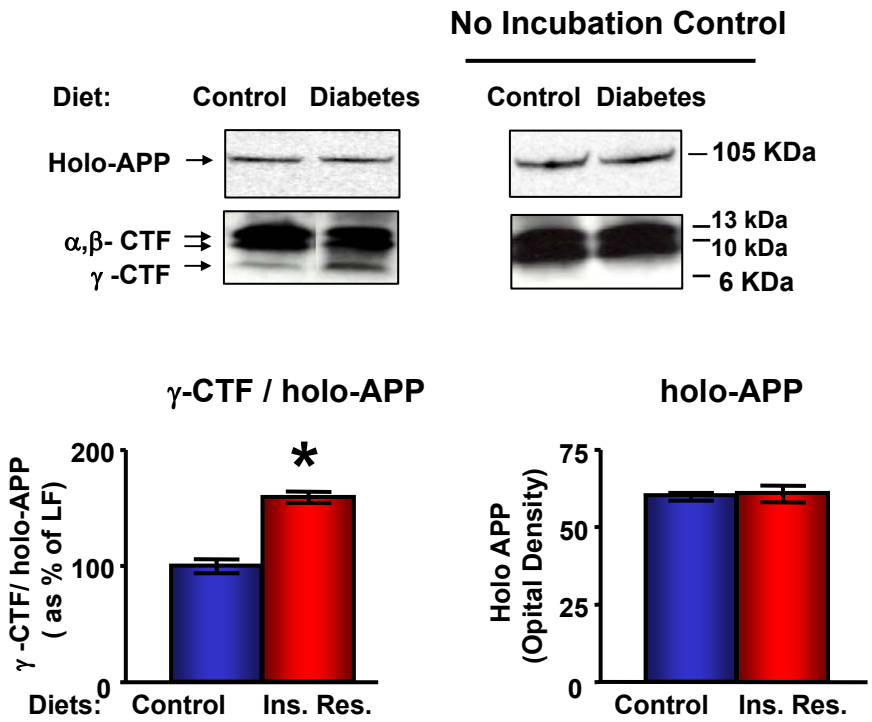
Electrophoretic detection of C8 immunoreactive γ–CTF

