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Conventional and novel anti-seizure medications reveal a particular role for $GABA_A$ in a North Sea progressive myoclonus Epilepsy *Drosophila* model

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ABSTRACT

Objective: North Sea Progressive Myoclonus Epilepsy (NS-PME) is a rare genetic disorder characterized by ataxia, myoclonus and seizures with a progressive course. Although the cause of NS-PME is known, namely a homozygous mutation in the *GOSR2* gene (c.430 G>T; p. Gly144Trp), sufficient treatment is lacking. Despite combinations of on average 3–5 anti-seizure medications (ASMs), debilitating myoclonus and seizures persist. Here we aimed to gain insight into the most effective anti-convulsive target in NS-PME by evaluating the individual effects of ASMs in a NS-PME Drosophila model.

Method: A previously generated *Drosophila* model for NS-PME was used displaying progressive heat-sensitive seizures. We used this model to test 1. a first-generation ASM (sodium barbital), 2. common ASMs used in NS-PME (clonazepam, valproic acid, levetiracetam, ethosuximide) and 3. a novel third-generation ASM (ganaxolone) with similar mode of action to sodium barbital. Compounds were administered by adding them to the food in a range of concentrations. After 7 days of treatment, the percentage of heat-induced seizures was determined and compared to non-treated but affected controls.

Results: As previously reported in the NS-PME *Drosophila* model, sodium barbital resulted in significant seizure suppression, with increasing effect at higher dosages. Of the commonly prescribed ASMs, clonazepam and ethosuximide resulted in significant seizure suppression, whereas both valproic acid and levetiracetam did not show any changes in seizures. Interestingly, ganaxolone did result in seizure suppression as well.

Conclusion: Of the six drugs tested, three of the four that resulted in seizure suppression (sodium barbital, clonazepam, ganaxolone) are primary known for their direct effect on $GABA_A$ receptors. This suggests that $GABA_A$ could be a potentially important target in the treatment of NS-PME. Consequently, these findings add rationale to the exploration of the clinical effect of ganaxolone in NS-PME and other progressive myoclonus epilepsies.

1. Introduction

Progressive myoclonus epilepsies (PME) are a heterogeneous group of conditions characterized by a combination of progressive myoclonus and epilepsy, sometimes combined with ataxia and cognitive deterioration (Marseille Consensus Group., 1990, Kälviäinen, 2015). One of the more recently discovered PMEs referred to as North Sea-Progressive Myoclonus Epilepsy (NS-PME) has, like most PMEs, an onset in young children. NS-PME is characterized by a typical sequence of symptoms: patients present around the age of 2 years with clumsiness, gait disorders and frequent falls, indicating coordination problems (ataxia). Thereafter, myoclonic jerks appear and subsequently, patients can develop generalized seizures (myoclonic, tonic-clonic seizures and absences) before their second decade (Corbett et al., 2011, Boissé Lomax et al., 2013, Van Egmond et al., 2014, Polet et al., 2020). The course of NS-PME is progressive; the intensity of myoclonic jerks and seizures

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increases and patients experience increasing difficulty with swallowing and speech. Consequently, patients require assistance with all daily activities, severely hampering their independence. NS-PME is caused by a homozygous missense mutation in the *GOSR2* gene (c.430 G > T, p. Gly144Trp) found in all NS-PME patients reported so far (Corbett et al., 2011; Polet et al., 2020; Van Egmond et al., 2014; Boissé Lomax et al., 2013). The *GOSR2* gene encodes a ubiquitous trafficking protein involved in the fusion of vesicles from the endoplasmic reticulum to the cis-Golgi (Lowe et al., 1997).

Treatment of PME is aimed at the suppression of myoclonus and seizures through combinations of anti-seizure medications (ASMs); however, because of the progressive nature of the disorders, myoclonus and seizures are often refractory to ASMs. Besides ASMs, dietary interventions such as ketogenic diet can be considered, which can be a meaningful addition to ASM treatment in some patients (McDonald and Cervenka., 2020, van Egmond et al., 2017). Additionally, three patients with NS-PME have undergone Deep Brain Stimulation (DBS), resulting in a reduction of seizures in all three patients and a reduction of myoclonus in one patient (Anderson et al., 2017). However, ASMs remain the mainstay of treatment in NS-PME; the most commonly prescribed ASMs in NS-PME include clonazepam (100 %), valproic acid (94 %), levetiracetam (65 %) and ethosuximide (24 %) (Polet et al., 2020). Unfortunately, despite combinations of 3–5 different ASMs, debilitating myoclonic jerks and seizures persist in NS-PME. Hence, new treatment options are urgently warranted. [1]

