1 **Lack of astrocytic glycogen alters synaptic plasticity but not seizure susceptibility**

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ABSTRACT

Brain glycogen is mainly stored in astrocytes. However, recent studies both *in vitro* and *in vivo* indicate that glycogen also plays important roles in neurons. By conditional deletion of glycogen synthase (GYS1), we previously developed a mouse model entirely devoid of glycogen in the central nervous system (GYS1$^{\text{Nestin-KO}}$). These mice displayed altered electrophysiological properties in the hippocampus and increased susceptibility to kainate-induced seizures. To understand which of these functions is related to astrocytic glycogen, in the present study we generated a mouse model in which glycogen synthesis is eliminated specifically in astrocytes (GYS1$^{\text{Gfap-KO}}$). Electrophysiological recordings of awake behaving mice revealed alterations in input/output curves and impaired long-term potentiation, similar, but to a lesser extent, to those obtained with GYS1$^{\text{Nestin-KO}}$ mice. Surprisingly, GYS1$^{\text{Gfap-KO}}$ mice displayed no change in susceptibility to kainate-induced seizures as determined by fEPSP recordings and video monitoring. These results confirm the importance of astrocytic glycogen in synaptic plasticity. (150 words)

Keywords: glycogen, long-term potentiation, plasticity, epilepsy, astrocyte, metabolism

1   INTRODUCTION

Most cell types in the body store glucose in the form of glycogen, a branched macromolecule containing up to 55,000 glucose units. The only enzyme able to form glycogen *in vivo* is glycogen synthase (GYS). There are two isoforms of glycogen synthase in mammals: the muscle isoform, GYS1, which is expressed in all tissues except the liver, and the liver-specific isoform, GYS2. In the brain, glycogen is estimated to comprise about 0.1% of tissue weight [1]. Both astrocytes and neurons express GYS1 and synthesize glycogen, although glycogen levels in astrocytes are much higher than in neurons [2, 3].

In the past decades, numerous studies have demonstrated that brain glycogen plays a role in memory consolidation and synaptic function [reviewed in [4–6]]. In histological studies of the healthy brain, glycogen granules are almost always confined to astrocytic cell bodies and processes [7]. Hence, there is a longstanding belief that the contribution of brain glycogen to cerebral functions is entirely due to its role in astrocytes. However, recent *in vitro* studies suggested an active glycogen metabolism in neurons [2, 8].

To study the role of brain glycogen *in vivo*, we previously developed a transgenic mouse line (GYS1$^{\text{Nestin-KO}}$) which lacked GYS1 and thus glycogen throughout the whole CNS, while GYS1 expression was normal in other tissues. Paired-pulse recordings at the CA3-CA1 synapse of the hippocampus showed that the GYS1$^{\text{Nestin-KO}}$ animals displayed increased facilitation, i.e. an increased response to the second pulse [9, 10]. The GYS1$^{\text{Nestin-KO}}$ animals also exhibited impaired long-term potentiation (LTP) evoked at the CA3-CA1 synapse. LTP is believed to be a primary molecular mechanism underlying long-term memory consolidation [9]. Additionally, the animals were more susceptible to hippocampal seizures induced by kainate or train stimulation [10]. These *in vivo* results demonstrate that brain glycogen plays a role in both short- and long-term synaptic plasticity as well as in the prevention of seizures.