Diet Induced Diabetes Coincides with Increased γ-Secretase Activity in the Brain

Factors affecting Aβ production

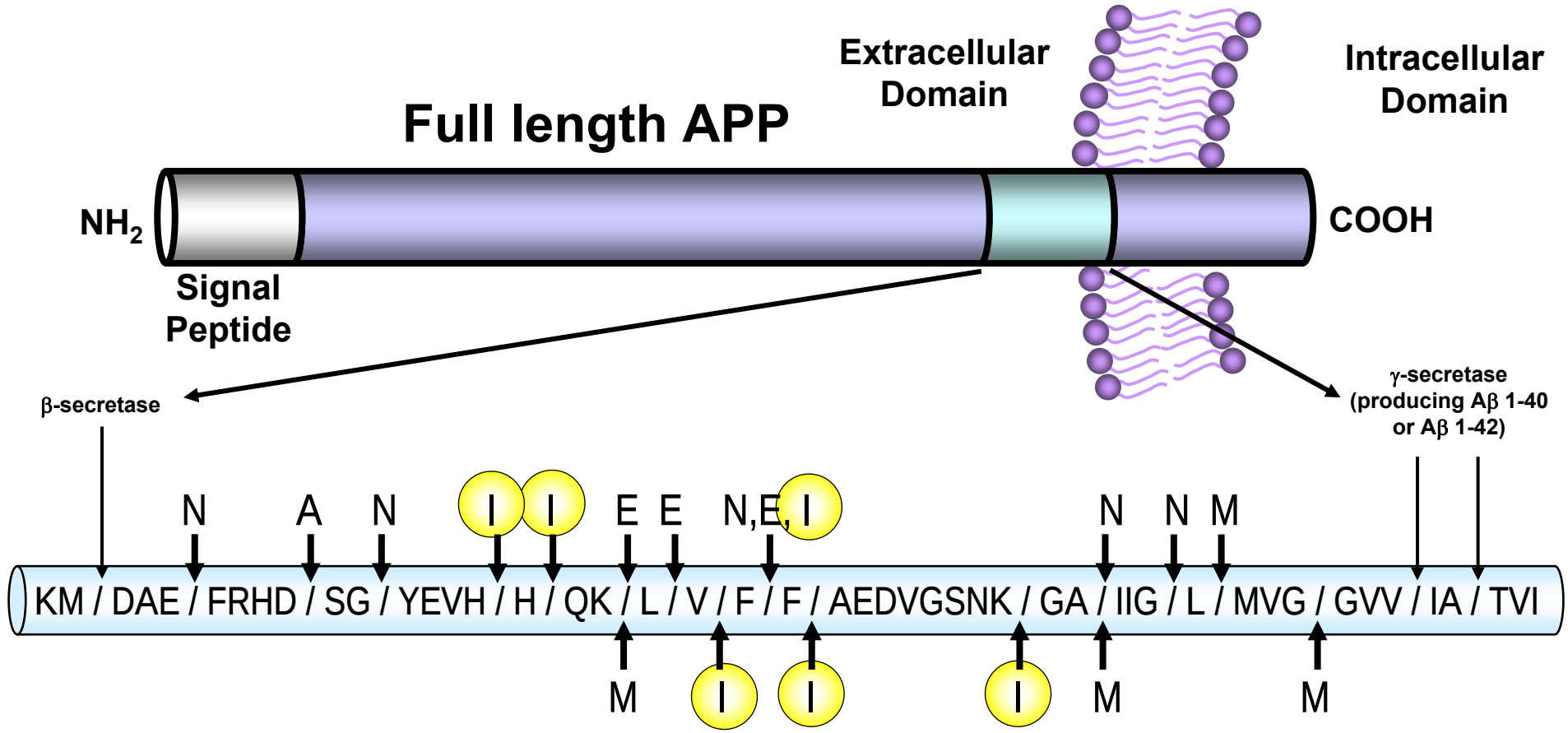


Generation of CTF-γ of APP as Index of γ-Secretase activity



- Diet induced insulin resistance lasted for 5 months starting at 3 month of age
- Means ± SEM, n= 3 per group; *P <0.01 vs control group

Major Cleavage Sites for Metallopeptidases in the holo-APP Protein May Predict Degradation



A = angiotensin-converting enzyme
E = ECE-1
M = MNP-9
N = NEP
I = IDE

Role of Insulin Degrading Enzyme in AD

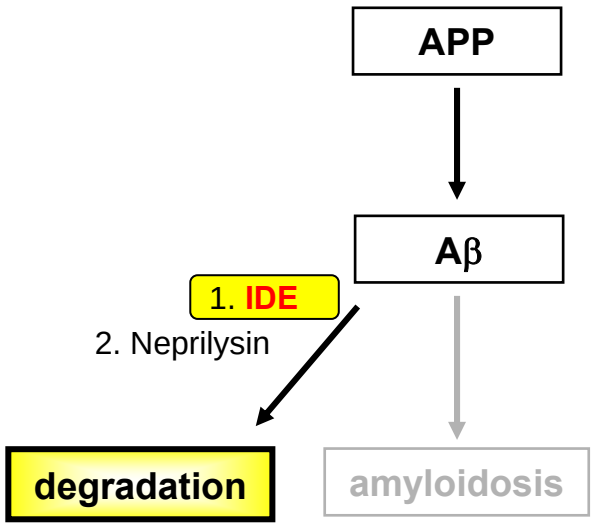
- Aβ peptide levels in brain are inversely correlated with IDE and IDE influence γ–CTF degradation (Miller et al., 2003).

- IDE regulates the elevation of insulin, and its hypofunction (IDE KO) promotes Aβ generation *in vivo* (Farris et al., 2003).

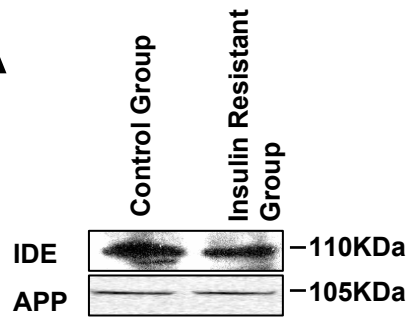
- Reduced hippocampal IDE in late onset AD associated most strongly with APOE4 allelic content (Cook et al., 2003).

