NMR Studies of Glu/GABA/Gln Cycling & GABA Synthesis Regulation in Rodent Cerebral Cortex

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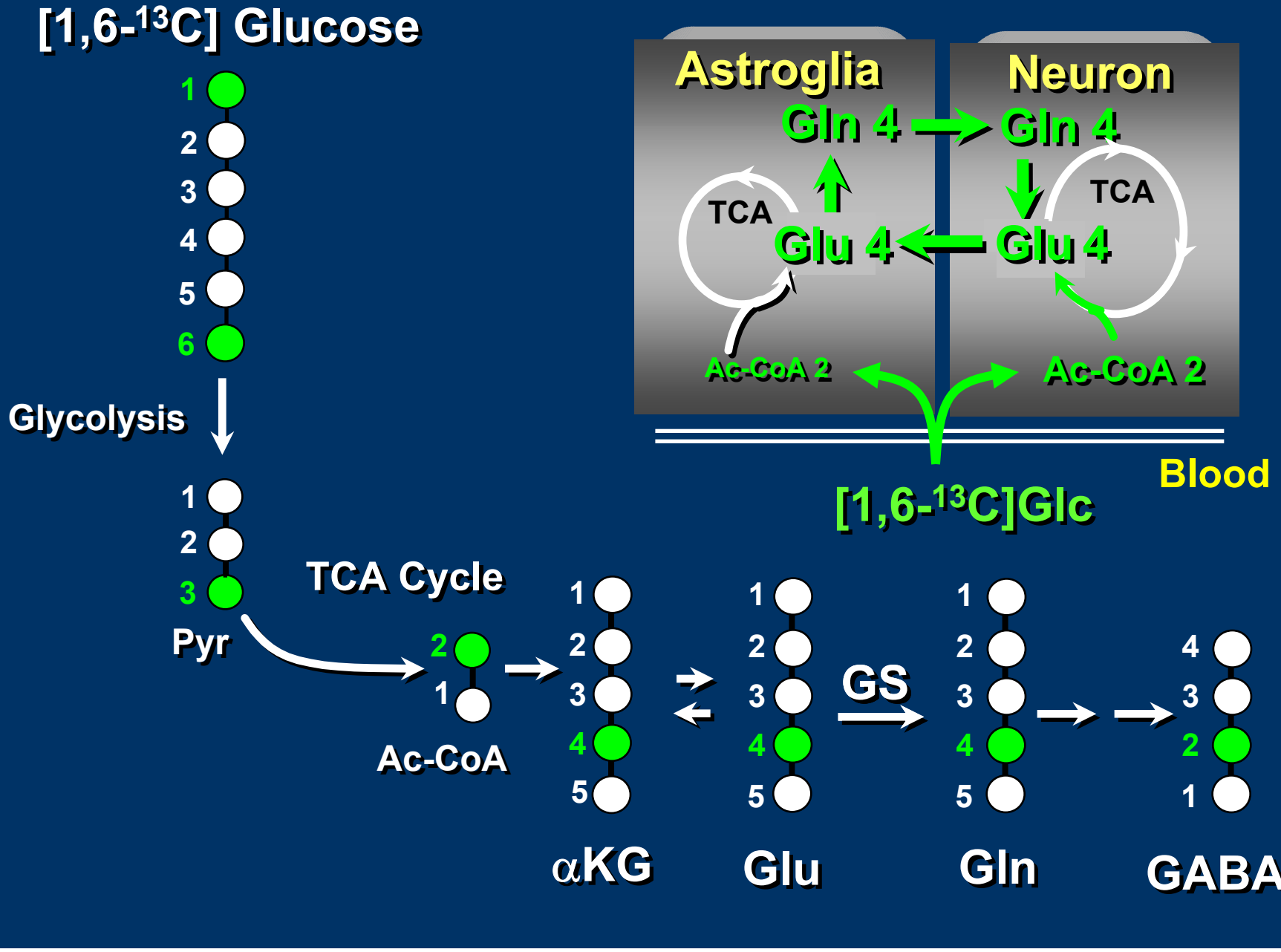
BACKGROUND

- Glutamate & GABA are the major excitatory & inhibitory neurotransmitters in cerebral cortex.
- Glu & GABA are synthesized from glutamine (Gln) in a "metabolic cycle" between neurons & astroglia.
- MRS findings indicate that Glu/GABA/Gln cycling is linked to brain glucose utilization & neuronal activity.

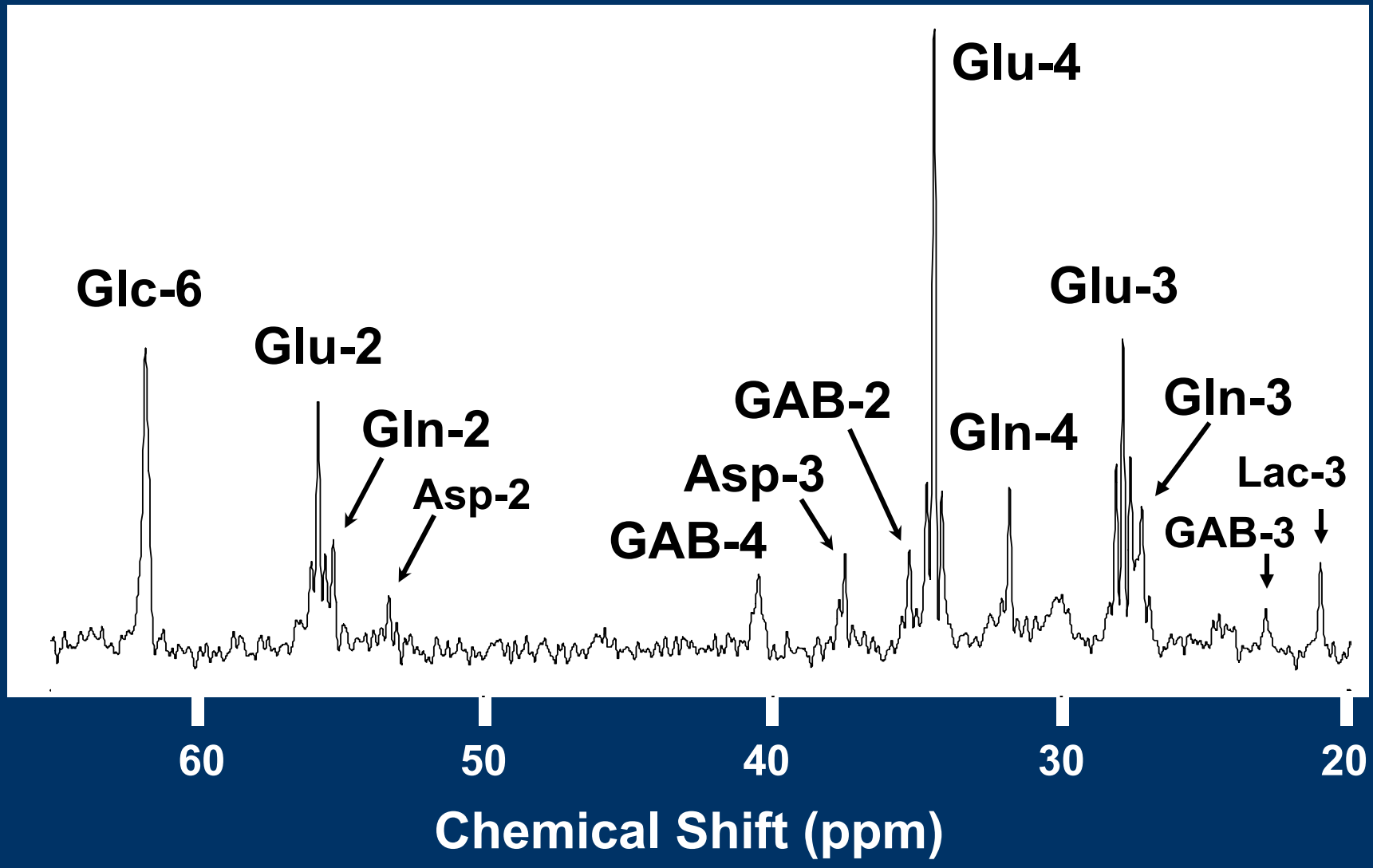
BACKGROUND

- MRS findings of low brain GABA levels in patients with epilepsy, depression, panic disorder, & alcohol dependence suggests that GABA synthesis is reduced.
- ^{13}C-labeled substrates combined with direct ^{13}C MRS or indirect ^{1}H MRS provides a non-invasive means to assess metabolic pathway fluxes