68 Since the GYS1[Nestin-KO] mice lacked both glial and neuronal glycogen, the differential
69 contribution of each glycogen pool to these results could not be determined with this
70 model. For this reason, we next generated a new model with greater cellular resolution
71 devoid of GYS1 in a subset of glutamatergic neurons, the excitatory Ca^{2+}/calmodulin-
72 dependent protein kinase 2 (Camk2a)-positive neurons of the forebrain [11], including the
73 pyramidal cells of the hippocampal CA3-CA1 synapse. Like the GYS1[Nestin-KO], these
74 animals presented altered LTP and an associative learning deficiency, although the
75 impairment was not as pronounced. However, unlike the GYS1[Nestin-KO] mice, GYS1[Camk2a-KO]
76 mice exhibited no statistically significant change in PPF and no difference in
77 susceptibility to kainate-induced seizures. This study corroborated the presence of an active
78 glycogen metabolism in neurons *in vivo* and illustrated the importance of neuronal
79 glycogen in LTP. However, since only a subset of neurons was affected in this model, it
80 remains unclear whether the differences between the GYS1[Nestin-KO] and GYS1[Camk2a-KO] lines
81 are due to the lack of astrocytic glycogen and/or glycogen in another subtype of neuron.
82 In the present study, we generated a new mouse model, lacking GYS1 specifically in
83 astrocytes (GYS1[Gfap-KO]). Our results clarify the specific contribution of astrocytic glycogen
84 to cerebral function, confirming its role in synaptic plasticity and discarding its role in the
85 prevention of epileptic seizures.
86
87 2 METHODS
88
89 2.1 Animals
90 Male and female mice aged 4 ± 1 months were used in this study. All experiments
91 were carried out following European Union (2010/63/EU) and Spanish (BOE 34/11370-
92 421, 2013) regulations for the use of laboratory animals. In addition, all experimental
93 protocols were approved by the Ethics Committee of the Pablo de Olavide University.
94 Animals were kept in collective cages (up to five animals per cage) on a 12-h light/dark
95 cycle with constant temperature ($21 \pm 1°C$) and humidity ($50 \pm 5\%$). After
96 electrophysiological studies were initiated, mice were kept in individual cages until the end
97 of the experiments. Animals were allowed access *ad libitum* to commercial mouse chow
98 and water. The Gfap-Cre transgenic line 77.6 used in this study was purchased from
99 Jackson laboratories (Stock #024098) and has been thoroughly characterized [12].
100
101 2.2 Biochemical analysis
102 Mice were euthanized by cervical dislocation and decapitation, and the brains were
103 removed and hemisected. The cerebellum, hippocampus and cortex from each hemisphere
104 were then dissected and frozen in liquid nitrogen. Samples were maintained at -80 °C until
105 use. Tissue lysates for Western blot were prepared as previously described [11] and sample
106 protein content was determined by Bradford assay (BioRad). Lysates were loaded in 10%
107 polyacrylamide gels and transferred to Immobilon membranes (Millipore) for Western blot.
108 The following antibodies were used: anti-glycogen synthase (Cell Signaling cat# 3886) and
109 anti-GFAP (Millipore cat# MAB360). The REVERT total protein stain was used as a
110 loading control and densitometry was performed using Image Studio[TM] Lite (LI-COR
111 BioSciences).
112

2.3 Animal preparation for the electrophysiological study

For electrode implantation, animals were anesthetized with 0.8–3% halothane from a calibrated Fluotec 5 (Fluotec-Ohmeda, Tewksbury, MA, USA) delivered via a homemade mask and vaporizer at a flow rate of 0.8 L/min oxygen. Briefly, animals were implanted with bipolar stimulating electrodes at the right Schaffer collaterals of the dorsal hippocampus and with a recording electrode in the ipsilateral CA1 area using stereotaxic coordinates [13]. Electrodes were made of 50 μm Teflon-coated tungsten wire (Advent Research Materials Ltd., Eynsham, England). The final location of the CA1 recording electrode was determined electrophysiologically, as described by some of us [11, 14]. Stimulating, recording and ground wires were soldered to a 6-pin socket. The socket was fixed to the skull with the help of three small screws and dental cement [11, 14].

2.4 Input/output curves and LTP procedures

Both input/output curves, paired-pulse facilitation (PPF), and LTP were evoked in behaving mice following procedures described elsewhere [11, 14]. For the electrophysiological study, the mouse was located in a small ($5 \times 5 \times 5$ cm) box, aimed to avoid over walking. For input/output curves, mice were stimulated at the CA3-CA1 synapse with single pulses of increasing intensities (0.02–0.4 mA). PPF was determined by applying double pulses with increasing inter-pulse intervals (10, 20, 40, 100, 200 and 500 ms) at a fixed intensity corresponding to ~40% of asymptotic values, as previously described [11]. Evoked field excitatory post-synaptic potentials (fEPSPs) were recorded with Grass P511 differential amplifiers, across a high impedance probe (2×10^{12} Ω; 10 pF), and with a bandwidth of 0.1 Hz-10 kHz (Grass-Telefactor, West Warwick, RI, USA).

For LTP measurements, baseline fEPSP values evoked at the CA3-CA1 synapse were collected 15 min prior to LTP induction using single 100 μs, square, biphasic pulses. Pulse intensity was set well below the threshold for evoking a population spike (0.15-0.25 mA); i.e., 30–40% of the intensity necessary for evoking a maximum fEPSP response [14, 15]. LTP was evoked with a high-frequency stimulus (HFS) protocol consisting of five 200 Hz, 100-ms trains of pulses at a rate of 1/s, repeated six times, at intervals of 1 min. The stimulus intensity during the HFS protocol was set at the same value as that used for generating baseline recordings to prevent the presentation of electroencephalographic seizures and/or large population spikes. After each HFS session, the same stimuli were presented individually every 20 s for 60 additional min and for 30 min on the following three days [11, 14]. Evoked fEPSPs were recorded as described above.