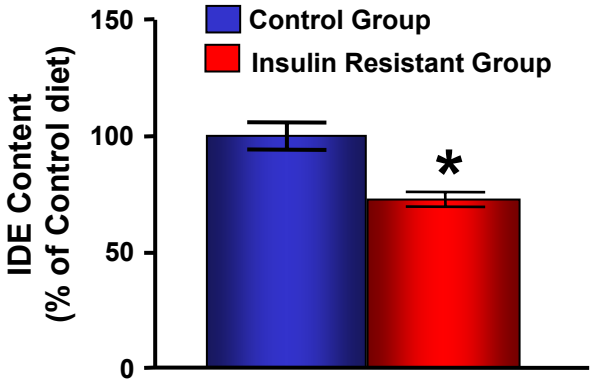
Diet induced diabetes in Tg2576 Mice Coincides with Decreased IDE Expression and Activity in the Brain

Factors affecting Aβ production



A

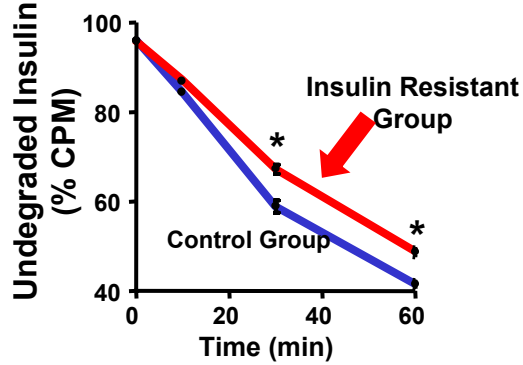




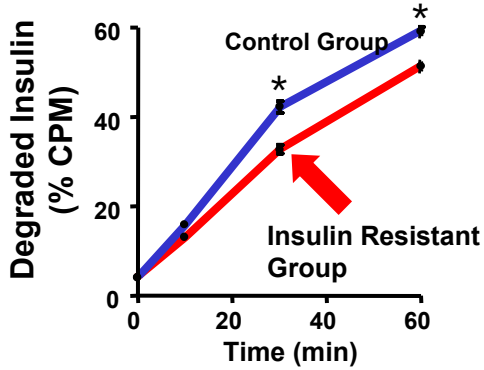
- Means ± SEM, n= 3 per group; *P <0.01 vs control group
- Diet induced insulin resistance lasted for 5 months starting at 3 month of age

B

Insulin resistance results in increased levels of undegraded insulin (Aβ)



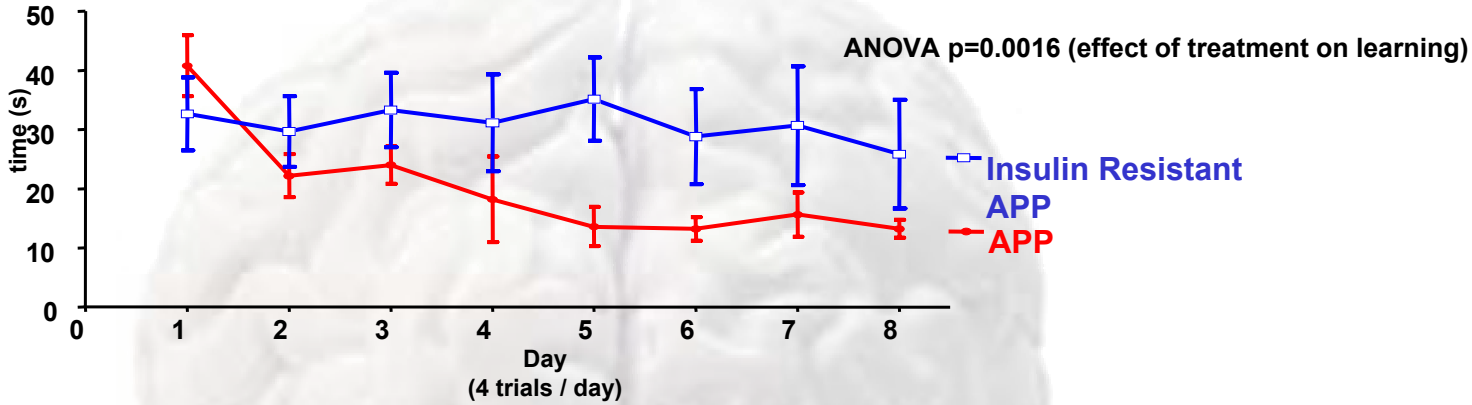
…while resulting in decreased degraded insulin (Aβ)



