Brain glycogen content is increased in the acute and interictal chronic stages of the mouse pilocarpine model of epilepsy

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INTRODUCTION

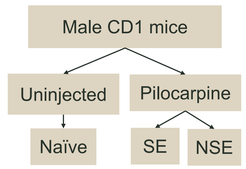
Astrocytic glycogen is increasingly recognized as an alternative brain fuel in stimulated states, such as in seizures [1]. Studies in rodent models have shown that glycogen is decreased shortly after seizures [2]. However, less is known about glycogen in chronic epilepsy.

This project explores the following hypotheses:

1. Glycogen is chronically elevated in epilepsy
2. Glycogen formed in chronic epilepsy is an insoluble, unmetabolisable type leading to accumulation [see 3]
3. Decreased glutamine synthetase activity underlies channelling of glucosyl units into glycogen synthesis via the TCA cycle [see 3]

METHODS

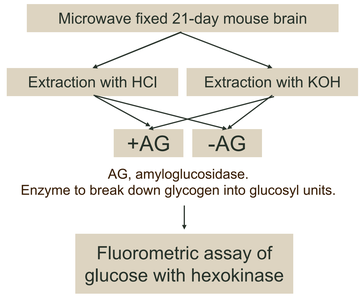
Pilocarpine model of epilepsy



SE, Status epilepticus
NSE, No status epilepticus
Induction of status epilepticus was judged clinically.

The chronic phase of the model refers to 21 days post-SE associated with spontaneous recurrent seizures.

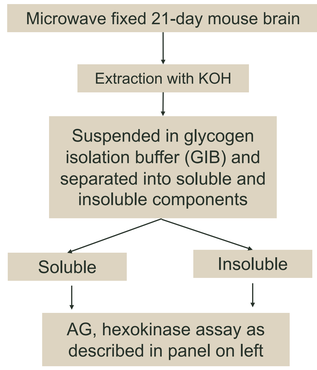
Verification of increased glycogen in chronic model



Amount of glycogen = (-AG) – (+AG)

Both methods of extraction verified increased glycogen in brains of chronic epilepsy mice.

Solubility



Glutamine synthetase activity

Cytosolic fraction isolated from hippocampi (1-day).

Glutamine synthetase (GlnS) activity measured via production of y-glutamyl hydroxamate at 540nm.

qPCR assessment of glycogen metabolism genes

Genes assessed were:

- ***Gfap*** (glial fibrillary acidic protein, marker of astrocytosis assoc with SE)
- ***CD11b*** (maker of SE)
- ***Gys1*** (glycogen synthase);
- ***Gsk3b*** (glycogen synthase kinase 3b)
- **Pygb** (glycogen phosphorylase brain)
- ***Pygm*** (glycogen phosphorylase muscle)

RESULTS

1. Glycogen is increased across the brain in the chronic stages of the mouse pilocarpine model of epilepsy, as demonstrated through two extraction methods.

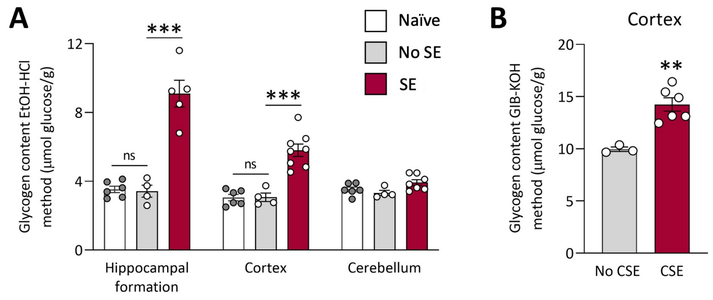


Fig 1. A, Glycogen as extracted with EtOH-HCl from hippocampi, cortices and cerebelli in naïve, No SE, and SE mice. Glycogen is high SE in hippocampi and cortices.
B, This increase in glycogen is observed in SE when extracted with GIB-KOH.

2. This accumulation is not associated with altered solubility of the glycogen.

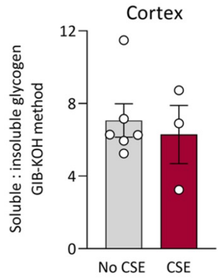


Fig 2. Ratio of soluble to insoluble glycogen in chronic epileptic mice, as assessed through the GIB-KOH method. No difference is noted in the proportion of insoluble glycogen in the SE mice.

3. Glycogen content is also increased acutely in this model, but this is not associated with a decrease in glutamine synthetase activity.

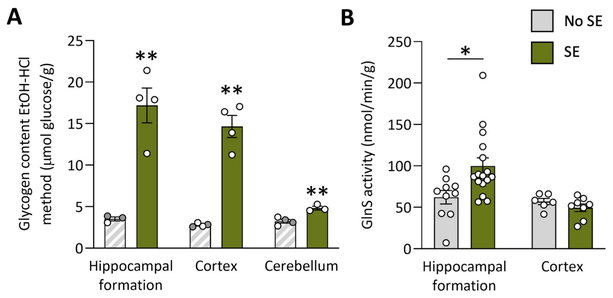


Fig 3. A, Glycogen is increased acutely in this model also, as demonstrated through EtOH-HCl extraction.
B, This is not accompanied with a decrease in glutamine synthetase activity.

4. In the chronic stages of the model, glycogen synthase kinase 3b expression is decreased.

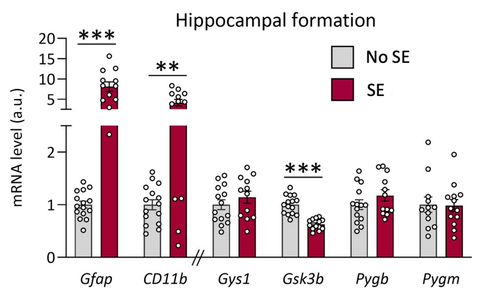


Fig 4. qPCR panel of glycogen metabolism genes. *Gfap* and *CD11b* are SE markers. There were no notable changes to expression of glycogen metabolism genes except for a decrease in glycogen synthase kinase 3b expression.

DISCUSSION

- Not only is glycogen high 1-day post-SE, interictal glycogen content is increased in the hippocampal formation and cerebral cortex.
  - This suggests long-lasting changes in glycogen metabolism.
  - Previous literature has suggested that glycogen accumulation may be a protective metabolic change in response to seizures; this is the first study to show an elevated interictal glycogen.
- Glycogen solubility in GIB in the cerebral cortex was unchanged.
  - The accumulation has been suggested in the literature to be a result of the glycogen being an unmetabolizable type; these results suggest that this is unlikely.
- *Gsk3b* mRNA levels were reduced chronically in the hippocampus.
  - The acutely increased glycogen is likely attributable to the decreased GSK3b expression, and enzyme that inactivates glycogen synthase.
- Alterations to glutamine synthetase function are unlikely to underlie the accumulation of glycogen.
- Further research should pursue the relevance of glycogen in seizure susceptibility.

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