2.5 Induction of hippocampal seizures with kainate injections in implanted mice

Following procedures described elsewhere [16], we determined the propensity of control and GYS1^{Gfap-KO} mice to generate convulsive seizures in the hippocampal area. For this, we intraperitoneally (i.p.) administrated the AMPA/kainate receptor agonist kainate (8 mg/kg; Sigma, St. Louis, MO, USA) dissolved in 0.1 M phosphate buffered saline (PBS) pH = 7.4. Local field potentials and electrically evoked fEPSPs were recorded in the hippocampal CA1 area from 5 min before to 60 min after kainate injections.

2.6 Video monitoring of seizures after kainate injections

159   Animals were placed in individual cages and were administered with three consecutive
160 i.p. injections of kainate (8 mg/kg per dose, 24 mg/kg total) one every 30 min from the
161 onset of the experiment in order to induce convulsive non-lethal seizures. Seizure stages
162 after kainate injections were evaluated as described previously [17–19]. After the first
163 kainate injections, the animals developed hypoactivity and immobility (Stage I–II). After
164 successive injections, hyperactivity (Stage III) and scratching (Stage IV) were often
165 observed. Some animals progressed to a loss of balance control (Stage V) and further
166 chronic whole-body convulsions (Stage VI). Extreme behavioural manifestations such as,
167 uncontrolled hopping activity, or "popcorn behaviour" and continuous seizures (more than
168 1 minute without body movement control) were included in Stage VI. All behavioural
169 assessments were performed blind to the experimental group (genotype) in situ, as well as
170 recorded and reanalysed blind to the first analysis. Analysis consisted in the record of the
171 time spent until the onset of the first seizure, the number of seizures per animal, the time
172 spent on each grade, as well as the maximum grade reached by each animal.
173

174   2.7 Data collection and statistical analysis

175   fEPSPs and 1-V rectangular pulses corresponding to brain stimulation were stored
176 digitally on a computer through an analog/digital converter (CED 1401 Plus, CED,
177 Cambridge, England). Data were analyzed off-line for fEPSP recordings with the help of
178 the Spike 2 (CED) program. Five successive fEPSPs were averaged, and the mean value of
179 the amplitude (in mV) was determined. Computed results were processed for statistical
180 analysis using the IBM SPSS Statistics 18.0 (IBM, Armonk, NY, United States). Data are
181 represented as the mean ± SEM. Statistical significance of differences between groups was
182 inferred by Two-way repeated measures ANOVA, followed by the Holm-Sidak method for
183 all pairwise multiple comparison procedures. The Fisher exact test for data collected from
184 kainate experiments. Statistical significance was set at $P < 0.05$. For the biochemical
185 analyses and behavioral assessment of seizure susceptibility after kainate administration,
186 computed results were processed for statistical analysis with PRISM 8.0 (GraphPAD
187 Software, San Diego, USA). Data are represented as the mean ± SEM. Normality of the
188 distributions was checked via the Shapiro–Wilk test; All tests performed were two-sided.
189 Statistical significance of differences between groups was inferred by Student's t-Test or
190 Two-way ANOVA, followed by the Bonferroni post-hoc comparison for all pairwise
191 multiple comparison procedures. Statistical significance was set at $P < 0.05$.

192   3 RESULTS

193   3.1 Generation of GYS1[Gfap-KO] mice

194   GFAP is a cytoskeletal protein found in nearly all astrocytes and a common marker for
195 this cell type. The astrocyte-specific inactivation of *Gys1* was achieved by crossing mice
196 homozygous for the conditional *Gys1* allele [9] with mice expressing Cre recombinase
197 under the control of the *Gfap* promoter [12]. Littermates that were homozygous for the
198 conditional *Gys1* allele and negative for Cre recombinase expression were used as controls.
199 To confirm the inactivation of *Gys1*, we measured GYS1 protein levels in cortex,
200 hippocampus, and cerebellum by Western blot. GYS1 protein was greatly diminished in all
201 regions (Fig. 1a). Quantification by densitometry showed that GYS1 expression was

