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The impact of heatwaves on families of children with Dravet syndrome.

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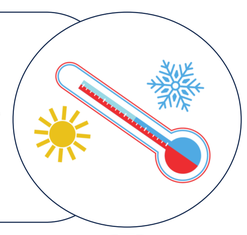
PURPOSE Dravet syndrome (DS) is as a severe early-onset developmental and epileptic encephalopathy. Individuals affected by DS present with refractory seizures, which may be triggered by fever and elevated environmental temperatures.
Our objective is to **delineate the experiences of parents with a child with DS during a heatwave**.

METHODS A **qualitative study** was conducted, with 10 Spanish DS caregivers being enrolled through purposive sampling. Data collection was carried out via in-depth interviews, and a thematic analysis was conducted to extract meaningful insights.

Caregivers:
8 mothers (80%) and 2 fathers
Mean age = 45.1 (± 4.4) years

RESULTS

Individuals with Dravet syndrome (DS) often experience **difficulty in regulating their body temperature**, which can result in seizures.



You go to the beach or the pool, and if you put her in the water straight away, it's a seizure. I can't suddenly put her in the water to cool her down, because if I put her in the water, it's a sure seizure. But if I take more than 5 minutes, it's also a seizure. What do I do now?

Heat and elevated temperature have an impact on **behaviour**, resulting in increased irritability and frustration.

She gets very irritable, very frustrated. (…) I take her out of the house, and she starts pulling at her clothes, like she wants to take everything off.

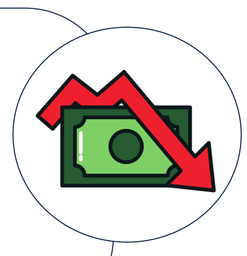
The **entire family is affected**, resulting in the curtailment of daily activities and the limitation of outdoor outings, even to the extent of preventing vacations.

From the start of the heat, sometimes in April or May, she practically doesn't leave the house until September. And we stay with her. In winter, we can live a normal life.

In response to these challenges, parents have to develop and devise **strategies** to mitigate these effects.

I've been thinking and considering the possibility of moving north. Many times, we've thought about moving north, but we can't. Our work is here, and we don't have enough resources to move elsewhere.

The **financial burden** of maintaining a constant air conditioning system increases, and parents encounter difficulties in maintaining employment, even resorting to leaving their jobs to care for their children.



I pay more for electricity than for the mortgage. That says it all. We can't stay in the house without air conditioning, because otherwise, we have to go straight to the hospital.

The **lack of effective therapeutic** options for temperature regulation and the **paucity of guidance** from healthcare professionals contribute to parental distress and frustration.

They told me that each child is different and that against her inability to regulate her temperature, they can't do anything. There is no medication, nothing. Who do you fight against? Who do you blame? Because it's unbearable.

School absences are frequent during the warmer months, and school infrastructure adaptations are often inadequate for the needs of these children.

When it starts to get hot, she practically doesn't go to school. Maybe she goes one day or two, but at the end of May and June, she doesn't go to school anymore.

CONCLUSIONS The impact of heatwaves on parents' experiences and their caregiving strategies for children with DS is significant. There is a pressing need to **raise awareness** about the impact of climate change on the health of individuals with DS and to **facilitate the implementation of measures aimed at ameliorating the effects of heatwaves** on this vulnerable population.

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Ambient temperature, body temperature, and seizures: A pilot study of seven individuals with developmental and epileptic encephalopathies

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Purpose

Climate change, dominated by global warming, is one of the **biggest global health threats of the 21st century[1].**

This is particularly important in some epilepsies where **raised body and/or ambient temperature may provoke seizures.**

To plan for provision of neurological health care with inevitably **rising global temperature**, we documented **ambient temperature, body temperature, and seizure frequency** in people with **developmental and epileptic encephalopathies** (DEEs) living in a long-term care facility in the UK.