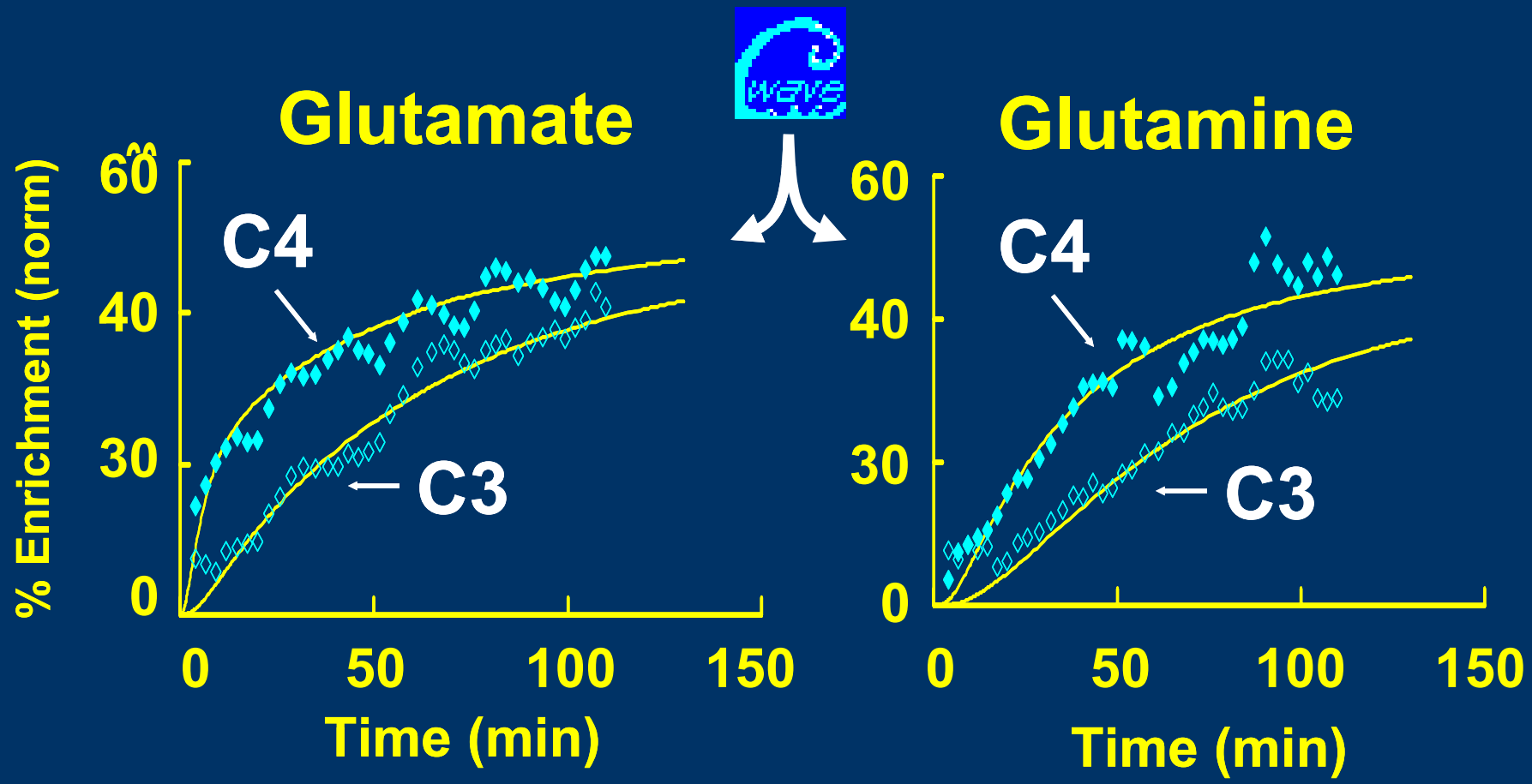
Isotopic Labeling from [1,6-^{13}C]Glucose



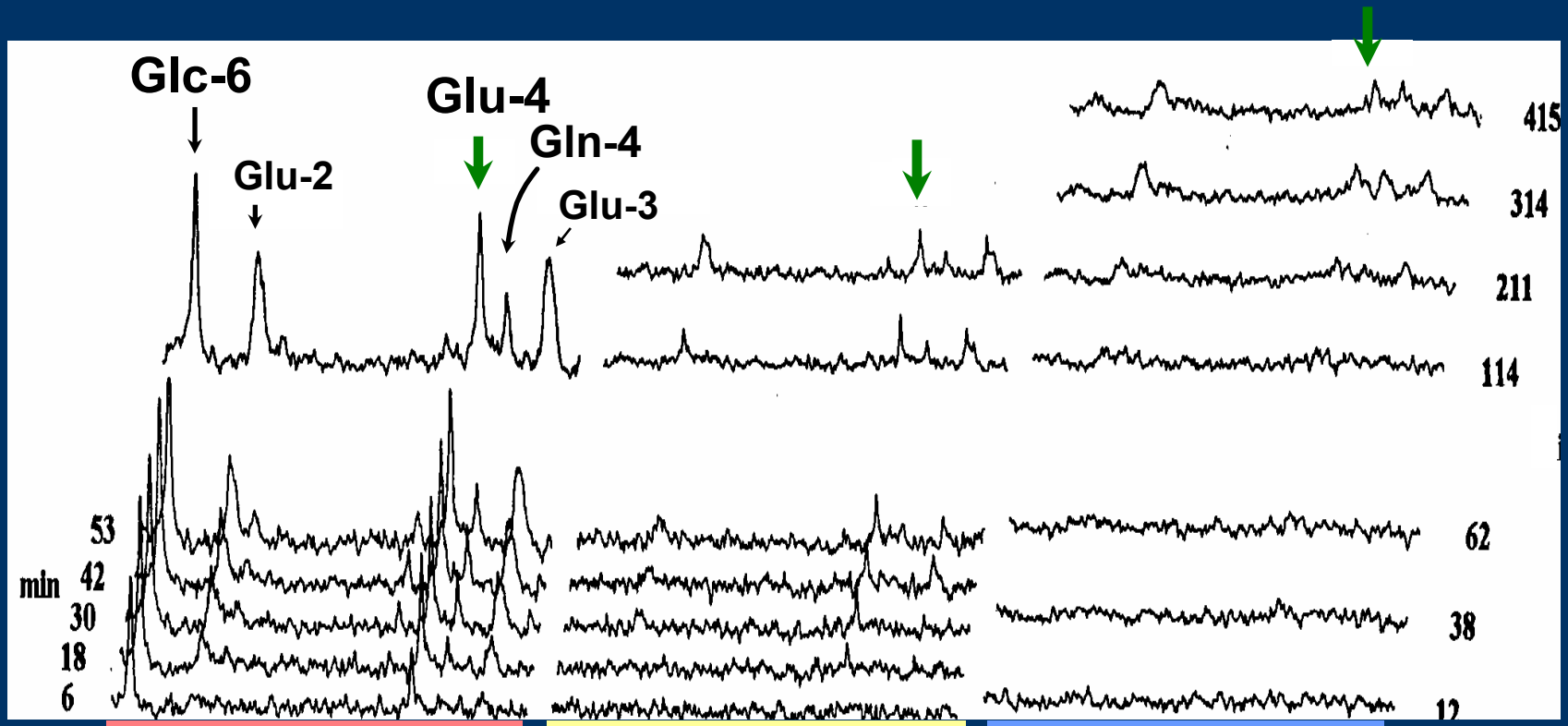
In Vivo ^{13}C MRS (7 Tesla) of Rat Cerebral Cortex during Infusion of [1,6-^{13}C]Glucose