To gain insight into the pathophysiology and explore treatment options for NS-PME, we previously generated a *Drosophila* model with a knockdown of membrin, the GOSR2 ortholog, to recapitulate the phenotype of NS-PME (Lambrechts et al., 2019). Knockdown of membrin specifically in glial cells, and not in neuronal cells, leads to a phenotype with heat-induced seizures. Heat sensitivity is also observed in clinical practice, as patients with NS-PME report factors such as heat, fever and intercurrent illness to worsen their symptoms (Lambrechts et al., 2019). Moreover, seizure incidence increases as the NS-PME *Drosophila* model ages, indicating a progressive phenotype. This demonstrates that the progressive, heat-induced phenotype observed in the *Drosophila* model shows similarities with the symptoms and disease progression seen in patients with NS-PME. This clinically relevant phenotype enables evaluating the effect of drugs on seizures in the NS-PME *Drosophila* model.

ASMs exert their anti-convulsive effect through different modes of action. To gain insight into the most effective anti-convulsive target, the individual effects of different ASMs need to be evaluated. The most commonly used ASMs in NS-PME (clonazepam) belongs to the group of benzodiazepines, which are known for their allosteric effect on $GABA_A$ receptors (Griffin et al., 2013). Valproic acid is the second most common ASMs in NS-PME and has a broad scope of actions; among those, it influences GABA concentrations both through inhibition of GABA degradation and increasing its synthesis. In addition, it blocks voltage-gated ion channels and modulates excitatory neurotransmission (Owens and Nemeroff., 2003, Mishra et al., 2021). Levetiracetam has a unique and novel mechanism with regard to underlying mechanisms of ASM. Its main effect is through binding to the synaptic vesicle protein 2 A (SV2A), thereby affecting presynaptic release of neurotransmitters (Contreras-García et al., 2022). In contrast to many other ASMs, levetiracetam has no direct effect on GABA or sodium channels. Lastly, ethosuximide acts by lowering the threshold of- and blocking T-type calcium channels (Gören and Onat., 2007). Thus, patients with NS-PME receive ASM polytherapy with diverse modes of action, but the individual effects of the ASMs cannot be distinguished.

Interestingly, although previously shown to be effective in the NS-PME *Drosophila* model, the first-generation ASM (sodium barbital) is not part of the current treatment in NS-PME. Sodium barbital is part of the class of barbiturates and one of the oldest ASMs which exert their anti-convulsive effect through stimulation of $GABA_A$ receptors (Löscher and Rogawski., 2012). Nowadays, the clinical use of barbiturates has been reduced due to its narrow therapeutic range and associated adverse effects such as dependence and respiratory depression (López-Muñoz et al., 2005). Recently, however, a third-generation ASM (ganaxolone) has been approved and similarly to barbiturates acts as a positive allosteric modulator of both synaptic and extrasynaptic $GABA_A$ receptors (Zolkowska et al., 2018). Until now, it has only been approved for the treatment of seizures in CDKL5 deficiency (Meng et al., 2023). Moreover, it has been studied in the context of neonatal seizures, as an alternative for phenobarbital (Miller et al., 2022). Ganaxolone acts on the δ-subunit of the $GABA_A$ receptors that differ from the modulatory sites of both benzodiazepines and barbiturates (Meng et al., 2023). Therefore, we hypothesize that ganaxolone may be an alternative to barbiturates in the treatment of NS-PME.

As described previously, combinations of 3–5 ASMs are used in NS-PME, all with different modes of action. Yet, in clinical practice, it is difficult to distinguish the individual effects of the prescribed drugs on hyperexcitability in NS-PME. Clinical trials determining the effect of ASMs are challenging, even more so in rare disorders; hence an animal epilepsy model can provide first clues towards the applicability of specific ASMs. Here, we aimed to determine the individual effects of commonly prescribed ASMs in NS-PME alongside a first- (sodium barbital) and novel third-generation ASM (ganaxolone) in the *Drosophila melanogaster* model. We found that when given as a single ASM, the drugs directly targeting $GABA_A$ are effective in suppressing seizures in the NS-PME *Drosophila* model. This supports that $GABA_A$ could be a potentially important target to treat seizures in NS-PME and specifically, ganaxolone is a novel and recently approved ASM that may prove useful in the treatment of NS-PME.

