Childhood-onset movement disorders
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Chapter 6

North Sea progressive myoclonus epilepsy is exacerbated by heat, a phenotype primarily associated with affected glia

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ABSTRACT

The progressive myoclonic epilepsies (PMEs) comprise a group of rare disorders of different genetic aetiologies, leading to childhood-onset myoclonus, myoclonic seizures and subsequent neurological decline. One of the genetic causes for PME, a mutation in the gene coding for Golgi SNAP receptor 2 (GOSR2), gives rise to a PME-subtype prevalent in Northern Europe and hence referred to as North Sea Progressive Myoclonic Epilepsy (NS-PME). Treatment for NS-PME, as for all other PME subtypes, is symptomatic; the pathophysiology of NS-PME is currently unknown, precluding targeted therapy.

Here, we investigated the pathophysiology of NS-PME. By means of chart review in combination with interviews with patients (n=14), we found heat to be an exacerbating factor for a majority of NS-PME patients (86%). We demonstrated that knockdown of the GOSR2-orthologue Membrin in Drosophila melanogaster also leads to a sensitivity to heat-induced seizures. Specific downregulation of Membrin in glia but not in neuronal cells resulted in a similar phenotype, which was progressive as the flies aged and was partially responsive to treatment with sodium barbital. Our data suggest a role for GOSR2 in glia in the pathophysiology of NS-PME.
INTRODUCTION

The progressive myoclonic epilepsies (PMEs) form a group of rare diseases of different genetic aetiologies. They are characterized clinically by a progressive neurological disorder starting in childhood with myoclonus and epilepsy. Mutations in the Golgi SNAP receptor 2 gene (GOSR2) cause a particular type of PME with a relatively high prevalence in countries bordering on the North Sea: aside from its systematic classification as PME, it is also known as North Sea progressive myoclonus epilepsy (NS-PME). Interestingly, nearly all patients known to date are homozygous for the same mutation (c.430G>T, Gly144Trp), leading to the amino acid change of an evolutionarily conserved residue of the GOSR2 protein. The clinical phenotype of these patients consists of early-onset ataxia around the age of two years, followed by generalised cortical myoclonus around the age of six with seizures often starting in the second decade of life. All these features are progressive, causing patients to become wheelchair-bound in adolescence or adulthood and having a reduced life-expectancy. Despite the progressive neurological decline, no neurodegeneration is observed in imaging studies of affected patients or in the single post-mortem neuropathological study that was performed. Also, cognitive function is relatively preserved in NS-PME.

As in other types of PME/PMA, treatment is symptomatic and aimed at minimizing invalidating myoclonus and epilepsy using (combinations) of antiepileptic drugs. Importantly, a large part of the antiepileptic armamentarium (e.g. phenytoin, carbamazepine, gabapentin) is known to potentially aggravate myoclonus and is therefore contraindicated in PME, limiting therapeutic options. Unfortunately, insight into the pathogenesis of NS-PME/PMA is lacking, hampering the development of more targeted treatment strategies.

The GOSR2 gene codes for a Qb-SNARE protein involved in traffic of proteins through the Golgi apparatus. The Gly144Trp amino acid change found in NS-PME/PMA patients most likely confers a loss of function, as this alteration in the yeast GOSR2 homologue bos1 yielded a protein unable to complement the Δbos1 knockout strain, contrary to the wild type bos1, supporting the notion that the mutation leads to a partial loss of function of the GOSR2 protein.

Model organisms provide insight into pathophysiology of disease, particularly in the case of a known genetic defect. Over the last decades, the fruit fly (Drosophila melanogaster) has emerged as a versatile model organism for many conditions, including neurodegenerative diseases and epilepsy. In a recently described Drosophila model of NS-PME/PMA, dendritic growth defects and discrete changes in larval neuromuscular junctions were reported. However, the exact pathophysiology of NS-PME/PMA remains unknown.

We collected retrospective data and analysed semi-structured interviews and we found heat to worsen symptoms in a large majority of patients. These findings were further substantiated in a novel Drosophila model for NS-PME/PMA by knockdown of the GOSR2-orthologue Membrin. Our results demonstrate that specifically upon glial loss of Membrin, epileptic seizures are progressive with age and partially corrected by sodium barbital.
MATERIALS AND METHODS

Patients and patient interviews
From the medical records of 14 patients with NS-PME/PMA (age 4-44 years; male: female 10:4), we retrospectively collected factors influencing symptoms, in particular myoclonic jerks and seizures, both positively and negatively. In addition, we interviewed these 14 patients and/or their caregivers using a semi-structured interview with a focus on factors that influenced NS-PME/PMA symptoms (supplementary information).