^{13}C Labeling of Glu & Gln: Determination of V_{tca} & V_{cycle}



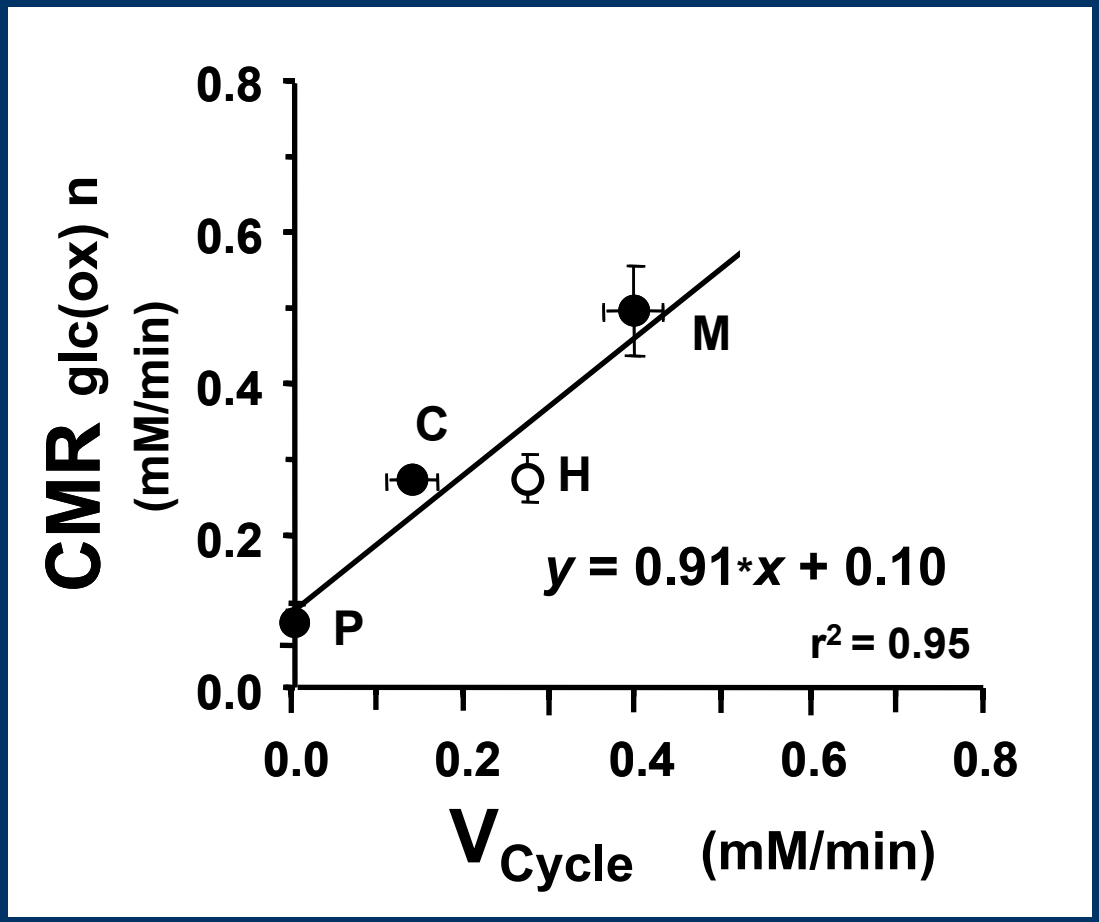
Glu & Gln ^{13}C Turnover from [1,6-^{13}C]Glc reflects Cortical Activity



Higher activity ⟷ Lower activity

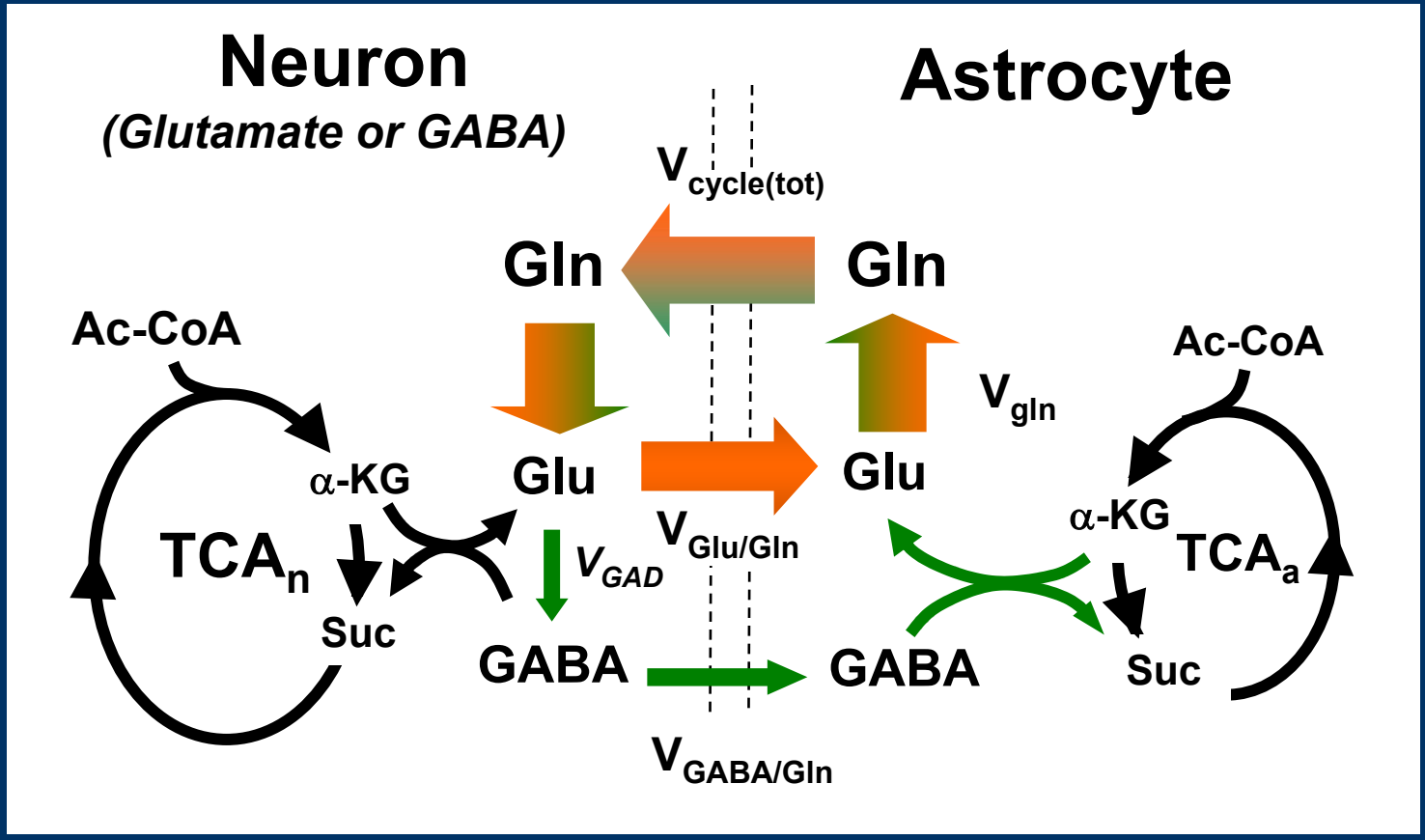
N. Sibson et al, 1998

Relationship between Neuronal $CMR_{glc(ox)n}$ & $V_{cycle(tot)}$ is Linear



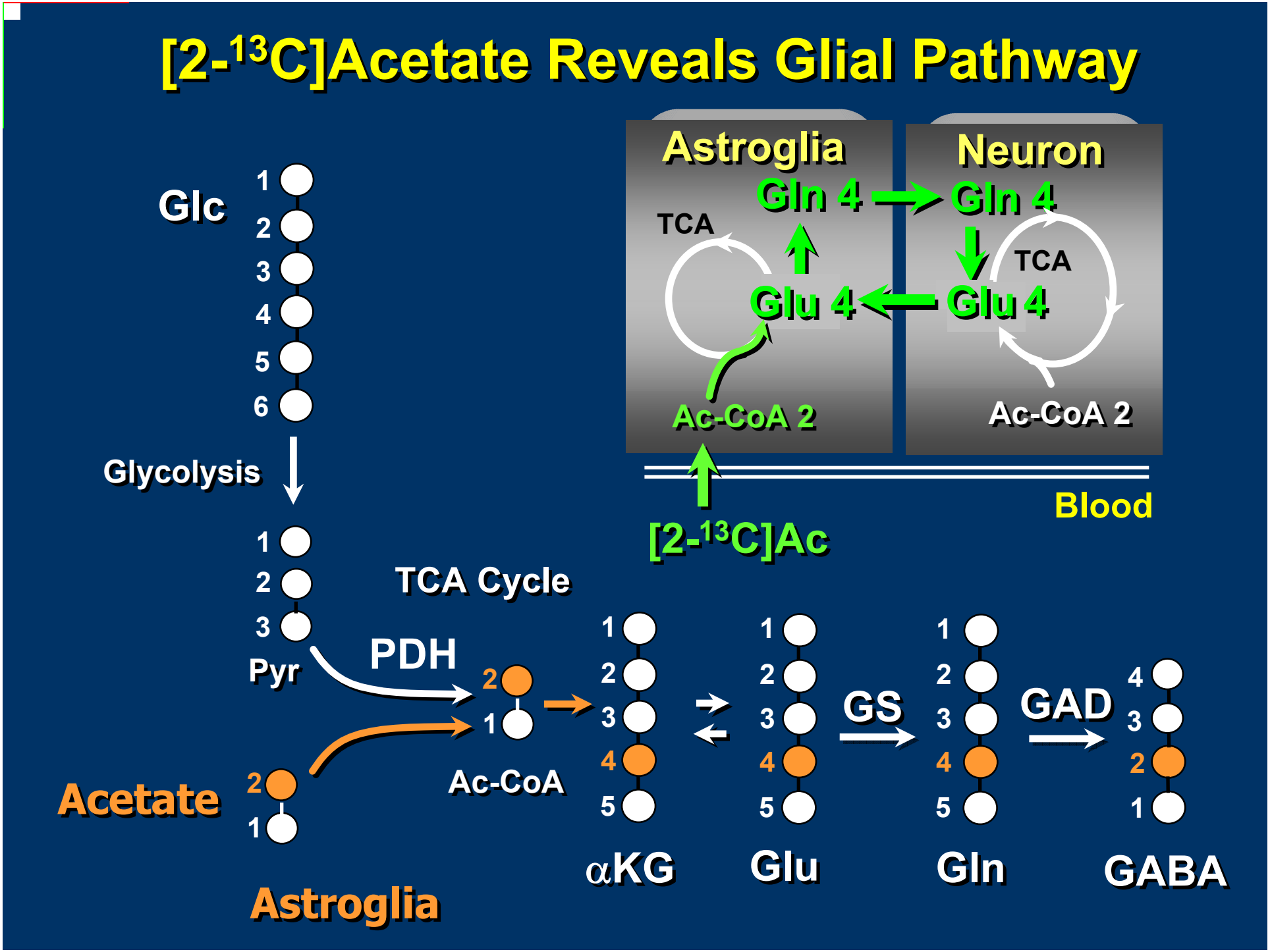
N. Sibson et al (1998); A. Patel et al (2003)