2. Material and methods

2.1. Drosophila strains and crossings

Flies were maintained at 25 °C on a 12-hour light/dark cycle and raised on Bloomington food (Nutri-Fly™ Bloomington Formulation (Genesee Scientific) with propionic acid and nipagin). We used the NS-PME model with membrin knockdown (UAS-membrin-RNAi) in glial cells (Repo-GAL4) as described previously (Lambrechts et al., 2019), hereafter referred to as the repo>membrin RNAi model. We obtained the Repo-GAL4 stock (#7415) from the Bloomington *Drosophila* Stock Center (BDSC) and the UAS-membrin-RNAi stock (#44534) from the Vienna *Drosophila* Resource Center (VDRC). Male and female offspring were selected on eclosion (day 1) and directly transferred to drug-containing vials on which they aged (see Section 2.2) in same-sex groups until day 8.

2.2. Chemicals and treatment procedure

We used the following ASMs: Sodium Barbital (CAS 144–02–5; Sigma-Aldrich), Levetiracetam (CAS 102767–29–2; LKT Laboratories), Valproic Acid (CAS 1069–66–5; Sigma-Aldrich), Clonazepam (CAS 1622–61–3; Sanbio), Ethosuximide (CAS 77–67–8; Sigma-Aldrich) and Ganaxolone (CAS 38398–32–2; Biosynth- Carbosynth). The ASMs were dissolved in the most optimal solvent (either water, ethanol or DMSO) and used in a range of concentrations. The used concentrations for the experiments were based on how soluble and tolerated the drugs were (lethality) in the fly model. Lethality was defined as ≥ 50 % of dead flies after the treatment period. For an overview of solvents and concentrations used per ASM, see supplementary 1. Per vial, 50 μL of solvent was equally distributed by pipet on top of the sugar-yeast food substrate (approximately 5 ml of food substrate per vial: 26,3 g/L yeast, 54,4 g/L

[1] PME= progressive myoclonus epilepsy; NS-PME= North Sea- Progressive Myoclonus Epilepsy; ASM= anti-seizure medication; SV2A= synaptic vesicle protein 2 A,

sugar, 17 g/L agar, 1 ml of 10 % nipagin). Concentrations are noted as the solution in mM that is pipetted on the food (e.g. 50 µL of 2 mM solution pipetted is referred to as a concentration of 2 mM). Vials were left for two hours before use for the solvents to be absorbed. Flies selected after eclosion were transferred to vials with the drug and aged for 8 days on the drug-containing vials. After 4 days, vials were refreshed to ensure adequate administration of the drug. For all experiments, the negative control was the solvent only.

2.3. Seizure assay

At day 8, flies were transferred to empty vials in groups of 5–10 flies. A heat-induced seizure assay was used, by placing the vials with flies for 120 seconds into a water bath of 40 °C. During the heat shock vials were observed and seizures were recorded in 5 second intervals. Seizures are defined as twitching of the legs and flapping of the wings, sometimes turned on their back, resulting in a phase of paralysis where they lie completely still on their back for the remaining time of heat shock. Only when the phase of paralysis occurred, flies were scored as having a seizure. The observers were blinded to the treatment condition during the heat-induced seizure assay.

2.4. Data analysis and statistics

All data was plotted and analyzed using Graphpad 8.0. Each condition was tested in three independent experiments, after which the data was combined per condition. Firstly, seizure activity determined as percentage of paralysis after 2 min heat shock were plotted as a bar graph and tested for significance with Fisher's exact test in which the seizure suppressing effects of each ASM concentration was separately compared with the negative control (solvent only). Secondly, seizure activity determined as cumulative paralyis during the 2 min heat shock were plotted as a survival curve and significance was tested with the Mantel-Cox log-rank test comparing the seizure suppressing effects of each ASM concentration with its respective negative control (solvent only). All p-values below 0.05 are considered significant and are reported in the results section. No post-hoc analysis/corrections were performed. The number of flies used for the seizure assays is indicated behind the condition in the graphs. Significance is noted in the figures as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$.

3. Results

3.1. Sodium barbital

Sodium barbital, a $GABA_A$ receptor agonist, was previously shown to be effective in the NS-PME *Drosophila* model (Lambrechts et al., 2019). Here, we tested sodium barbital in a range of concentrations from 2 mM up to 200 mM in the NS-PME *Drosophila* model and evaluated the seizure activity in percentages after 120 s. Fig. 1a shows that in females, only the highest concentration of 200 mM resulted in a significant seizure suppression compared to the control (p=0.0046). Fig. 1b shows that males show increasing seizure suppression with increasing concentrations. Both 20 mM and 200 mM sodium barbital resulted in a significant seizure reduction compared to the control (p resp. <0.0001 and <0.0001). We did not observe lethal effects up to a concentration of 200 mM. These data demonstrate that sodium barbital is well tolerated, and dose dependently suppresses seizures in the NS-PME *Drosophila* model, both in males and females.