Drosophila husbandry
Drosophila flies were maintained on Bloomington food at 25 degrees. Stocks that were obtained from the Bloomington Drosophila Stock Center were Actin-GAL4 (#4414), nSyb-GAL4 (#51941), Elav-GAL4 (#8765), Repo-GAL4 (#7415). The membrin RNAi strain was obtained from the Vienna Drosophila Resource Center (#44534).

Heat-induced seizure assay
The heat-induced seizure paradigm was performed as previously described12.

Chemicals and administration
Sodium barbital was obtained from Sigma-Aldrich. Administration of barbital was achieved 24 hours prior to the heat induced seizure assay by feeding the flies apple juice with 0.5 mg/mL sodium barbital by transferring them to a Whatman filter soaked in 600 µL of solution. Apple juice was used for the control condition. Administration of barbital over an extended period was achieved by supplementing the fly food with barbital in the concentration of 0.5mg/mL food.

RNA isolation, quantitative real-time PCR, and primers.
Membrin qPCR was performed as previously described8. Primer sequences: membrin fp: 5′-TGGGTCTGTCCAATCACACG-3′, rp: 3′-CAAGGTGACCACCACTCCTC-5′; rp49 fp: 5′-CCGCTTCAAGGGACAGTATC-3′, rp: 5′-GACAATCTCCTTGCGCTTCT-3′.

Western blot
Protein levels were compared between Act>membrin RNAi and Act>GFP flies using Western Blot with anti-Membrin antibody (Abcam, ab115642, 1:1000) as a primary antibody and HRP-linked anti-rabbit IgG as a secondary antibody. Anti-tubulin was used to detect the loading control (Sigma, T5168, 15000). The images were obtained using a ChemiDoc MP (BioRad).
Statistics
Data was visualised and analysed using GraphPad Prism version 5. Statistical analysis was performed using Fisher’s exact test or Mantel-Cox log-rank-test as appropriate using Graphpad Prism version 5.

RESULTS

Heat causes exacerbation of symptoms in NS-PME/PMA patients
We interviewed 14 NS-PME/PMA patients with a specific emphasis on factors influencing their symptoms, both positively and negatively. The full results are displayed in Supplementary Table 1. Interestingly, heat was reported to exacerbate symptoms in a majority of patients (Table 1). This not only included fever and intercurrent illness (11/14 patients, 79%), but also exogenous factors such as hot showers/baths (5/14 patients, 36%) and increased environmental temperature (7/14 patients, 50%). In total, 12 patients (86%) reported at least one form of exogenous heat as exacerbating their NS-PME/PMA symptoms. Other, more well-known exacerbating factors such as (unexpected) noise, lights/flashes and stress were also frequently reported by patients (Supplementary Table 2).

Table 1 | Distribution of reported heat-related factors negatively influencing NS-PME/PMA symptoms

<table>
<thead>
<tr>
<th>Age and gender</th>
<th>Fever</th>
<th>Any exogenous heat source</th>
<th>Environmental heat</th>
<th>Shower/bath</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 24M</td>
<td>x</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 25M</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 17M</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 4M</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 7M</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 18F</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 44F</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 35M</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 30M</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 7F</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 40F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 12M</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 31M</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 31M</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In this table, only the presence or absence of factors associated with heat are displayed; all reported factors can be found in Supplementary Table 1.
Figure 1: Ubiquitous or glia-specific knockdown of Drosophila membrin causes seizure-like behaviour in (female) flies and barbital suppresses seizure-like behaviour associated with glial loss of membrin

(A) 5 day old female flies Act-Gal4 UAS-membrin-RNAi (ubiquitous membrin-downregulated) flies or Act-Gal4 UAS-GFP (control) flies were tested for sensitivity to heat-induced seizures during exposure of 120 seconds in a 40 °C water bath. The vials were scored in a cumulative manner at 5 second intervals for seizing flies. While the control flies never seized, the membrin-downregulated flies showed seizure-like behaviour in about 30% of the flies after 120 seconds.