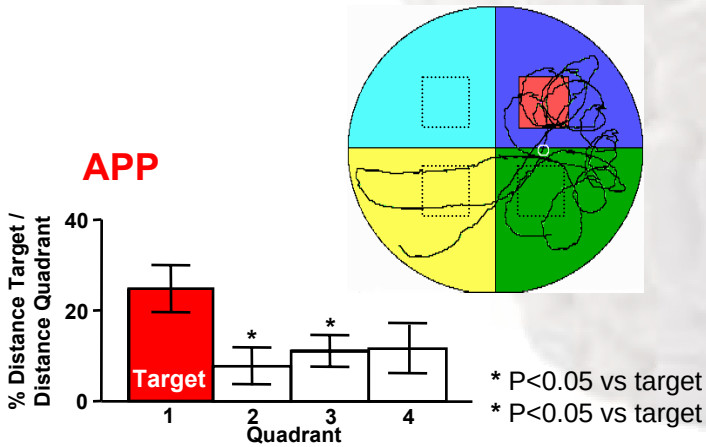
Diet Induced Diabetes Coincides with Spatial Memory Impairment in a Water Maze Behavior Test

(8 months old Tg 2576 mice following 5 month diet leading to insulin resistance)

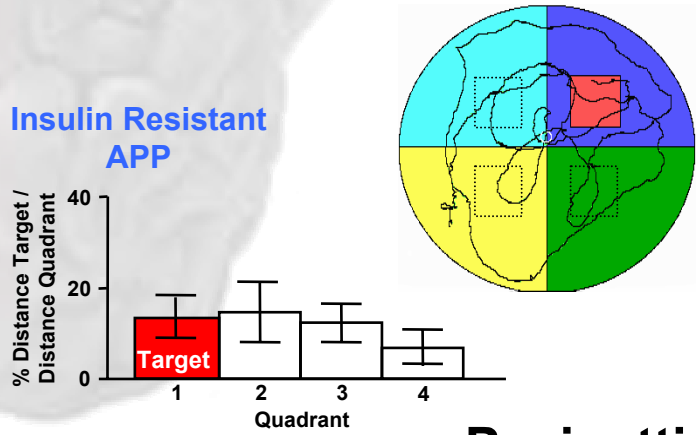
Escape latencies for APP mice improved during the the learning phase of water maze testing (consistent at this age) while Insulin Resistant APP mice maintained longer latencies (suggesting that insulin resistance coincided with memory impairment).



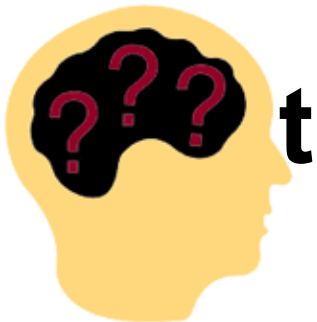
APP mice showed a preference for the former platform location during the spatial probe test



While Insulin Resistant APP mice swam randomly across the 4 quadrants, suggesting impaired spatial learning



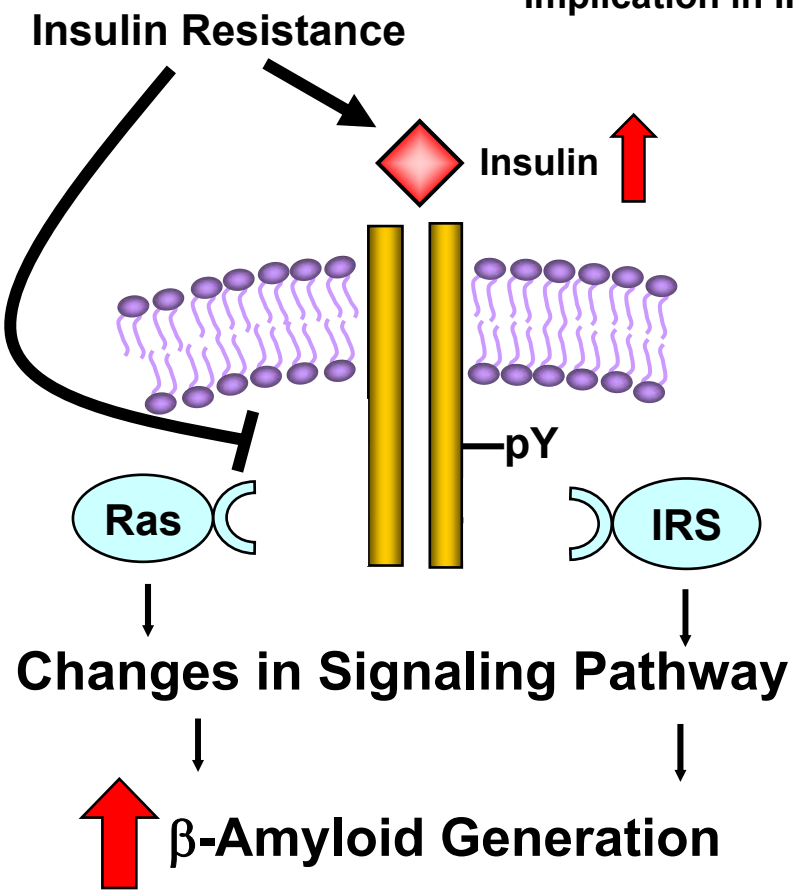
The Role of Diet Induced Diabetes in Alzheimer's Disease