202 reduced by approximately 80% in the cortex and in the hippocampus, and 60% in the
203 cerebellum (Fig. 1b). Total brain glycogen was decreased by more than 80% (Fig. 1c),
204 which is consistent with the reduction in GYS1 protein. No changes in GFAP levels were
205 observed (Fig. 1a, quantification not shown).
206
207 **3.2 Electrophysiological alterations at the CA3-CA1 synapse in GYS1$^{\text{Gfap-KO}}$ mice**

208   To study the consequences of the lack of astrocytic glycogen on synaptic function, we
209 performed recordings of input/output curves, PPF and LTP evoked at the CA3-CA1
210 synapse of the hippocampus (Fig. 2a). In a first experimental step, we examined the
211 response of CA1 pyramidal neurons to single pulses of increasing intensity (0.02–04 mA)
212 presented to the ipsilateral Schaffer collaterals. Both control and GYS1$^{\text{Gfap-KO}}$ mice
213 presented similar increases in the amplitude of fEPSPs evoked at CA1 pyramidal neurons
214 by the stimuli presented to Shaffer collaterals (Fig. 2b). These two input/output
215 relationships were best fitted by sigmoid curves ($r \geq 0.9$; $P \leq 0.001$; not illustrated),
216 suggesting the normal functioning of the CA3-CA1 synapse in both groups. However, the
217 experimental group reached lower maximal fEPSP amplitudes than their littermate controls.
218 No significant differences [Two-way repeated measures ANOVA; $F_{(19,266)} = 1.222$; $P =$
219 0.239] were observed overall between control and GYS1$^{\text{Gfap-KO}}$ groups. However, fEPSPs
220 evoked by three increasing intensities presented significant differences (All pairwise
221 multiple comparison procedures; $P < 0.05$). We also performed an analysis of PPF at the
222 CA3-CA1 synapse by applying double pulses at a fixed intensity with increasing inter-
223 stimulus intervals (10, 20, 40, 100, 200, 500 ms). Both groups displayed facilitation at 20
224 and 40 ms intervals (Fig. 2c). GYS1$^{\text{Gfap-KO}}$ exhibited no statistical difference in PPF
225 compared to control animals [Two-way repeated measures ANOVA; $F_{(5,95)} = 0.726$; $P =$
226 0.606].
227   In a following experimental step, we evoked LTP at the CA3-CA1 synapse of the two
228 genotypes as an indication of long-term synaptic plasticity. It is well known that the
229 hippocampus is involved in the acquisition of different types of associative [20, 21] and
230 non-associative [22, 23] learning tasks and that the CA3-CA1 synapse is often selected for
231 evoking LTP in behaving mice [14, 24]. For baseline values, animals were stimulated every
232 20 s for ≥ 15 min at the implanted Schaffer collaterals (Fig. 2d). Afterward, they were
233 presented with a high frequency stimulus (HFS) protocol. Immediately after the HFS
234 session, the same single stimulus used to generate baseline records was presented at the
235 initial rate (3/min) for another 60 min. As illustrated in Fig. 2d, recording sessions were
236 repeated for three additional days (30 min each). The control group presented a significant
237 LTP when comparing baseline values with those collected following the HFS session
238 (Holm-Sidak method, all pairwise multiple comparison procedures; $P \leq 0.041$). Although
239 the amplitude of fEPSPs also increased in GYS1$^{\text{Gfap-KO}}$ mice following the HFS session,
240 only a tendency was presented ($P \geq 0.671$) (Fig. 2d). In addition, the amplitude of fEPSPs
241 evoked in the control group was significantly [Two-way repeated measures ANOVA;
242 $F_{(32,512)} = 6.277$; $P < 0.001$] larger and longer lasting than that evoked in the experimental
243 group (Fig. 2d). In summary, GYS1$^{\text{Gfap-KO}}$ mice display no change in PPF but a
244 significantly impaired LTP compared to the littermate controls.
245
246 **3.3 Seizure susceptibility in GYS1$^{\text{Gfap-KO}}$ mice**