3.2. Anti-seizure medications with GABAergic mode of action

Other ASMs that primarily act GABAergic and are frequently prescribed in NS-PME are valproic acid and clonazepam (Polet et al., 2020). Valproic acid has a broad scope of actions, where it influences GABA concentrations as well as ion concentrations and excitatory neurotransmission (Owens and Nemeroff., 2003, Mishra et al., 2021). In this experiment, the concentrations ranged from 10 mM to 200 mM (supplementary table 1). No significant improvement in seizure outcome was found for valproic acid in both females and males. In males, we observed lethality from 20 mM and in females from 100 mM onwards with no surviving flies at 200 mM. These data suggest that valproic acid does not effectively suppress seizures in the NS-PME *Drosophila* model.

Similarly, to sodium barbital, clonazepam primarily affects $GABA_A$ receptors. Here we tested clonazepam up to 50 mM; poor solubility prevented dose escalation above 50 mM (supplementary table 1). In both females (Fig. 2c) and males (Fig. 2d), we observed that the higher the concentration, the more seizure suppression is obtained. Significant seizure reduction was seen in both females and males at 50 mM (respectively p=0.0017 and p=0.0245). We did not observe lethality in any of the tested dosages. Thus, clonazepam at the highest dose possible suppresses seizures in the NS-PME *Drosophila* model.

3.3. Anti-seizure medications with other modes of actions

Next, we tested ASMs that do not rely on $GABA_A$ agonism for their effect in the *Drosophila* model. Of this category of ASMs, levetiracetam and ethosuximide are most commonly prescribed in NS-PME (Polet et al., 2020). Levetiracetam has its main effect through binding of the synaptic vesicle protein 2 A (SV2A), thereby affecting the presynaptic release of neurotransmitters (Contreras-García et al., 2022). Here, we tested levetiracetam in concentrations of 2 mM to 2 M (supplementary table 1). Fig. 3a and b demonstrate that in both females and males, none of the levetiracetam concentrations significantly suppressed seizures. Supplementary figures 1a and 1b demonstrate that even higher concentrations of levetiracetam did not lead to seizure suppression; no lethality was observed at the highest concentration of 2 M. These data suggest that levetiracetam is not effective in suppressing seizures in the NS-PME *Drosophila* model.

Ethosuximide acts by lowering the threshold and blocking of the T-

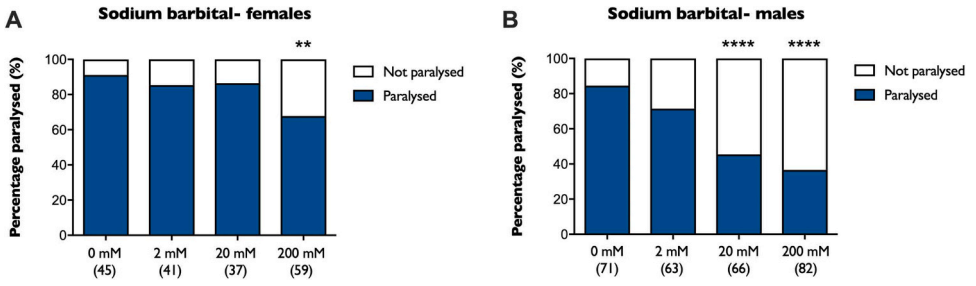

Fig. 1. Percentage of paralyzed flies after 120 s heat-stimulus as a measurement of seizure activity with 8-day exposure of sodium barbital or control only. (a) seizure outcomes of female *Drosophila* repo>membrin RNAi on sodium barbital 2–200 mM (b) seizure outcomes of *Drosophila* male repo>membrin RNAi on sodium barbital 2–200 mM. Significance of seizure suppressing effect between sodium barbital versus negative control is noted in the figures as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$. Numbers between parentheses indicate the numbers of flies tested.