$V_{cycle(tot)}$ Includes Glu/Gln & GABA/Gln Cycling



- **What fraction of total Glu/GABA neurorotransmitter cycling is contributed by GABA?**
- **What is the energetic cost of GABAergic function?**

[2-^{13}C]Acetate Reveals Glial Pathway



Experiment (i)

- Measure steady-state ^{13}C enrichment of glu-4, gln-4 & GAB-2 from [2-^{13}C]Ac during low (PB) & high (Hal) cortical activity.

- Use ^{13}C-enrichment values to calculate Glu & GABA "cycling flux-to-oxidation" ratios from their steady-state equations:

Glutamate Neurons:

$$V_{glu/gln} / V_{TCA(glu)} = \text{glu-4} / (\text{gln-4} - \text{glu-4})$$

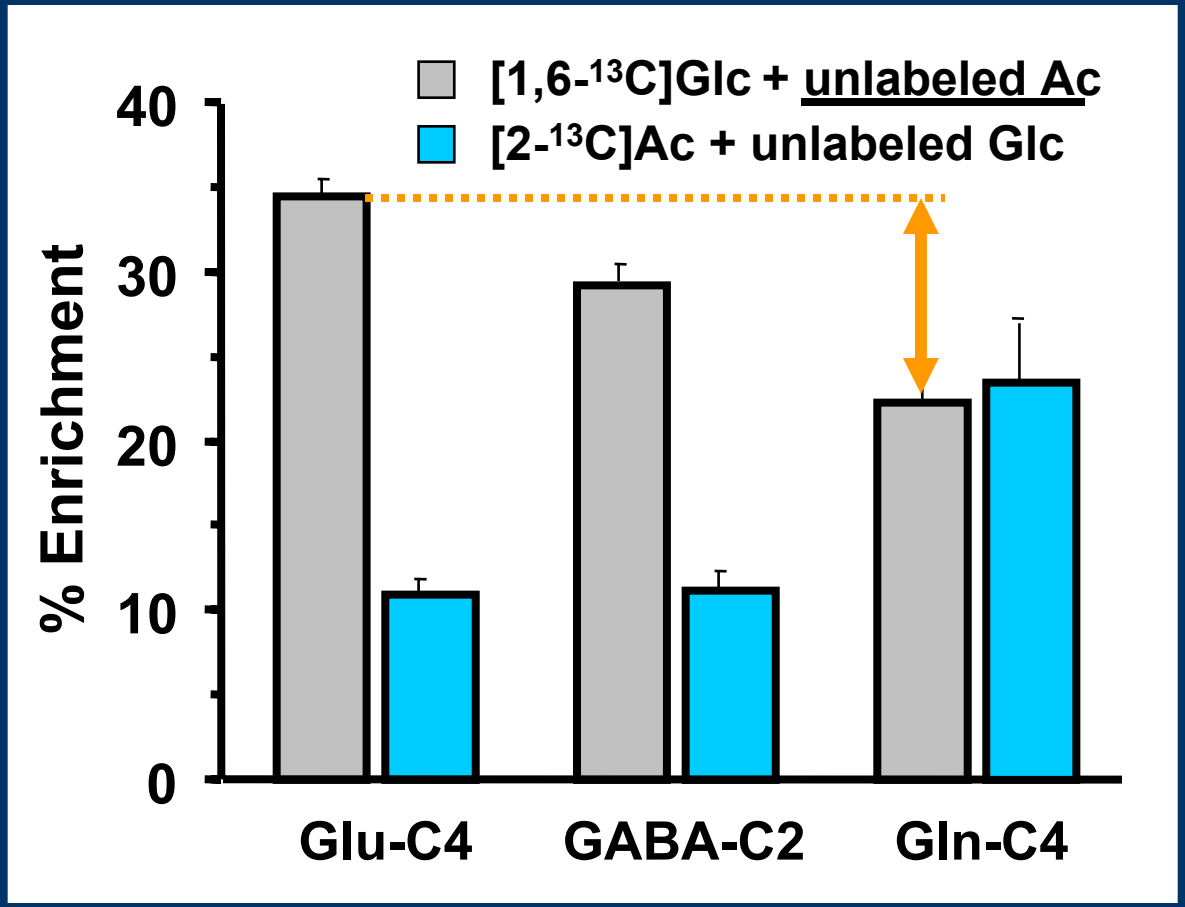
GABA Neurons:

$$V_{GAB/gln} / V_{TCA(GAB)} = \text{GAB-2} / (\text{gln-4} - \text{GAB-2})$$

Experiment (ii)

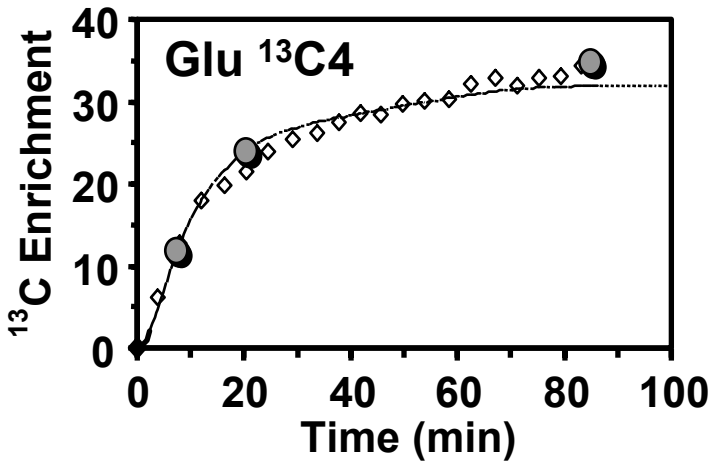
- Measure time courses of ^{13}C labeling of Glu-4 & -3, Gln-4 & GABA-2 from [1,6-^{13}C]glucose at low (PB) & high (Hal) cortical activity.
- Fit metabolic/cycling model to ^{13}C enrichment time courses constrained by the flux ratios determined in the [2-^{13}C]Ac experiment.