247   Kainate is a widely used chemoconvulsant used to study seizure susceptibility in
248   rodents [25]. We previously demonstrated that mice devoid of cerebral glycogen
249   (GYS1$^{Nestin-KO}$) are more susceptible to kainate-induced seizures [10]. To understand the
250   participation of astrocytic glycogen in seizure susceptibility, we also assessed the response
251   of GYS1$^{Gfap-KO}$ mice to kainate-induced seizures in the pre-implanted animals. Evoked
252   seizures in control and GYS1$^{Gfap-KO}$ mice presented similar durations and profiles (Fig. 3a).
253   Both groups presented a noticeable depression in the amplitude of evoked fEPSP recorded
254   following a kainate-dependent seizure (Fig. 3b). Overall, there was no difference in the
255   number of seizures observed per genotype (Fisher exact test; $P = 0.657$) (Fig. 3c).
256   To corroborate these results, we employed an alternative seizure assessment protocol in
257   a new cohort of mice. Mice were given three kainate injections (8 mg/kg, i.p. every 30 min)
258   and video-recorded for 180 minutes to monitor their behavior (i.e. epileptic events)
259   following the first injection. Mice from both genotypes reached similar severity stages (Fig.
260   4a).   In the majority of the mice of both genotypes, seizures began approximately 15
261   minutes after the third dose of kainate ($P = 0.9501$, Student's t-test; Fig. 4b). There were no
262   significant differences between groups in the prioritary stage (the behavioral stage in which
263   an animal spends the most time after kainate administration throughout the duration of the
264   experiment) ($P = 0.5441$, Student's t-test; Fig. 4c) nor the maximum stage (the most severe
265   stage reached during the experiment) ($P = 0.9644$, Student's t-test; Fig. 4d). Furthermore,
266   there were no significant differences in the time spent per stage (Two-way ANOVA: Stage
267   factor: $P = 0.0007$, Genotype factor: $P = 0.2370$; Fig. 4e) or in the number of seizures per
268   animal after each injection (Two-way ANOVA: Administration factor: $P < 0.0001$,
269   Genotype factor: $P = 0.7980$; Fig. 4f). These results unequivocally confirmed that
270   GYS1$^{Gfap-KO}$ animals present a similar seizure susceptibility compared to control
271   littermates.
272
273   **4   DISCUSSION**

274
275   In this study, we analyze for the first time the physiological consequences of removing
276   glycogen specifically from astrocytes by means of transgenic tools. By comparing this
277   mouse to previous models lacking glycogen in the entire CNS [9, 10] or only in Camk2a-
278   positive excitatory neurons of the forebrain [11], we are able to define the specific
279   physiological roles of glycogen in astrocytes versus neurons.
280   Using Cre/Lox technology, we deleted *Gys1* in GFAP-positive cells to eliminate only
281   astrocytic glycogen synthesis. Western blot analyses confirmed a clear reduction in GYS1
282   protein levels in hippocampus, cortex and cerebellum of GYS1$^{Gfap-KO}$ mice (Fig. 1, a and
283   b). Total brain glycogen content was also greatly reduced relative to littermate controls
284   (Fig. 1c). These results are consistent with the well-documented observation that the
285   majority of brain glycogen is stored in astrocytes, but also point to a remaining significant
286   fraction of non-astrocytic GYS1 expression and glycogen synthesis, likely in neurons.
287   We also studied synaptic function in the GYS1$^{Gfap-KO}$ model via stimulation of the
288   CA3-CA1 synapse in the hippocampus. Input/output curves showed no overall significant
289   difference between groups, but at some intensities, evoked fEPSPs were statistically higher
290   in the control group compared to the GYS1$^{Gfap-KO}$ animals, and the latter group reached a
291   lower maximum fEPSP (Fig. 2b). These results suggest that the absence of astrocytic
292   glycogen may reduce basal synaptic strength. A similar situation is obtained with inhibitors

7

293   of astrocytic glutamate transport, which also reduce basal EPSPs since the accumulation of
294   glutamate causes presynaptic inhibition [26]. Astrocytes take up synaptic glutamate and
295   convert it to glutamine for transfer to neurons, where it is recycled into glutamate and
296   repackaged into synaptic vesicles, a process known as the glutamate/glutamine cycle [27,
297   28]. Since astrocytic glycogen has been shown to play a role in both glutamate uptake and
298   recycling [29, 30], the lack of astrocytic glycogen could cause impaired glutamate uptake
299   leading to presynaptic inhibition and therefore lower synaptic strength. While PPF
300   experiments with increasing inter-pulse intervals showed significantly greater facilitation in
301   GYS1$^{\text{Nestin-KO}}$ mice [10], in the present study, GYS1$^{\text{Gfap-KO}}$ mice only lack astrocytic
302   glycogen displayed only a trend toward increased PPF, with no statistical difference (Fig.
303   2c). PPF is typically attributed to presynaptic mechanisms such increased $[Ca^{2+}]$ in the
304   presynaptic terminal [31]. The altered PPF observed in GYS1$^{\text{Nestin-KO}}$ mice could be
305   exclusively neuronal in origin.