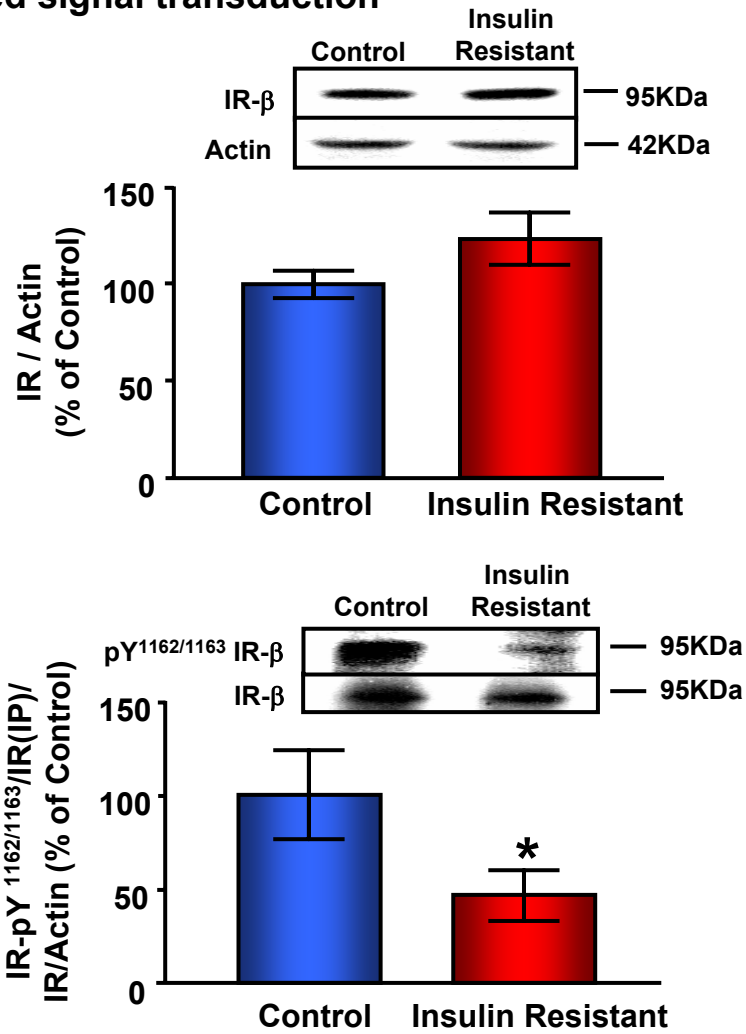


What is the mechanism through which diabetes may promotes Aβ processing?

Diet Induced Diabetes Influences Insulin Receptor PY $^{1162/1163}$-IR in Absence of Detectable Change in Insulin Receptor Expression in the Cerebral Cortex

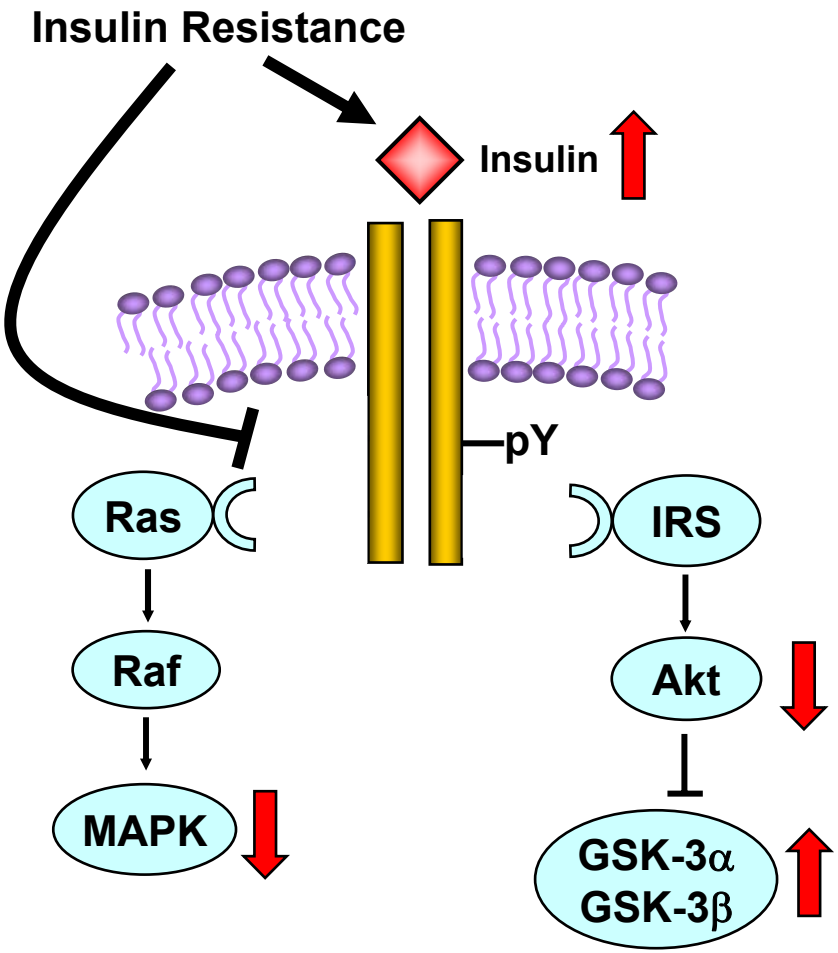
Implication in IR mediated signal transduction