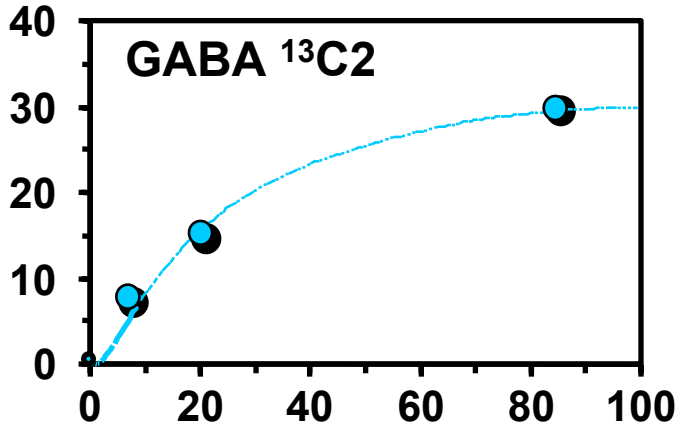
Steady State Isotopic Enrichment from [1,6-^{13}C]Glc & [2-^{13}C]Ac

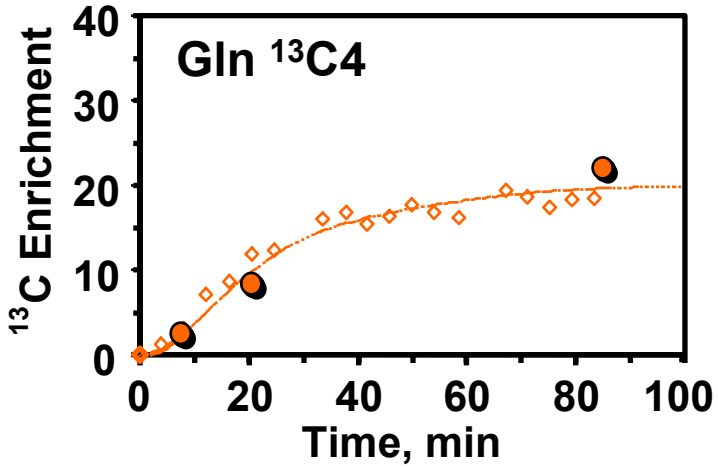


A. Patel et al (2003)

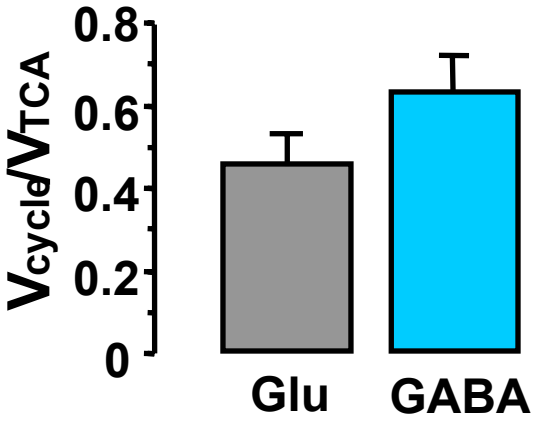
^{13}C Enrichment Timecouses of Glu, Gln & GABA from [1,6-^{13}C]Glc



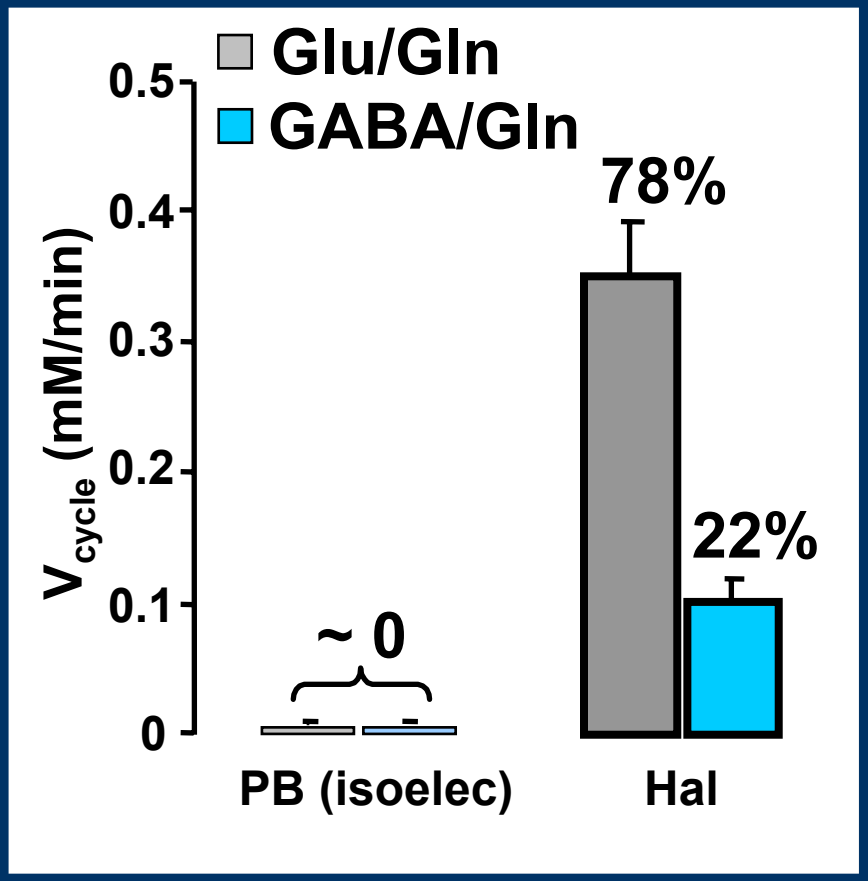


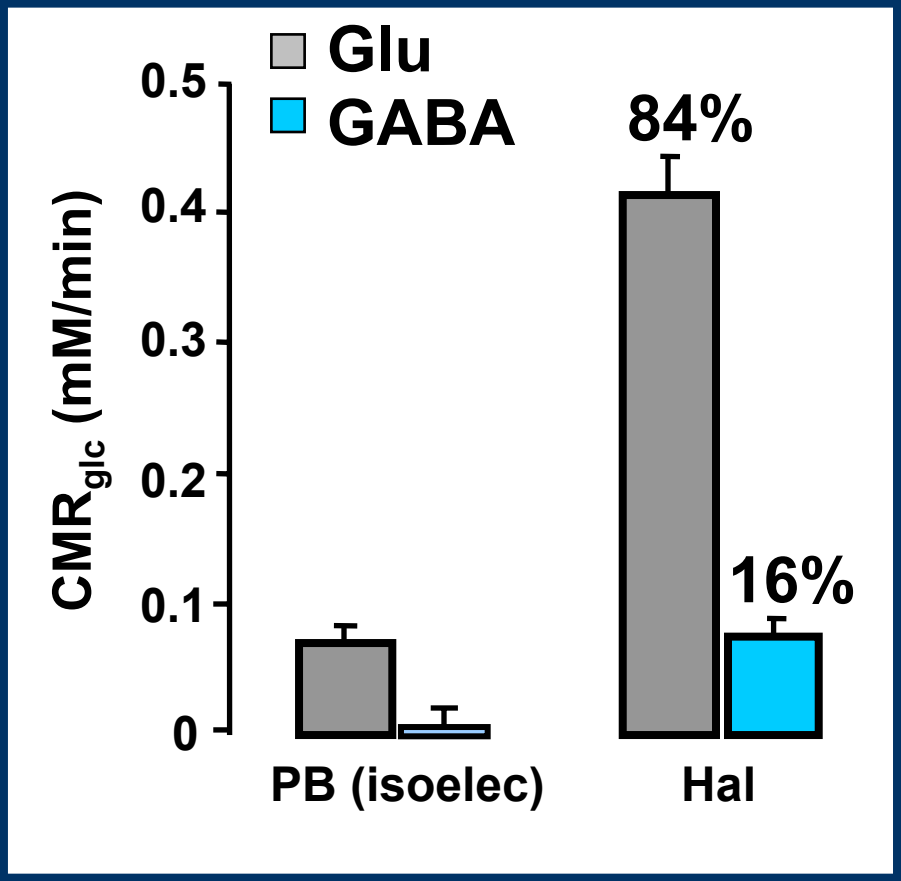


[2-^{13}C]Ac (Steady State)

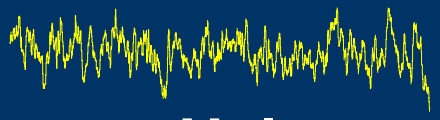


Energetics of GABA/Gln & Glu/Gln Cycling





EEG



PB (iso)