306   We also observed an impairment in hippocampal LTP in GYS1$^{\text{Gfap-KO}}$ animals (Fig.
307   2d). However, the LTP impairment in the GYS1$^{\text{Gfap-KO}}$ animals was not as pronounced as
308   was observed in the GYS1$^{\text{Nestin-KO}}$ mice [9]. The present results are reminiscent of those
309   from the GYS1$^{\text{Camk2a-KO}}$ mice, in which LTP was still observed, although it was
310   significantly impaired [11]. Collectively, these three mouse models demonstrate that both
311   astrocytic and neuronal glycogen contribute to LTP. Previous studies using
312   pharmacological agents have shown that astrocytic glycogen is important for long-term, but
313   not short-term, memory formation [32, 33]. The presence of a normal PPF, a measure of
314   short-term plasticity, and the major impairment in LTP that we observed in the GYS1$^{\text{Gfap-KO}}$
315   model are consistent with these observations.

316   The most surprising result regarding the GYS1$^{\text{Gfap-KO}}$ line is its susceptibility to
317   kainate-induced epilepsy. We previously showed that GYS1$^{\text{Nestin-KO}}$ mice were more
318   susceptible to seizures induced by a single convulsive dose of kainate (8mg/kg, i.p.) [10].
319   However, using the same protocol, we detected no statistical difference in the GYS1$^{\text{Gfap-KO}}$
320   line (Fig. 3). Utilizing a second experimental protocol with three consecutive doses of
321   kainate (8mg/kg, i.p., every 30 minutes) we found no statistical differences between the
322   groups in the seizure stages achieved (Fig. 4a), seizure onset (Fig. 4b), priorary or
323   maximum stage reached (Fig. 4, c and d), time spent per stage (Fig. 4e) or number of
324   seizures per animal (Fig. 4f). These results unequivocally show that GYS1$^{\text{Gfap-KO}}$ animals
325   are not more susceptible to kainate than their littermate controls. Altered glycogen
326   metabolism has been linked to seizures, as reviewed elsewhere [34–37]. A commonly held
327   view is that altered astrocytic glycogen metabolism induces neuronal excitability via
328   impaired glutamate and K^+ uptake. However, herein we show mice lacking astrocytic
329   glycogen do not have more kainate-induced seizures. Since GYS1$^{\text{Camk2-KO}}$ mice also show
330   unaltered kainate susceptibility [11], collectively these mouse models suggest that seizure
331   susceptibility in the GYS1$^{\text{Nestin-KO}}$ line is a consequence of the lack of glycogen in another
332   cell type. Inhibitory neurons play a critical role in suppressing excitability, and their
333   dysfunction is associated with epilepsy in rodent models and humans [38]. Therefore, our
334   results suggest that glycogen in inhibitory neurons might be critical for their regulatory
335   role. This possibility will be addressed in future studies.

336   In summary, the GYS1$^{\text{Gfap-KO}}$ mouse model illustrates the specific contribution of
337   astrocytic glycogen to the physiological roles of glycogen in the brain, further clarifying
338   how brain glycogen is involved in memory and epilepsy. Our results confirm that astrocytic
339   glycogen plays an active role in long-term synaptic plasticity. However, the lack of