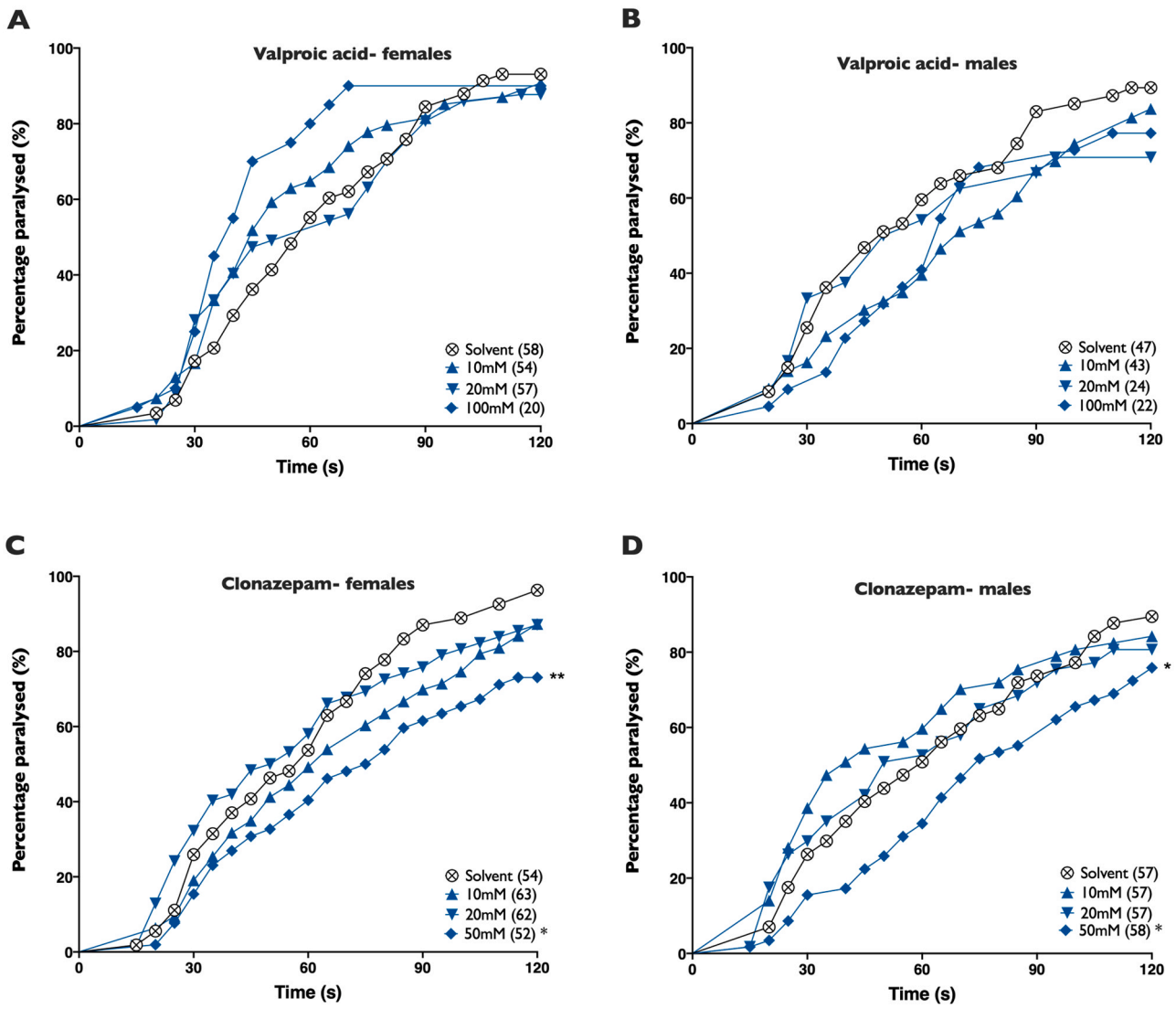

Fig. 2. Time course of cumulative paralysis as a measurement of seizure activity with 8-day exposure of ASMs directly acting on $GABA_A$ (valproic acid and clonazepam). (a) seizure outcomes of female *Drosophila* repo>membrin RNAi on valproic acid 2 mM – 100 mM (b) seizure outcomes of male *Drosophila* repo>membrin RNAi on valproic acid 2 mM – 100 mM (c) seizure outcomes of female *Drosophila* repo>membrin RNAi on clonazepam 10 mM – 50 mM (d) seizure outcomes of male *Drosophila* repo>membrin RNAi on clonazepam 10 mM – 50 mM. Significance of seizure suppressing effect between valproic acid and clonazepam versus negative control are noted in the figures as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$. Numbers between parentheses indicate the numbers of flies tested.

type calcium channels (Gören and Onat., 2007). Here, we tested ethosuximide in concentrations of 2 mM to 200 mM. In females, no significant seizure reduction was observed, (Fig. 3c). In males (Fig. 3d), ethosuximide showed a significant seizure reduction at the highest concentration of 200 mM (p 0.0043). No lethality was observed at any of the concentrations. Thus, in these concentrations, ethosuximide is effective only in males at the highest concentration of treatment.