(B-C) 3, 5 and 8 day old female flies expressing UAS-membrin-RNAi under the control of neuronal drivers Elav-Gal4 and nSyb-Gal4, to downregulate membrin in neuronal cells, were treated as in A. 8 day old flies expressing UAS-GFP under the control of the same drivers are shown as controls. Seizure-like behaviour is absent in control flies and in flies in which membrin is downregulated in neuronal cells.

(D) 3, 5 and 8 day old female flies expressing UAS-membrin-RNAi under the control of glial driver Repo-Gal4, to downregulate membrin in glial cells, were treated as in A. 8 day old flies expressing UAS-GFP under the control of the same driver are shown as controls. Seizure-like behaviour is absent in control flies and in flies in which membrin is downregulated in glial cells.

(E-F) 8 days and 24 hrs Barbital Treatment

*** p < 0.001, * p < 0.05
A Drosophila model implicates glia in NS-PME

RNAi-mediated knockdown of Membrin causes a decrease of membrin mRNA and protein in vivo

Next a *Drosophila melanogaster* model for NS-PME/PMA was developed. Using RNAi-mediated knockdown, we downregulated the *Drosophila* GOSR2 orthologue Membrin in all cells and we verified that, respectively, *membrin* mRNA and Membrin protein levels were reduced (Supplementary Figure 1). As reported previously, ubiquitous knockdown of Membrin was observed to cause a drastic reduction in the amount of offspring as compared to the controls (Supplementary Figure 2). This demonstrated that adult flies expressing reduced levels of *membrin* are viable, although their numbers are reduced.

Ubiquitous knockdown of membrin is associated with sensitivity to heat-induced seizures

We investigated whether the flies with reduced Membrin levels showed sensitivity to heat-induced seizures. We subjected control flies and Membrin-reduced flies to a water bath of 40 °C for up to 120 seconds, as previously described. Seizure-like behaviour, characterised by twitching, wing flapping and loss of standing position, was not observed in control flies. However, in 5 day old Membrin-reduced flies, this seizure-like behaviour is observed in approximately 30% of flies in an accumulative manner, the longer the heat shock lasted, the more flies showed seizure-like behavior (Figure 1A). These data are consistent with the clinical data.

Heat-induced seizure sensitivity is recapitulated by glial, but not neuronal knockdown of membrin and is progressive with age

Both in humans and in *Drosophila*, the central nervous system (CNS) consists of neuronal cells and glial cells. We proceeded to investigate in which cell type in the *Drosophila* central nervous system membrin plays a role in preventing heat-induced seizures. We expressed the membrin RNAi construct using either an exclusively neuronal or an exclusively glial driver, to induce the downregulation of membrin in each cell type separately. In contrast to ubiquitous downregulation of Membrin using Act-GAL4 as a driver, specific downregulation of Membrin in neuronal cells or glia did result in normal numbers of viable male and female offspring. Neuronal downregulation of Membrin using either the Elav-GAL4 or the nSyb-GAL4 driver did not cause any seizure-like behaviour in response to heat (Figure 1B,C (females), Supplementary Fig 3 (males)). In contrast, we did find an increased sensitivity to heat-induced seizure behaviour using the pan-glial driver Repo-GAL4. (Figure 1D (females), Supplementary Figure 3 (males), video 1-2), remarkably similar to what is observed with the ubiquitous knockdown of membrin using Actin-GAL4. In concordance
with the progressive nature of NS-PME/PMA, we observed that the incidence of seizures induced by heat increased as flies aged. Whereas no seizures were observed at 3 days, 20% of the flies seized at 5 days and when tested at the age of 8 days, approximately 40% of these flies seized within 120 seconds in response to the heat stimulus (Figure 1D). This suggests that membrin expression in glial cells and not in neuronal cells is required to prevent heat-induced seizures during aging.

**Barbital suppresses seizure-like activity associated with glial loss of membrin**

Seizure-like behaviour in Drosophila shares not only electrophysiological but also pharmacological features with human epilepsy, including responsivity to anticonvulsant drugs used in humans. In order to further investigate that the seizure-like behaviour induced by glial downregulation of membrin resembles human epilepsy, we treated these flies with barbital, a GABA-agonist known to potently suppress seizures in humans. Indeed, we observed a potent suppression of seizure-like behaviour of these flies when barbital was added to the food during 8 days prior to seizure induction (Figure 1E). This effect was also observed in flies that were only treated with barbital in the 24 hours prior to exposure to heat (Figure 1F).