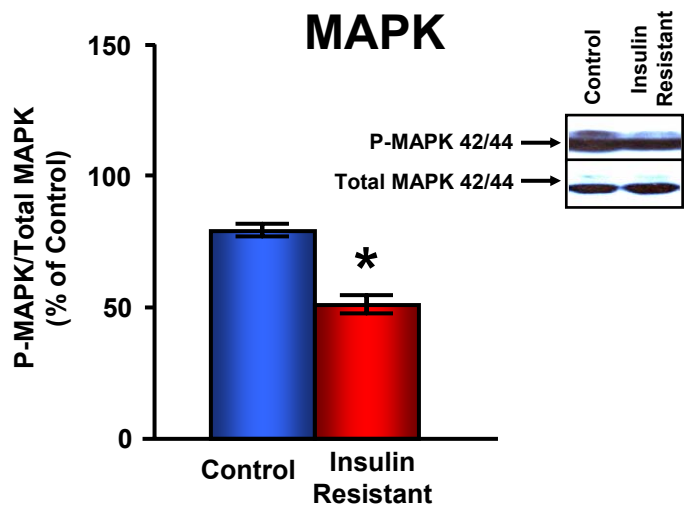


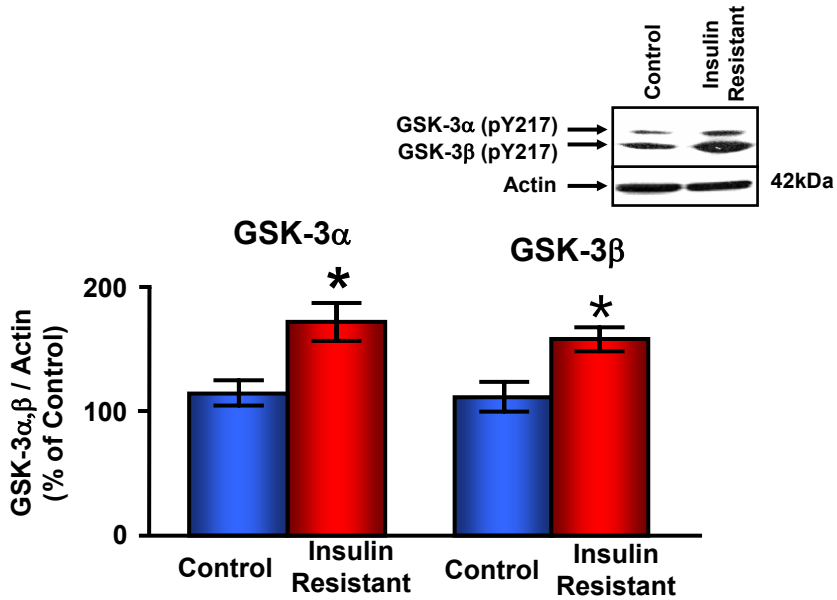


8 month old Tg 2576 AD mice, 5 months of diet

Altered Insulin Receptor PY $^{1162/1163}$-IR in Diabetic Tg 2576 Mice Coincides with Decreased MAP Kinase Phosphorylation and and Increase GSK-α and GSK-β Phosphorylation in the Cerebral Cortex