3.4. Ganaxolone: a novel GABAergic anti-seizure medication

In view of the previously observed effects of sodium barbital on the NS-PME *Drosophila* model, we included a novel ASM with a similar mode of action, namely ganaxolone. Ganaxolone acts as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors (Zolkowska et al., 2018). Ganaxolone was tested at a range of 2 mM to 20 mM; similar to clonazepam it was not possible to dissolve ganaxolone at higher concentrations (supplementary table 1). In both females and males, treatment with ganaxolone resulted in seizure suppression with a significant effect at a concentration of 20 mM for females and 10 mM for males (Fig. 4; respectively p 0.0014 and p 0.0443). No lethality was observed. These data demonstrate that ganaxolone, a novel ASM, can effectively suppress seizures in the NS-PME *Drosophila* model.

4. Discussion

In the North Sea- Progressive Myoclonus Epilepsy (NS-PME) *Drosophila* model several anti-seizure medications were tested. Although four out of six drugs tested are commonly prescribed in NS-PME, only one out of the four (clonazepam) resulted in significant seizure suppression in the NS-PME *Drosophila* model and one (ethosuximide) that showed a mild effect in males. Moreover, both a first-generation ASM (sodium barbital) and third-generation ASM (ganaxolone) resulted in significant seizure suppression. Ganaxolone is a novel ASM currently undergoing investigation for various epilepsy indications and has never been used in NS-PME patients.

The three ASMs (sodium barbital, clonazepam and ganaxolone) that showed significant seizure suppression in the NS-PME *Drosophila* model all act primarily through $GABA_A$. This overlap helps with understanding the underlying basis of the specific disorder. For example, in a different *Drosophila* seizure model, *easily shocked[2]*, phenytoin and gabapentin

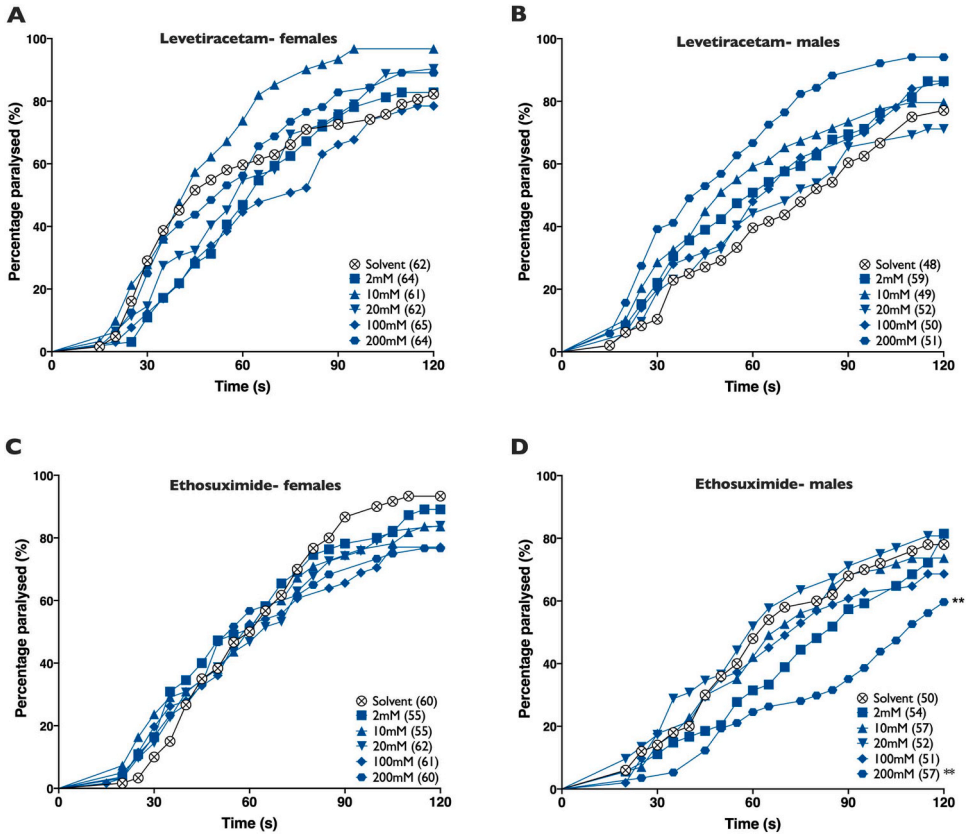

Fig. 3. Time course of cumulative paralysis as a measurement of seizure activity with 8-day exposure of ASMs not primarily acting on $GABA_A$ (levetiracetam or ethosuximide). (a) seizure outcomes of female *Drosophila* repo>membrin RNAi on levetiracetam 2 Mm – 200 Mm (b) seizure outcomes of male *Drosophila* repo>membrin RNAi on levetiracetam 2 mM – 200 mM (c) seizure outcomes of female *Drosophila* repo>membrin RNAi on ethosuximide 2 mM – 200 mM (d) seizure outcomes of male *Drosophila* repo>membrin RNAi on ethosuximide 2 Mm – 200Mm. Significance of seizure suppressing effect between levetiracetam and ethosuximide versus negative control are noted in the figures as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$. Numbers between parentheses indicate the numbers of flies tested.