340 astrocytic glycogen does not increase susceptibility to kainate-induced seizures in these
341 mice. These data point to a role of neuronal glycogen in cerebral functions, most
342 importantly in the regulation of excitability. A thorough understanding of these processes is
343 essential for better management and treatment of neurological disorders.
344
345 **Figure Legends**
346
347 **Fig. 1** Analysis of GYS1, GFAP and glycogen levels in GS$^{Gfap-KO}$ mice and controls. (a)
348 Representative Western blot of GYS1 and GFAP protein levels in cortex (Cx),
349 hippocampus (Hp) and cerebellum (Cb). REVERT protein stain (Li-COR BioSciences) was
350 used as a loading control. (b) Quantification of GYS1 and GFAP protein levels by region
351 normalized to total protein determined by REVERT. (c) Total brain glycogen in control
352 versus GS$^{Gfap-KO}$ animals. All data are expressed as average ±SEM (n = 4-6 per group).
353 Significant differences were calculated using student's t-test (∗, $P < 0.05$; ∗∗∗, $P < 0.001$;
354 ****, $P < 0.0001$).
355
356 **Fig. 2** Electrophysiological properties of hippocampal synapses in behaving control and
357 GYS1$^{Gfap-KO}$ mice. (A) Animals were chronically implanted with bipolar stimulating (St.)
358 electrodes in the right CA3 Schaffer collaterals and with a recording (Rec.) electrode in the
359 ipsilateral CA1 area. DG, dentate gyrus; Sub., subiculum. (B) Input/output curves of
360 fEPSPs evoked at the CA3-CA1 synapse via single pulses of increasing intensities (0.02–
361 0.4 mA) in control and GYS1$^{Gfap-KO}$ mice. Although no significant differences [Two-way
362 repeated measures ANOVA; $F_{(19,266)} = 1.222$; $P = 0.239$] were observed between groups,
363 fEPSPs evoked by three different intensities presented significant differences (All pairwise
364 multiple comparison procedures; $P < 0.05$). (C) Paired-pulse facilitation in control and
365 GYS1$^{Gfap-KO}$ animals with increasing inter-stimulus intervals. No significant differences
366 between the two groups were observed [Two-way repeated measures ANOVA; $F_{(5,95)} =$
367 1.222; $P = 0.606$]. (D) LTP evoked at the CA3-CA1 synapse of control and GYS1$^{Gfap-KO}$
368 mice following the HFS session. The HFS was presented after 15 min of baseline
369 recordings, at the time marked by the dashed line. LTP evolution was followed for four
370 days. At the right are illustrated representative examples of fEPSPs collected from control
371 and GYS1$^{Gfap-KO}$ mice at the times indicated in the bottom graph. fEPSP amplitudes are
372 given as a percentage of values measured from baseline recordings, and statistical
373 differences between control and GYS1$^{Gfap-KO}$ from two-way repeated measures ANOVA
374 are shown (*, $P \leq 0.01$). All data are expressed as average ± SEM (n= 7-9 mice/group).
375
376 **Fig. 3** Kainate susceptibility of GYS1$^{Gfap-KO}$ mice compared to controls. (a) Representative
377 examples of hippocampal seizures evoked in control and GYS1$^{Gfap-KO}$ mice following the
378 administration of 8 mg/kg i.p. of kainate. (b) Representative examples of fEPSPs evoked
379 before and immediately after a kainate-evoked seizure. (c) Percentage of control (n = 14)
380 and GYS1$^{Gfap-KO}$ (n = 9) mice presenting spontaneous seizures at the CA1 area during the
381 recording period (60 min). No significant differences between groups (Fisher exact test; P
382 = 0.657) were observed.
383
384 **Fig. 4** Comparison of kainate-induced seizure profile in control and GYS1$^{Gfap-KO}$. 3-4
385 months old mice were subjected to three kainate injections (8 mg/kg every 30 min) and
386 epileptic responses were analyzed for 180 minutes after the first injection. (a) Percentage of

387   mice reaching seizure stages I to VI and kainate-induced mortality. (b) Onset of the
388   epileptic activity. Student's t-test ($P = 0.9501$). (c) Prioritary stage displayed by each
389   animal during the course of the experiment. Student's t-test ($P = 0.5441$). (d) Maximum
390   stage reached by each animal during the course of the experiment. Student's t-test ($P = 0.9644$). (e) Percentage of time spent on each stage during the course of the experiment.
391   0.9644). (e) Percentage of time spent on each stage during the course of the experiment.
392   Two-way ANOVA (Stage factor: $P = 0.0007$; Genotype factor: $P = 0.2370$). (f) Number of
393   seizures experimented per animal divided on time segments after the first, second and third
394   kainate administrations. Two-way ANOVA (Administration factor: $P < 0.0001$; Genotype
395   factor: $P = 0.7980$). All data are expressed as average ± SEM ($n = 6\text{-}7$ mice/group).

396
397 **AUTHOR CONTRIBUTIONS**
398
399   JD and JJG conceived the study. JD generated and maintained the GYS1$^{\text{Gfap-Cre}}$ line. JD and
400   MKB collected brain tissues and performed biochemical analyses. AG and JMD-G
401   performed electrophysiological studies before and after single kainate injections. AH and
402   JAR performed seizure video-monitoring with multiple kainate injections. All authors
403   analyzed data and contributed to the writing of the manuscript.

404
405 **CONFLICT OF INTEREST**
406
407   All authors declare they have no conflicts of interest.