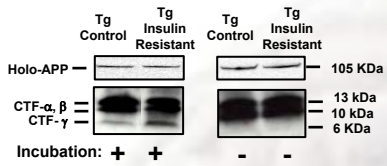






8 month old Tg 2576 AD mice, 3 months of diet

Activation of GSK-3 Correlates With Induction of γ-secretase activity in the brain of "diabetic" Tg2576 mice

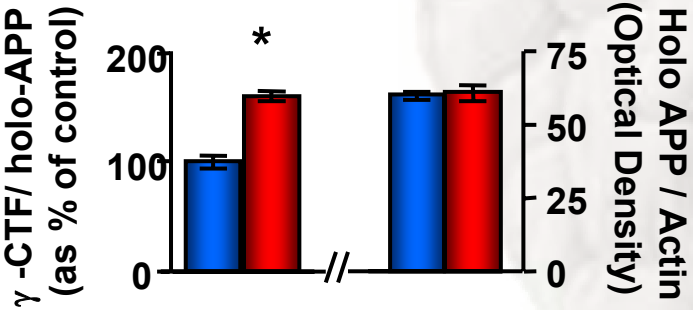


Tg control

Tg insulin resistant

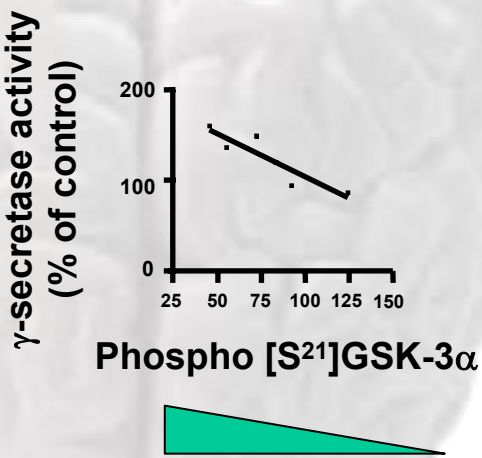
A

γ-secretase activity

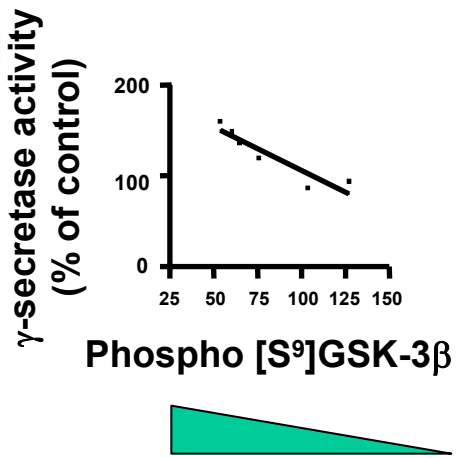


B

Correlation of γ -secretase activity with GSK-3 activities

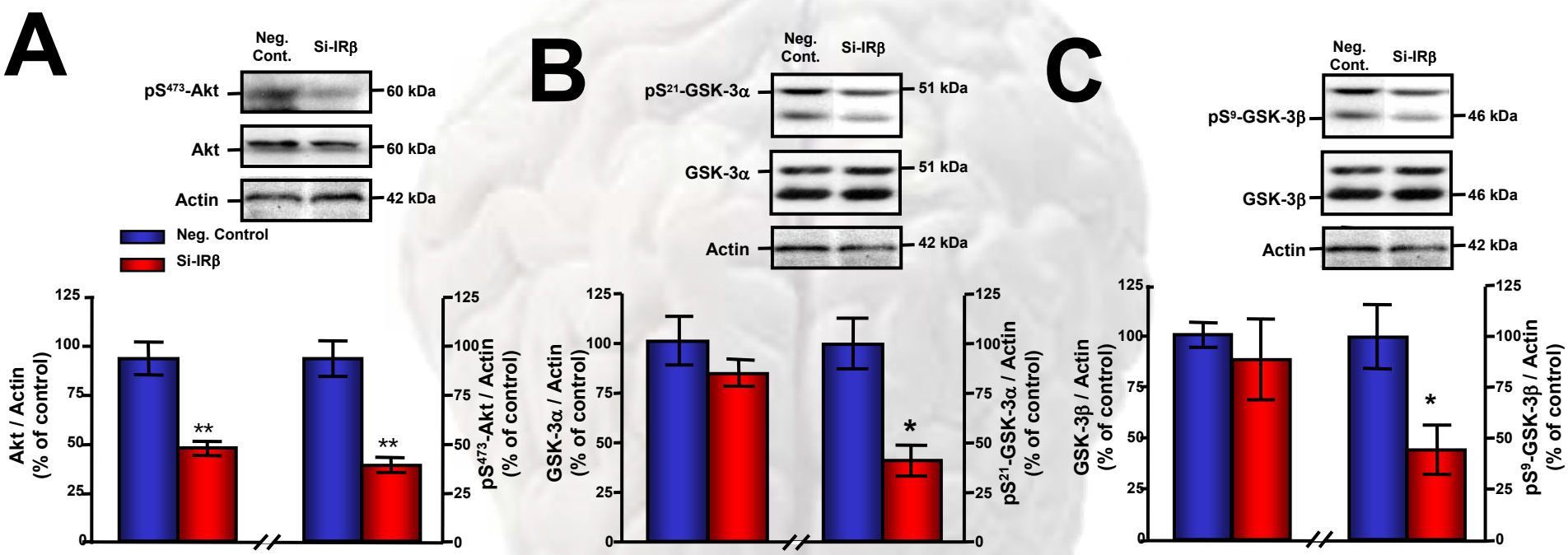


"Active" GSK-3α