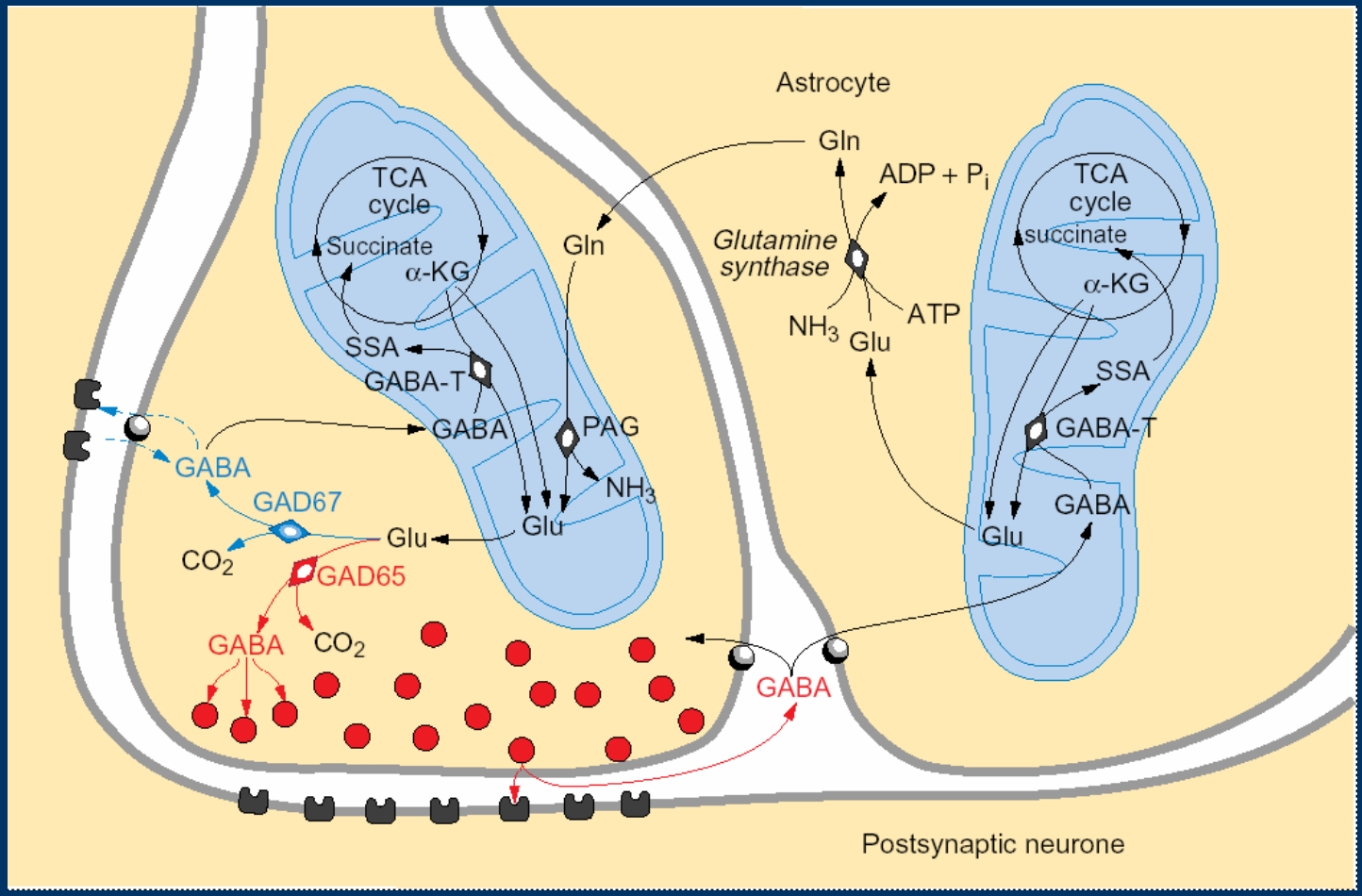
Hal

Regulation of GABA synthesis involves two isoforms of Glutamic Acid Decarboxylase (GAD_{67} & GAD_{65})

- GAD activity requires PyP.
- GAD_{67} is mostly bound with PyP & active.
- GAD_{65} is mostly unbound (apo) & inactive.
- GAD_{65} >> GAD_{67}. Majority of isolated GAD apoGAD_{65}.

- What role do the GAD Isoforms play in GABA synthesis?

Functional Specialization of GAD's in GABA Metabolism



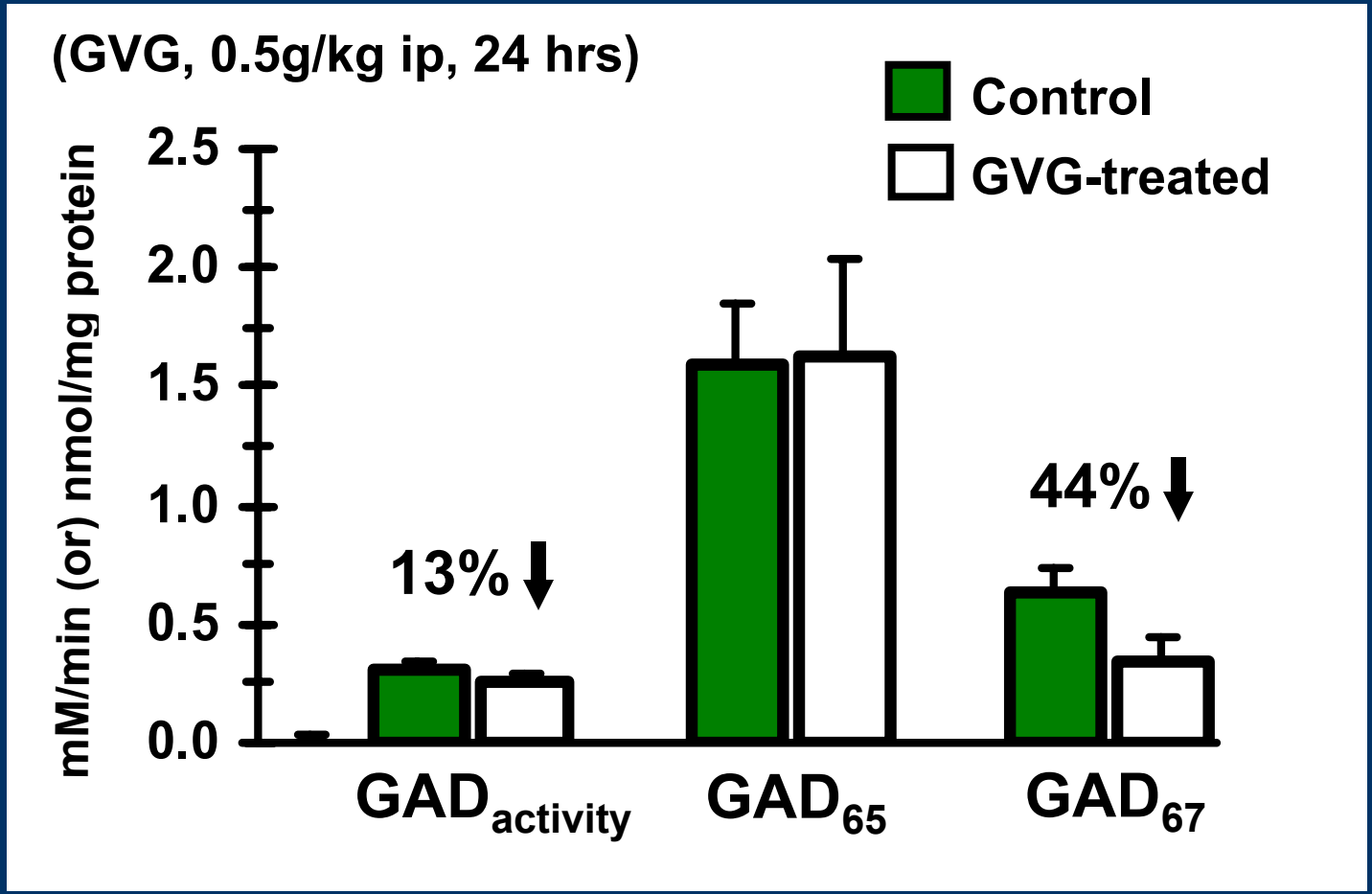
Shoghomonion and Martin, TiPS 1998

Background & Strategy

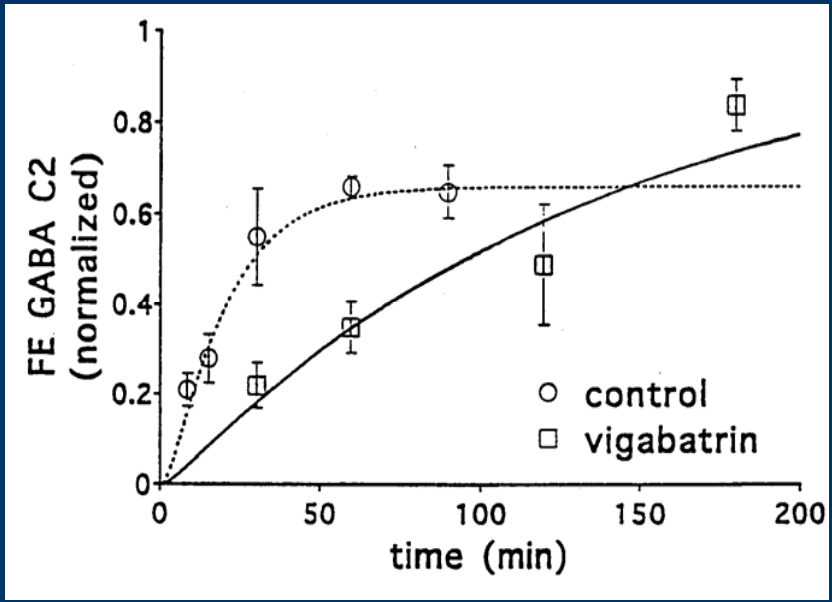
- GAD_{67} is selectively decreased by a rise in GABA level
- GABA levels rise when the catabolic pathway is blocked by inhibition of GABA-transaminase (e.g., γ-Vinyl GABA, gabaculine)