Methods

- Prospective, observational study
- From 22nd February – 21st July 2022 at the Chalfont Centre for Epilepsy, Buckinghamshire, UK
- Seven adult residents with monogenic DEEs
  - Two had *SCN1A*-related Dravet syndrome (DS)
  - One each with *ARX, CDKL5, PURA, SPTAN1, and SMARCB1*-related DEE.

"Body temperature"
- Measured 1-3 times per day
- Forehead skin temperature using non-contact infrared thermometry

Bedroom temperature
- Measured at 5-15 minute intervals

Outside temperature
- Measured at 15 minute intervals

Seizure frequency
- Recorded daily by care staff
- "Seizure-days" = 24-hour periods when at least one seizure was recorded.

Definitions:

Heatwave temperature threshold[2] – a regionally defined elevated temperature threshold used to define a heatwave. 28°C for Buckinghamshire, UK.

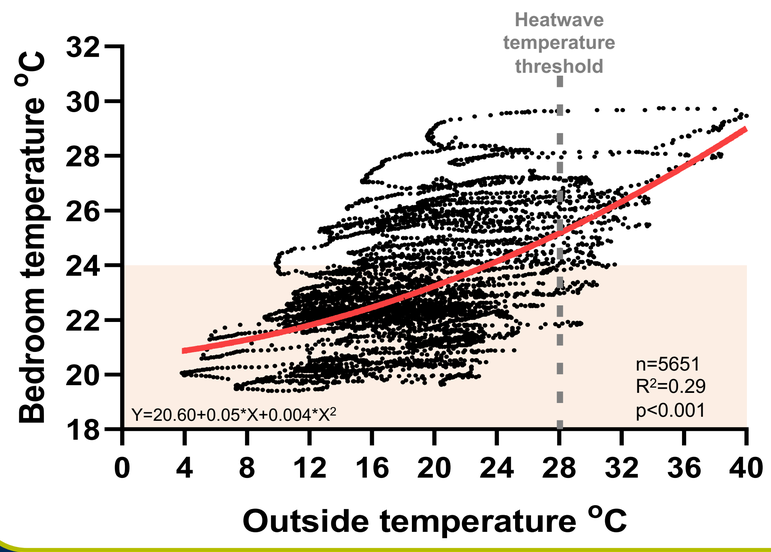
Heatwave[2] - at least three consecutive days with temperatures equal to or greater than the heatwave temperature threshold.

Thermal comfort range[3]: A room temperature range of 18-24°C which is recommended by the World Health Organization (WHO) as posing "minimal risk to the health of sedentary people".

Results

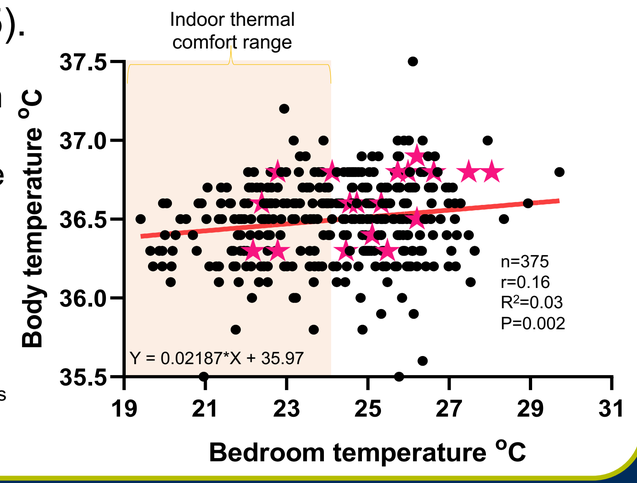
There was a significant relationship between **outside temperature and resident bedroom temperature,** ($p<0.001$, quadratic regression).