**DISCUSSION**

We found heat to be an exacerbating factor of symptoms in a majority of PME/PMA patients. Heat-sensitive epilepsy is observed in febrile seizure syndromes as well as in Dravet syndrome, a syndrome associated with mutations in sodium channel SCN1A. Interestingly, it has been reported that a novel type of PME/PMA associated with neuronal potassium channel KCNC1 features the opposite effect, with improvement of symptoms during fever. These observations suggest the presence of various distinct underlying epileptogenic mechanisms in this group of disorders, while simultaneously underlining the influence of temperature on channelopathies.

Our findings that loss of Membrin specifically in glia but not specifically in neurons leads to seizure-like behaviour in adult flies elaborates further on earlier findings in Drosophila, where changes in neuronal architecture, synaptic composition and electrophysiological seizure sensitivity at the larval neuromuscular junction were observed upon ubiquitous Membrin loss of function. Our study dissects the primarily neuronal from the primarily glial component of the phenotype caused by loss of Membrin. Taken together, this now suggests that the neuronal phenotypes mentioned earlier may be secondary to glial loss of Membrin function.

Glia emerge as important contributors to the pathophysiology of some types of epilepsy, due to the control they exert over the neuronal microenvironment. Derailment of this function leads to disequilibrium of ions and/or neurotransmitters, and as such may promote epilepsy. Whether these mechanisms underlie the neurological features of NS-PME/PMA remains to be investigated, in Drosophila as well as in other models. The Drosophila model reported here may be useful as a platform to identify pathophysiological
intermediates in NS-PME/PMA: these may include the glial partners of Membrin, as well as the crucial glia-neuron interaction involved in the seizure model. In addition, the model may aid the discovery of potential novel therapeutics that may benefit NS-PME/PMA patients.

ACKNOWLEDGMENTS

We want to thank the patients that participated in this study, and without whom this result could not have been achieved. Stocks obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537) were used in this study. Transgenic fly stocks and/or plasmids were obtained from the Vienna Drosophila Resource Center. The work was supported by the Dutch NS-PME/PMA foundation (Stichting Noordzееziekte)
REFERENCES

SUPPLEMENTARY MATERIALS AND METHODS

Patients and patient interviews
From the medical records of 14 patients with NS-PME/PMA (age 4-44 years; male: female 10:4), we retrospectively collected factors influencing symptoms, in particular myoclonic jerks and seizures, both positively and negatively. In addition, we interviewed these 14 patients and/or their caregivers using a semi-structured interview with a focus on factors that influenced NS-PME/PMA symptoms (supplementary information). The interview was based upon factors identified in literature on NSPME/PMA and from our retrospective data collection. All patients carry the same homozygous c.430G>T mutation in the GOSR2 gene. The effects of medication, diet, environmental conditions, internal factors (e.g. stress, anxiety) and intercurrent illness were systematically assessed. All patients consented to participate; the study was performed in accordance with the regulations of the Human Research Ethics Committee and the University Medical Centre Groningen (UMCG) (Review board number UMCG M17.215724, Erasmus MC MEC-2018-1136).
## Supplemental Table 1 | Positive and negative influences on NS-PME/PMA symptomatology as reported by patients