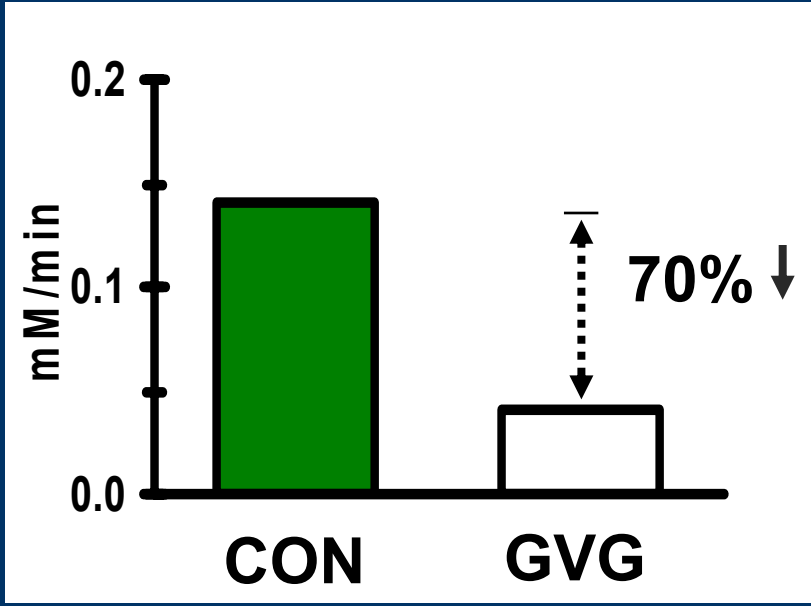
- **Approach:** Measure GABA turnover from [1-^{13}C]glucose in the absence & presence of GABA-T inhibition to raise GABA levels
- Relate change in GAD composition to the observed flux

GABA-T Inhibition leads to Reduced GAD_{67} Protein



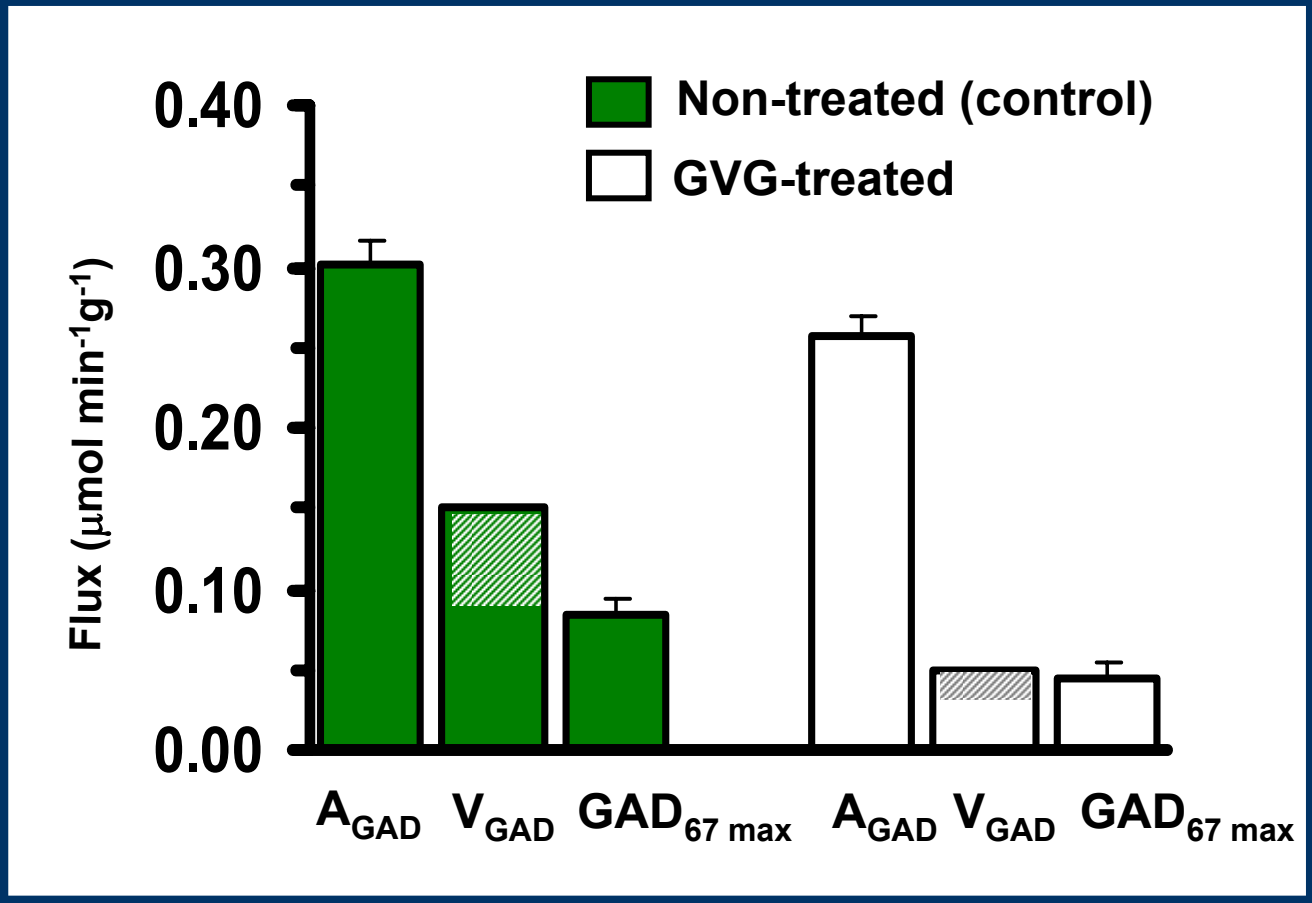
Basal GABA Synthesis is sensitive to GAD_{67} level





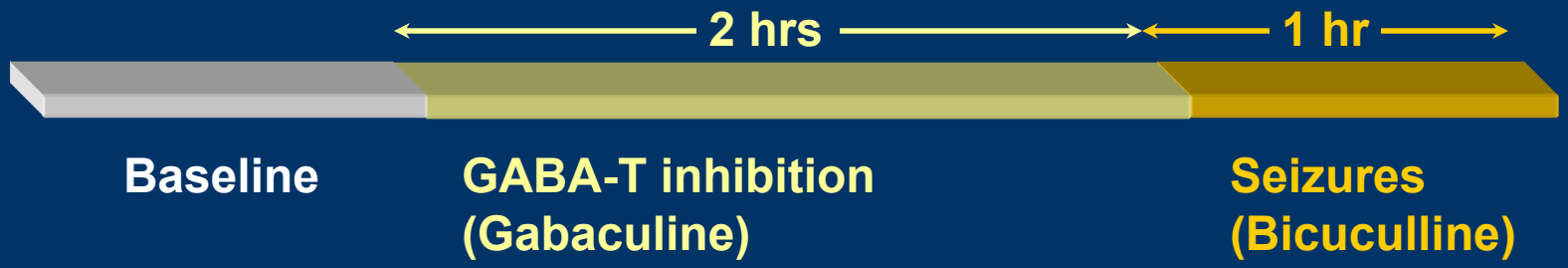
Manor et al, Neurochem. Res.