An example from a resident's bedroom is shown

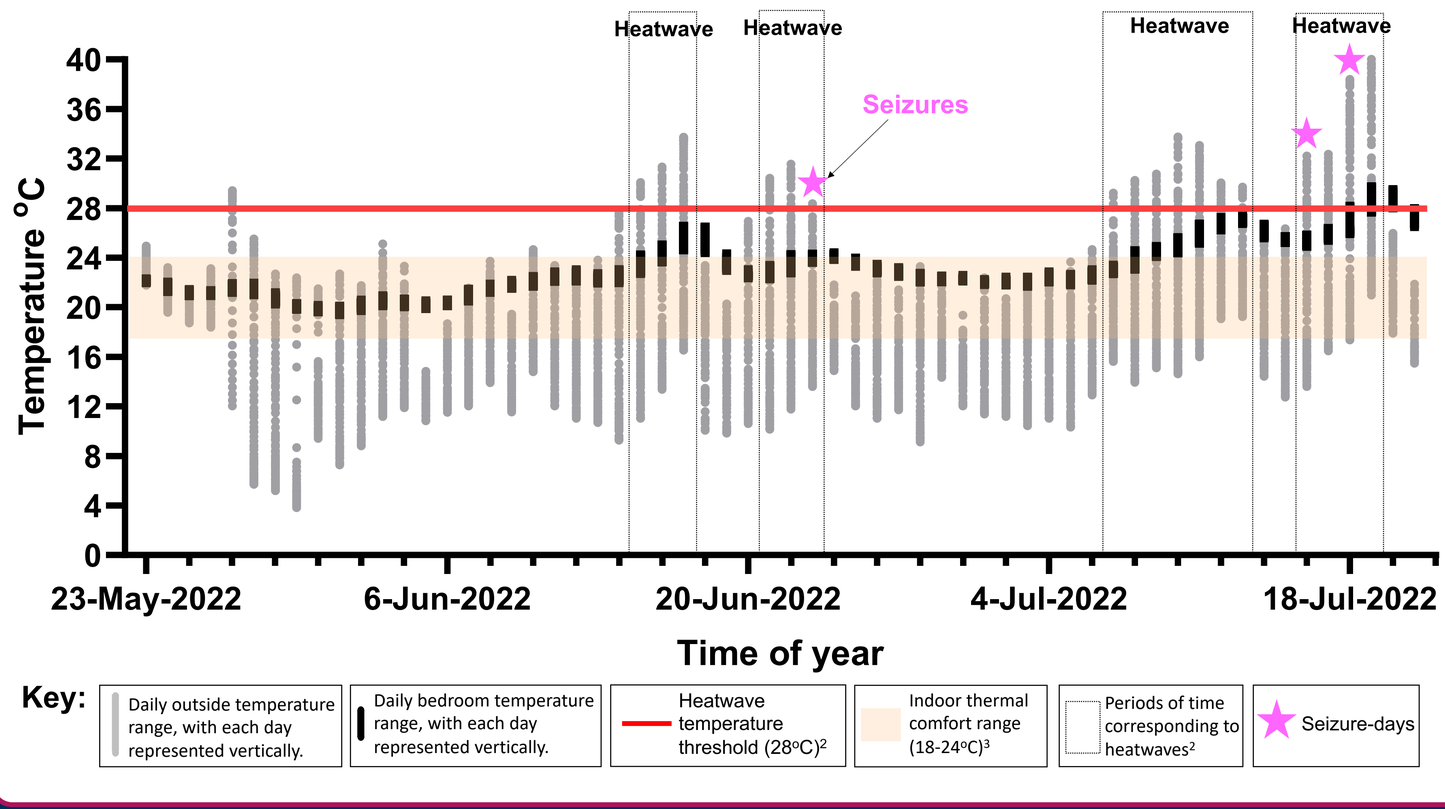


There was a significant **positive correlation between bedroom temperature and "body temperature"** for individuals with Dravet syndrome and *SMARCB1*-related DEE (Spearman correlation coefficient: r=0.16; r=0.22; r=0.31. p<0.05).

An example from a resident with Dravet syndrome is shown:



Outside temperature and bedroom temperature of a resident with Dravet Syndrome recorded between 23rd May - 21st July 2022



Outside temperature and seizures

One resident (with Dravet syndrome) had significantly more seizure-days when outside temperatures ≥28°C (Fisher's exact test; p=0.02).