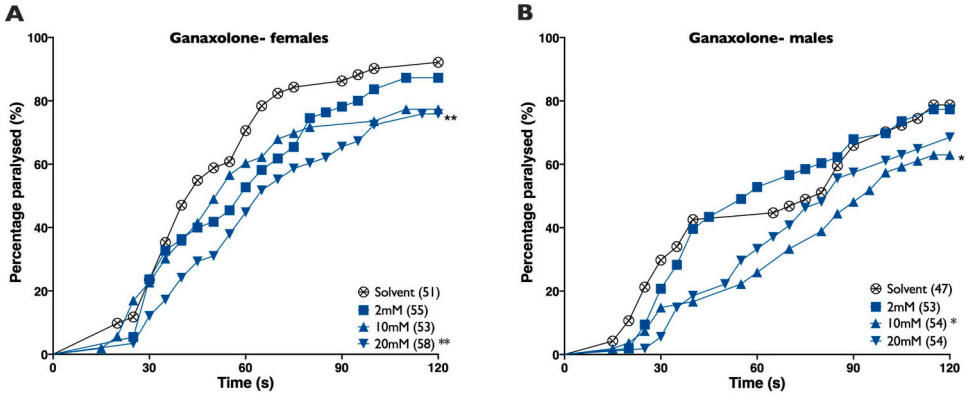

Fig. 4. Time course of cumulative paralysis as a measurement of seizure activity with 8-day exposure of ganaxolone or control only. (a) seizure outcomes of female *Drosophila* repo>membrin RNAi on ganaxolone 2–20 mM (b) seizure outcomes of male *Drosophila* repo>membrin RNAi on ganaxolone 2–20 mM. Significance of ganaxolone versus negative control is noted in the figures as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$. Numbers between parentheses indicate the numbers of flies tested.

were found to suppress seizures (Reynolds et al., 2004). Effects of these two drugs suggest that the *easily shocked[2]* mutants are mainly affected by altered sodium channel kinetics. Here, our results suggest a particular effectiveness of stimulating the $GABA_A$ receptor in seizure suppression in NS-PME.

GABA is the main inhibitory neurotransmitter in both the human and *Drosophila* central nervous system; its effect is mediated through $GABA_A$ and $GABA_B$ receptors (Smart and Stephenson., 2019). $GABA_A$ receptors are ligand-gated chloride channels responsible for inhibitory synaptic neurotransmission (Ghit et al., 2021). One of the oldest $GABA_A$ receptor agonists is the class of barbiturates (e.g. barbital). They are known to enhance synaptic GABA responses but can also influence extrasynaptic $GABA_A$ receptors (Löscher and Rogawski., 2012). Due to severe side-effects, other first-generation ASMs were developed such as ethosuximide and benzodiazepines (e.g. clonazepam) (Rho and White., 2018). The latter acts as $GABA_A$ agonist as well, but only on the synaptic

$GABA_A$ receptor (Griffin et al., 2013, Tan et al., 2011). One of the novel third-generation ASMs acting upon the $GABA_A$ receptor is ganaxolone. Similarly to barbiturates, it works as a positive allosteric modulator of both synaptic and extrasynaptic $GABA_A$ receptors (Zolkowska et al., 2018). Interestingly, both clonazepam and ganaxolone were effective only in their higher dosages and with lesser magnitude than sodium barbital, potentially suggesting a lower efficacy of these ASM. However, neither of these ASM could be tested in equal dosages to sodium barbital due to limitations in solubility. Alternatively, synaptic and extrasynaptic $GABA_A$ -receptors may play different roles in the suppression of the seizure phenotype in *Drosophila*, explaining possible differences between the effect of the GABAergic drugs tested here.