Comparison of GABA Synthesis with Total GAD & GAD_{67} Activity



- **If GAD_{67} mediates a major fraction of basal GABA synthesis, could GAD_{65} play a role in activity-dependent GABA synthesis?**

Experiment



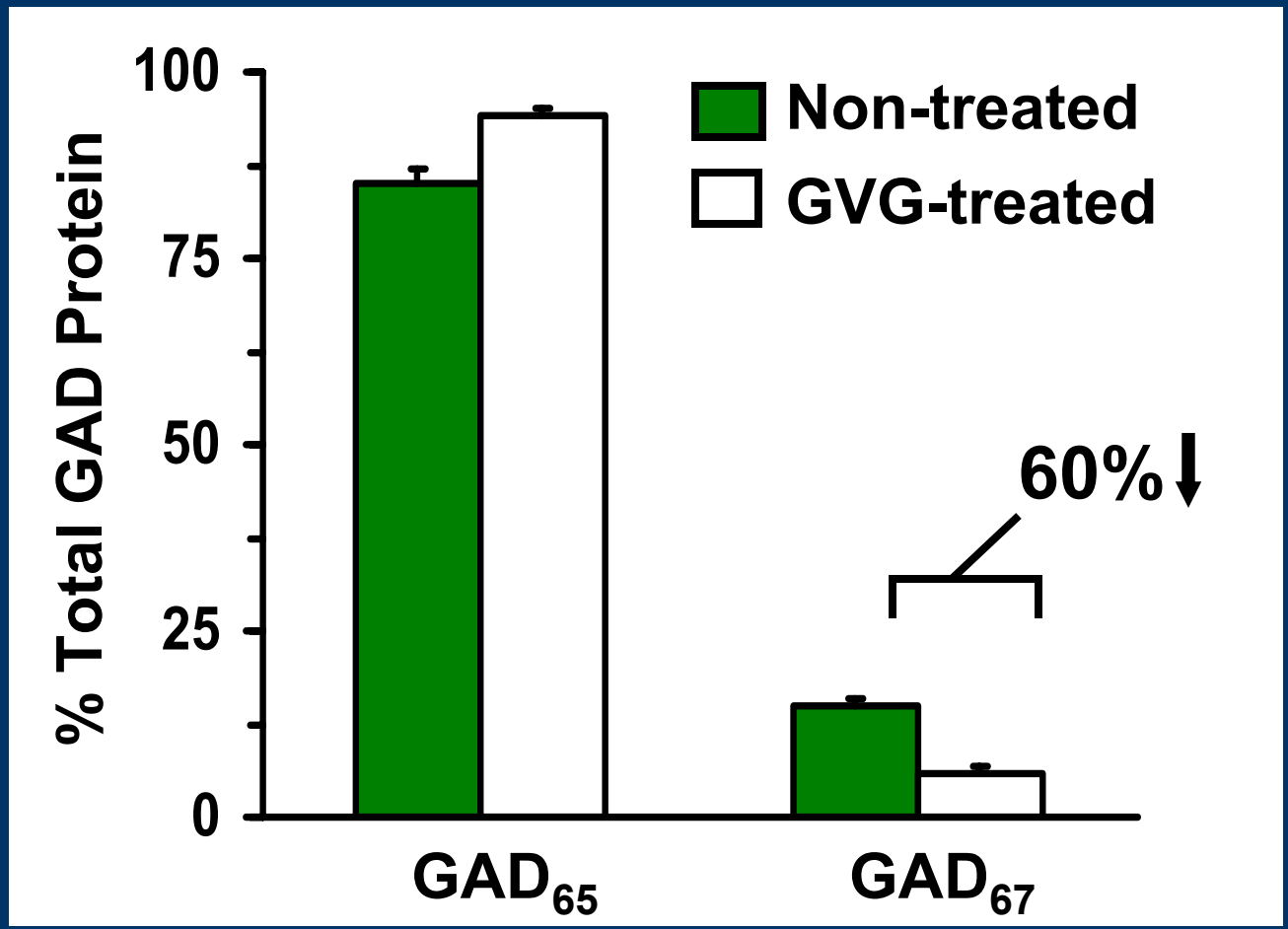
- Measure [GABA] by ^{1}H MRS in vivo.
- Block GABA catabolism with gabaculine: *GABA synthesis rate = Δ[GABA] /Δtime*.
- Measure rates before & during seizures.

Comparison Groups:

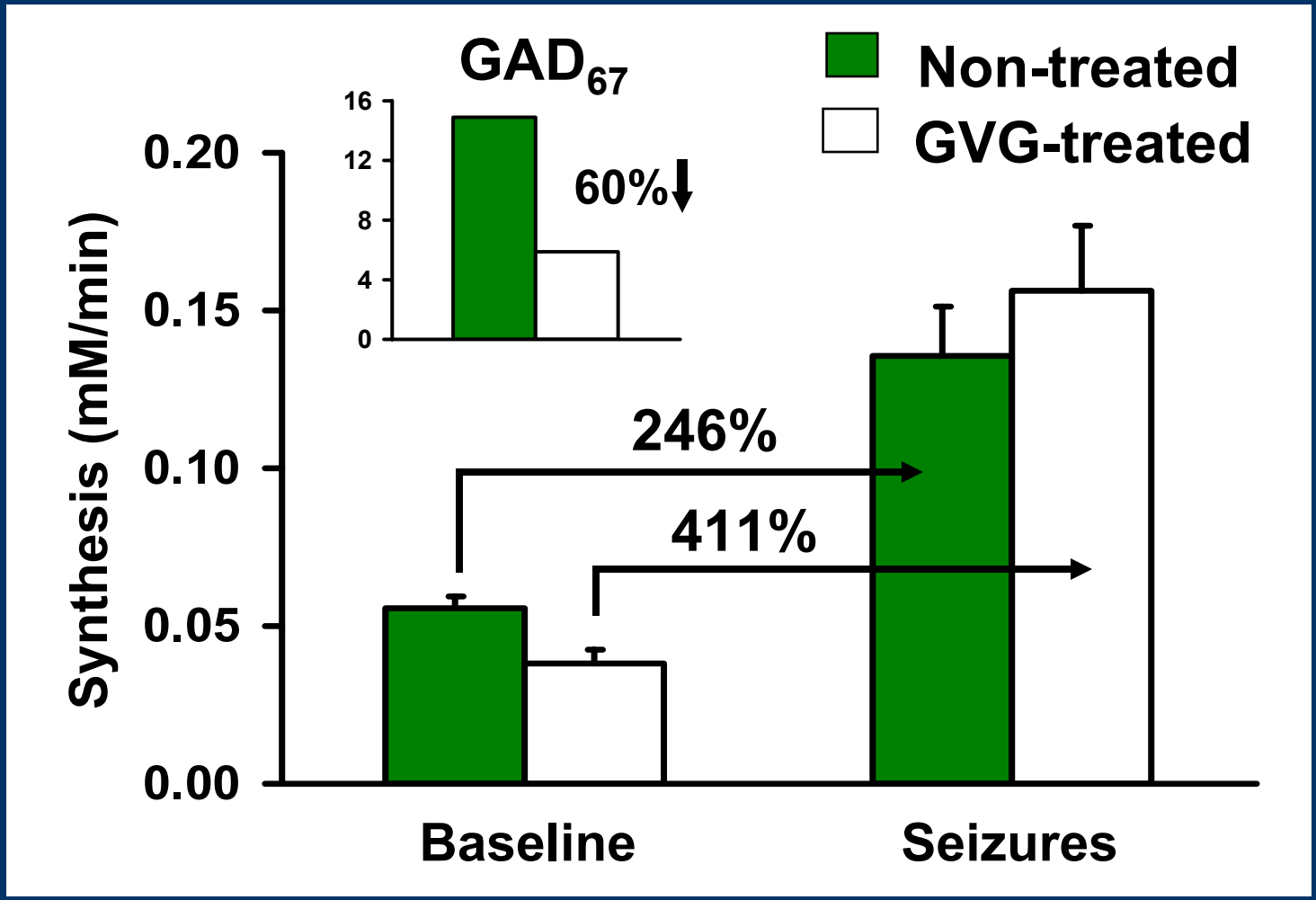
i) GVG-treated (0.5g/kg i.p., 24 hrs before)

ii) Non-treated (saline-injected)

GAD Isoform Composition in GVG-treated & non-treated rats



The Change in GABA Synthesis during seizures is not Suppressed by GAD_{67} Depletion



Conclusions (I)

- The Glu/GABA/Gln cycle (V_{cycle}) is a major pathway flux comprising ~75-80% of Gln synthesis.
- $\Delta V_{cycle(tot)}$ is coupled to $\Delta CMR_{glc(ox)n}$ in ~1:1 relationship.
- The GABA/Gln cycle comprises ~22% of total (Glu + GABA) cycling flux & 16% of neuronal oxidation.
- GABAergic and glutamatergic activity increase *together* & not in opposite directions.

Conclusions (II)

- GAD_{67} mediates the majority of basal GABA synthesis, possibly the "cytosolic" (non-vesicular) pool.
- The large increase in GABA synthesis during seizures, despite GAD_{67} depletion, strongly suggests the involvement of GAD_{65}.

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