<table>
<thead>
<tr>
<th>Age and gender</th>
<th>GOSR2 mutation</th>
<th>Situational factors</th>
<th>Environmental factors</th>
<th>Internal factors</th>
<th>Medication/ substance</th>
<th>Situational factors</th>
<th>Medication/ substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 24M</td>
<td>Gly144Trp</td>
<td>Before seizure, flanking sleep</td>
<td>Busy environment, lights, noises, blankets, touch</td>
<td>Illness, stress, fatigue, exertion</td>
<td>CBD oil, milk/dairy</td>
<td>Directly after seizure, during fever, relaxation, distraction, well-rested</td>
<td>Valproic acid</td>
</tr>
<tr>
<td>2 25M</td>
<td>Gly144Trp</td>
<td>Before seizure, flanking sleep</td>
<td>Busy environment, lights, showering</td>
<td>Fever, fatigue, emotion</td>
<td>Clonazepam</td>
<td>Relaxation, distraction, well-rested</td>
<td>Levetiracetam, valproic acid, ethosuximide, alcohol</td>
</tr>
<tr>
<td>3 17M</td>
<td>Gly144Trp</td>
<td>Before seizure, flanking sleep</td>
<td>Busy environment, lights, heat, noises, warm meal</td>
<td>Fever, fatigue, exertion, emotion</td>
<td>Milk/dairy, egg, citrus fruits, chocolate, banana, coconut, peanuts</td>
<td>Relaxation</td>
<td>CBD oil, Levetiracetam, Ketogenic diet</td>
</tr>
<tr>
<td>4 4M</td>
<td>Gly144Trp</td>
<td>Waking up</td>
<td>Lights, showering and hot bath, noises, touch</td>
<td>Fever, illness, stress</td>
<td>Relaxation</td>
<td>Valproic acid, Levetiracetam, Ketogenic diet</td>
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</tr>
<tr>
<td>5 7M</td>
<td>Gly144Trp</td>
<td>Waking up</td>
<td>Busy environment, lights, showering and hot bath, noises, touch</td>
<td>Stress</td>
<td>Relaxation</td>
<td>Clonazepam</td>
<td></td>
</tr>
<tr>
<td>6 18M</td>
<td>Gly144Trp</td>
<td>Before seizure</td>
<td>Lights, showering, touch</td>
<td>Fever, fatigue, emotion</td>
<td>Relaxation, well-rested</td>
<td>Levetiracetam, Valproic acid, Ethosuximide, Alcohol, CBD oil, Ketogenic Diet</td>
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<tr>
<td>7 44F</td>
<td>Gly144Trp</td>
<td>Menses</td>
<td>Busy environment, heat, lights, noises</td>
<td>Illness, fever, fatigue, stress, exertion, emotion</td>
<td>Relaxation, during fever lying still</td>
<td>Clonazepam</td>
<td></td>
</tr>
<tr>
<td>8 35F</td>
<td>Gly144Trp</td>
<td>Waking up</td>
<td>Busy environment, heat, warm bath, blankets, noises, touch</td>
<td>Fever, fatigue, stress, excitement</td>
<td>CBD oil</td>
<td>CBD oil</td>
<td></td>
</tr>
<tr>
<td>9 30F</td>
<td>Gly144Trp</td>
<td>Waking up, day after alcohol consumption</td>
<td>Busy environment, heat, lights, noises</td>
<td>Illness, fever, fatigue, emotion, anxiety</td>
<td>Clonazepam</td>
<td>Distraction</td>
<td>Alcohol, cannabis</td>
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<tr>
<td>10 7F</td>
<td>Gly144Trp</td>
<td>Waking up</td>
<td>Heat, lights, noises, touch</td>
<td>Illness, fever, fatigue, stress, anxiety</td>
<td>Distraction</td>
<td>Levetiracetam, Clonazepam</td>
<td>CBD oil</td>
</tr>
<tr>
<td>11 40F</td>
<td>Gly144Trp</td>
<td>Waking up</td>
<td>Busy environment, lights, weather</td>
<td>Illness, fatigue, anxiety, fever</td>
<td>During sleep, relaxation, well-rested, distraction</td>
<td>Clonazepam, CBD oil, Clonazepam</td>
<td></td>
</tr>
<tr>
<td>12 12M</td>
<td>Gly144Trp</td>
<td>Waking up</td>
<td>Busy environment, lights, noises, touch</td>
<td>Illness, fever, fatigue, excitement</td>
<td>Relaxation, well-rested</td>
<td>Clonazepam</td>
<td></td>
</tr>
<tr>
<td>13 31M</td>
<td>Gly144Trp</td>
<td>Oversleeping</td>
<td>Busy environment, heat, cold, lights</td>
<td>Illness, fever, fatigue, stress, anxiety</td>
<td>Relaxation, well-rested</td>
<td>Acetazolamide</td>
<td></td>
</tr>
<tr>
<td>14 31M</td>
<td>Gly144Trp</td>
<td>Oversleeping</td>
<td>Busy environment, heat, cold, lights</td>
<td>Illness, fever, fatigue, stress, anxiety</td>
<td>Relaxation, well-rested</td>
<td>Acetazolamide</td>
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### Supplementary Table 2 | Incidence of factors negatively influencing NS-PME/PMA symptoms