408
409 **ACKNOWLEDGEMENTS**
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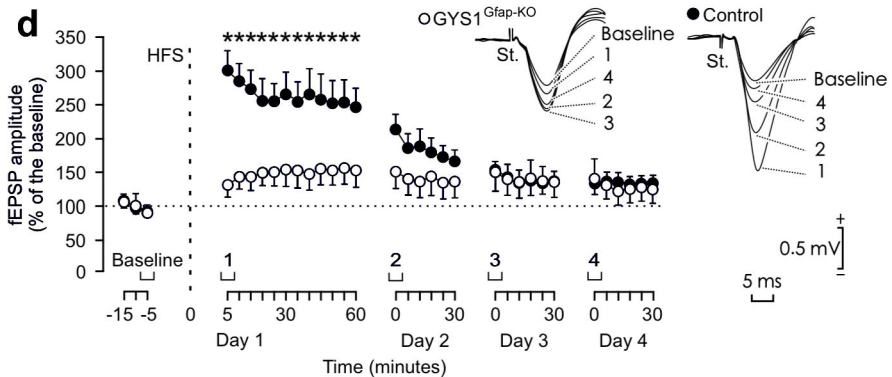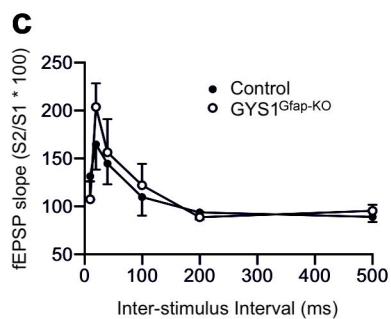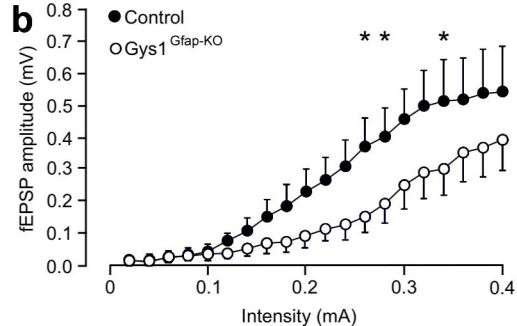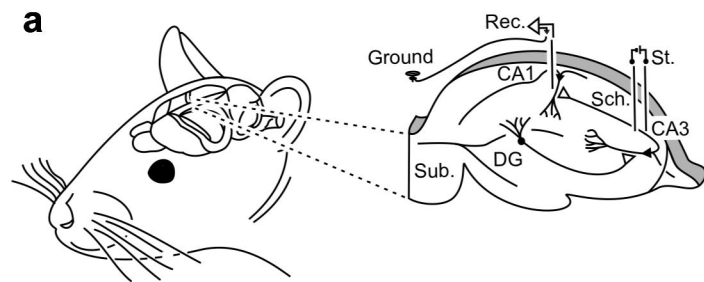
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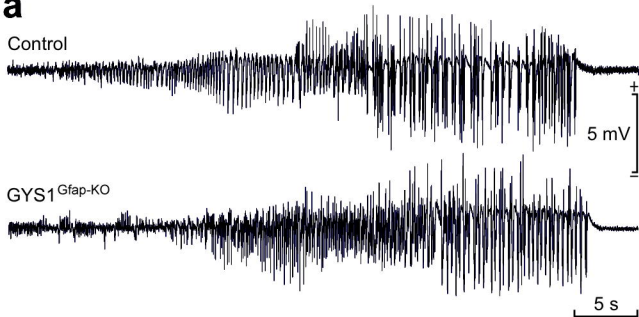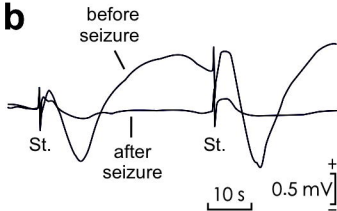542   epileptic human hippocampus. Trends in Neurosciences 28:334–340.
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544
545

a

	Cx	Hp	Cb
	Control GS$^{Gfap-KO}$	Control GS$^{Gfap-KO}$	Control GS$^{Gfap-KO}$

GYS1

GFAP

REVERT

b GYS1 (Western Blot)

Arbitrary Units

■ Control
□ GS$^{Gfap-KO}$

Cx: ****
Hp: ***
Cb: *

c Brain Glycogen

Glycogen (μg/mg tissue)

Control   GS$^{Gfap-KO}$ ****

a

Control

GYS1^Gfap-KO

5 mV

5 s

b

before
seizure

St.

after
seizure

St.

10 s

0.5 mV

c

■ Control
□ GYS1^Gfap-KO

71.4%

55.5%

Mice with
seizures (%)