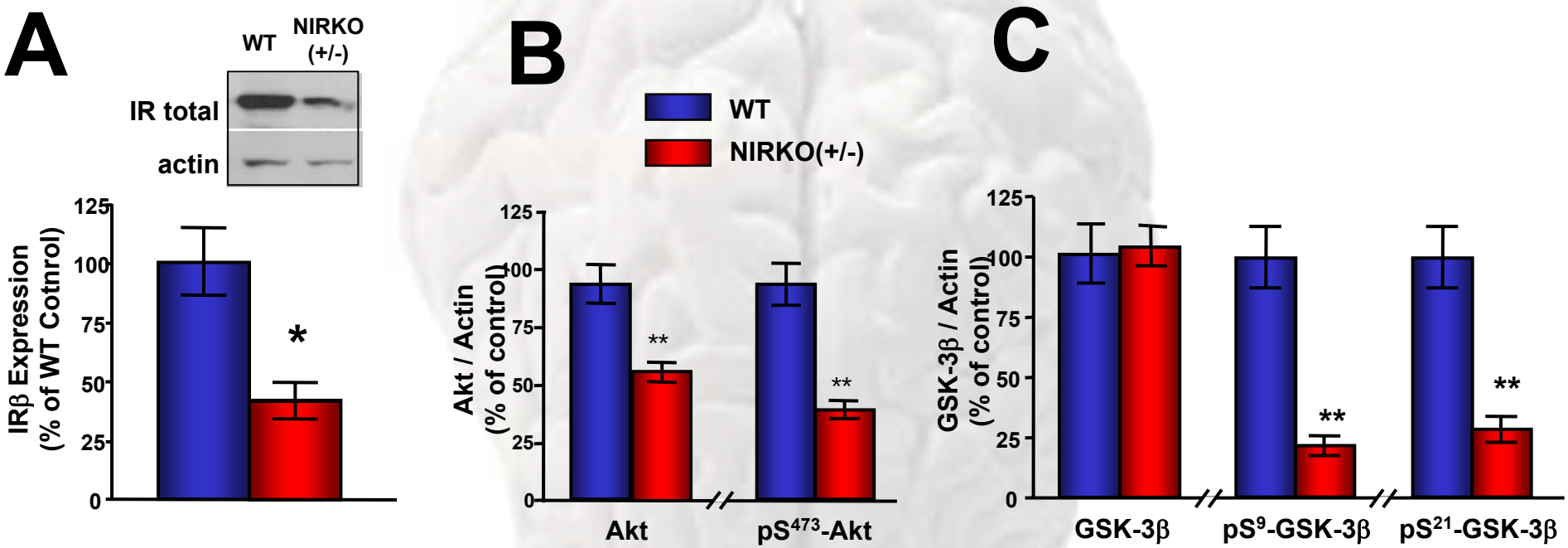
"Active" GSK-3β

IRβ silencing decreases AKT and promotes GSK (decreased S9 and S21 phosphorylation) consistent with the evidence in "diabetic" Tg2576 mice



Means ± SEM, n= 3 per group
in 2 independent studies
***P <0.01 vs control group**

Neuronal IR - KO in "NIRKO" mice recapitulates altered signalling in the brain observed in "diabetic" Tg2576 mice



- Hemizygous NIRKO, collaboration with Dr. Accili
- Did not detect for GSK3α

The Role of Diabetes in AD