<table>
<thead>
<tr>
<th>Factor</th>
<th>Proportion of patients reporting exacerbation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>12/14 (86%)</td>
</tr>
<tr>
<td>Any exogenous heat</td>
<td>12/14 (86%)</td>
</tr>
<tr>
<td>Environmental heat</td>
<td>7/14 (50%)</td>
</tr>
<tr>
<td>Shower/bath</td>
<td>5/14 (36%)</td>
</tr>
<tr>
<td>Blankets</td>
<td>2/14 (14%)</td>
</tr>
<tr>
<td>Bright/flashing lights</td>
<td>12/14 (86%)</td>
</tr>
<tr>
<td>Busy environment</td>
<td>11/14 (79%)</td>
</tr>
<tr>
<td>Stress/anxiety</td>
<td>10/14 (71%)</td>
</tr>
<tr>
<td>Noise</td>
<td>9/14 (64%)</td>
</tr>
<tr>
<td>Illness</td>
<td>9/14 (64%)</td>
</tr>
</tbody>
</table>

### SUPPLEMENTARY FIGURES

(A) mRNA expression levels of Drosophila membrin, normalized to housekeeping gene rp49 expression levels in 3 day old adult females, ubiquitously expressing UAS-membrin-RNAi (Act-Gal4/UAS-membrin-RNAi), to downregulate membrin in all cells. Act-Gal4/UAS-GFP females were used as age-matched controls.

(B) Western blot depicting Drosophila Membrin protein levels in Act-Gal4/UAS-membrin-RNAi (Membrin downregulated) females and Act-Gal4/UAS-GFP females as controls. α-Tubulin was used as loading control. Data shows mean ± SEM (n=3) and two-tailed unpaired Student’s t-test was used (*p ≤ 0.05)

**Supplementary Figure 1: Validation of the UAS-membrin-RNAi line by qPCR and Western blot**

(A) mRNA expression levels of Drosophila membrin, normalized to housekeeping gene rp49 expression levels in 3 day old adult females, ubiquitously expressing UAS-membrin-RNAi (Act-Gal4/UAS-membrin-RNAi), to downregulate membrin in all cells. Act-Gal4/UAS-GFP females were used as age-matched controls.

(B) Western blot depicting Drosophila Membrin protein levels in Act-Gal4/UAS-membrin-RNAi (Membrin downregulated) females and Act-Gal4/UAS-GFP females as controls. α-Tubulin was used as loading control. Data shows mean ± SEM (n=3) and two-tailed unpaired Student’s t-test was used (*p ≤ 0.05)
Supplementary Figure 2: Act-GAL4/UAS-membrin-RNAi flies show decreased eclosion compared to controls.

(A/B) Crossing scheme to obtain control flies (CyO/UAS-GFP; Act-GAL4/UAS-GFP; CyO/UAS-membrin-RNAi) and flies in which Membrin is downregulated (Act-GAL4/UAS-membrin-RNAi) using the GAL4-UAS binary system. (C) Control adult flies eclose from their pupal cases in expected ratios according to Mendelian inheritance. Act-GAL4/UAS-membrin-RNAi males are lethal and Act-GAL4/UAS-membrin-RNAi females eclose with reduced numbers. Groups were compared using Fisher’s exact test.
A Drosophila model implicates glia in NS-PME

Supplementary Figure 3: Glial, but not neuronal knockdown of Drosophila membrin causes seizure-like behaviour in male flies

(A/B) 8 day old male flies expressing UAS-GFP (control flies) or UAS-membrin-RNAi under the control of neuronal drivers Elav-GAL4 and nSyb-GAL4 (both inducing Membrin downregulation in neuronal cells) were tested for heat-sensitivity. As in females, Membrin downregulation in neuronal cells did not induce seizure-like behaviour in males.

(C) 3, 5 and 8 day old male flies expressing UAS-membrin-RNAi under the control of glial driver RepoGAL4 (inducing Membrin downregulation in glia) were treated as in Figure 1. Comparable to females, downregulation of Membrin in glia induced an age-dependent increase in seizures, while the control flies never seized.

Number in brackets depicts the number of flies. Groups were compared using the Mantel-Cox log-rank test.

SUPPLEMENTARY VIDEO

Supplementary video 1 | Induction of seizure-like behaviour in glial membrin hypomorphs by heat shock (repo>membrin RNAi)

Glial knockdown of membrin leads to prominent heat-induced seizure-like behaviour followed by paralysis in 8-day old flies. A copper chamber heated to 40 °C was used to facilitate video recording.

(The video can be found at http://ees.elsevier.com/nsc/download.aspx?id=1425850&guid=1f2058a0-270f-4482-b177-4cee492b8080&scheme=1)