We did not find any effect of valproic acid and levetiracetam in the NS-PME *Drosophila* model. This is especially interesting for valproic acid since its antiepileptic effect also partially depends on GABA-signaling (Owens and Nemeroff., 2003, Mishra et al., 2021). However, the molecular mechanism underlying the anticonvulsive action of valproic acid is complex and most likely multifactorial (Ghodke-Puranik et al., 2013). This may explain why valproic acid is not as effective as other, more strictly GABA-ergic medicaments such as the ones used in this study (barbiturates, benzodiazepines). Interestingly, in a seizure- and ataxia *prickle* mutant *Drosophila* model, feeding valproic acid and levetiracetam actually could improve the seizure phenotype (Tao et al., 2011, Ehaideb et al., 2016). Levetiracetam could also suppress the ataxia phenotype (Ehaideb et al., 2016). The concentrations of valproic acid and levetiracetam used in this study were in the same range of treatment as these previous studies. However, the findings in the *prickle Drosophila* model differ from the findings here in the NS-PME *Drosophila* model, as feeding levetiracetam and valproic acid did not appear effective in the NS-PME model. This makes the response to $GABA_A$ agonists in our NS-PME model even more striking.

Besides ASMs acting primarily through $GABA_A$, ethosuximide also suppressed seizures in males in the NS-PME *Drosophila* model. Ethosuximide lowers the threshold of T-type calcium channels and blocks T-type calcium channels. Low-voltage activated type T (transient) channels play a role in the oscillatory activity of the thalamocortical circuitry, which underlies the spike-wave discharges seen in generalized absence epilepsy. Therefore, ethosuximide prevents the synchronized firing of spike-and-wave discharges (Gören and Onat., 2007). Ethosuximide is used only in a minority of NS-PME patients (Polet et al., 2020); however, since it shows some potency in the *Drosophila* model of NS-PME, it may be considered in the treatment of these patients.

This study is one of the first to evaluate the effect of several anti-seizure medications with various modes of actions, using a *Drosophila* model with a distinct phenotype and associated with a human-epilepsy gene. The NS-PME *Drosophila* model shows a striking phenotypic overlap with features observed in NS-PME patients, including progressive disease course and heat-induced symptoms. We have shown a particular effectiveness of GABAergic compounds in the NS-PME *Drosophila* model, including the novel ASM ganaxolone, which could potentially be used in the treatment of NS-PME. Future studies should be focused on the effectiveness of ganaxolone as well as other ASMs with a similar mode of action for myoclonus and seizure suppression in NS-PME and PMEs in general.

However, there are also some limitations to this study. Where some studies evaluated multiple phenotypes in *Drosophila*, we have only focused on the seizure phenotype because other assays such as climbing assays are confounded by seizures. It is therefore possible that less effective ASMs here do still have an effect on other symptoms such as myoclonus. Nonetheless, with the seizure assay, we were able to characterize in detail the effects of ASMs in the NS-PME *Drosophila* model. Additionally, here we focused solely on treatment starting early in life, whereas in clinical practice certain ASMs are only added later in life. Therefore, administering compounds during different life phases, to determine efficacy of ASMs during the life course, could be of additive value. Moreover, in view of rational polytherapy, it would be interesting to elaborate on testing the synergistic effect of GABAergic compounds together with other ASMs. Rational polytherapy implies that combinations of ASMs with different modes of actions are more effective than polytherapy with similar modes of action (St Louis., 2009).

5. Conclusions

The findings highlighted here give insight into the effect, optimal dosages and toxicity of anti-seizure medications in a *Drosophila* seizure model. Furthermore, it is a starting point for the finetuning of treatment in NS-PME. Interestingly, this study showed significant seizure suppression mainly by compounds acting through $GABA_A$. Together with the fact that ASMs with other modes of action such as levetiracetam and valproic acid did work in other *Drosophila* seizure models, but not in the NS-PME *Drosophila* model, this suggests that $GABA_A$ could be a potentially important target when it comes to treating seizures in NS-PME. Specifically, ganaxolone is a novel and recently approved ASM that has never been applied in PME and could be evaluated for its clinical applicability through a clinical trial for NS-PME.

CRediT authorship contribution statement

Sjoukje Polet: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, visualization, writing- original draft. **Tom J de Koning:** Funding acquisition, Validation, Visualization, Supervision, Writing- Reviewing and Editing. **Roald A. Lambrechts:** Visualization, validation, writing-reviewing and editing. **Marina AJ. Tijssen:** Validation, Visualization, Writing- Reviewing and Editing. **Ody C.M. Sibon:** Resources, Validation, Visualization, Supervision, Writing- Reviewing and Editing. **Jenke A. Gorter:** Conceptualization, Data curation, Planning, Formal analysis, Investigation, Methodology, Funding acquisition, Project administration, Software, Writing- Review & Editing, Supervision

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eplepsyres.2024.107380.

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