Body temperature and seizures

One resident (with Dravet syndrome) had a significantly higher median body temperature on seizure-days compared to non-seizure days (Mann-Whitney U test; p=0.003).

Conclusions

- These pilot data highlight how climate change, in particular global warming, may impact seizure frequency in some epilepsies.
- The mechanisms by which raised ambient temperatures may provoke seizures is unknown.
- We need to plan now for the inevitable rise in global temperatures caused by climate change.
- For those who care for people with epilepsy, this includes gaining a better understanding of the relationship between ambient temperature, body temperature, and seizure risk.

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Body temperature variance in relation to spontaneous seizures in the intra-amygdala kainic acid model of drug-resistant temporal lobe epilepsy in mice

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Introduction

The climate change emergency has renewed interest in the relationship between temperature and epilepsy. The relationship between body temperature and seizure risk is well established in preclinical research. For example, hyperthermia induces seizures in immature rodent pups and in mice deficient in Scn1a. Elevated body temperature has also been reported to exacerbate seizures in excitotoxin models and there is histologic and imaging evidence of neuropathology in the hypothalamus, which contains the coordinating centre for temperature control, in some models. The intra-amygdala microinjection of kainic acid (IAKA) into mice is a well-characterised and clinically-relevant model for studying the mechanisms and treatment of drug-resistant temporal lobe epilepsy. Here we explored the relationship between temperature and the epilepsy phenotype in the model.

Materials & Methods

Induction of Status epilepticus (SE):

Male C57BL/6J mice (26-30 g) underwent surgery where a telemetry device (DSI) was implanted under the skin to allow continuous EEG recording. Furthermore, a guide cannula (coordinates from bregma: A/P= -0.95mm; L= -2.85mm) was placed above the amygdala and fixed in place with dental cement (Fig. 1A).
SE was induced by intra-amygdala microinjection of kainic acid. All animals received lorazepam (8 mg/kg: i.p.) when they showed a clear sign of SE.

The neuronal damage in the ipsilateral hippocampal CA3 region is one of the main characteristics of the intra-amygdala (i.a.) kainic acid (KA) model (Fig. 1B).

Fig. 1. Intra-amygdala kainic acid model: A Surgery schematic. B Neuronal damage in the ipsilateral CA3

Detection of SRS in i.a KA model:

Two weeks after SE, all mice were displaying regular spontaneous recurrent seizures (SRS). Brain and blood samples were collected on day 15 (Fig. 2A). Digitized EEG recordings and SRS counts were analyzed offline (Fig. 2B).

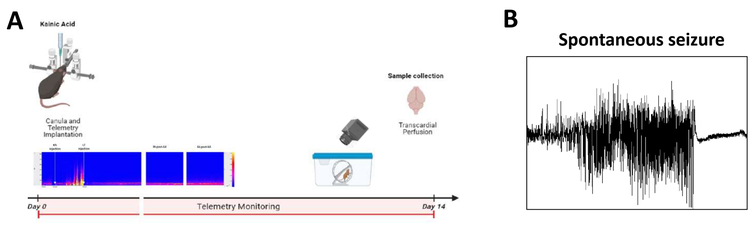


Fig. 2: Experimental design: A Implanted telemetry device for continuous EEG recording. B Example of Spontaneous recurrent seizure (SRS)

Results

1. Temporal changes and differences in SRS burden

The epilepsy phenotype progressed during monitoring, with a significant increase in SRS number during the second week compared to the first week post-KA (Fig 3B). The number of seizures exhibited per individual mouse was measured and graphed with respect to days (Fig. 3A) and weeks (Fig. 3B) post-KA.

The number of seizures that took place during 6pm – 6am versus 6am – 6pm (Fig. 3C) was also compared. As mice are nocturnal animals, the active period was considered from 6pm – 6am and non-active from 6am – 6pm. No difference was found in the number of seizures recorded during the active compared to inactive period of the day. Immunostaining on hypothalamus brain region in KA show a neuron damage also (Fig. 3D).

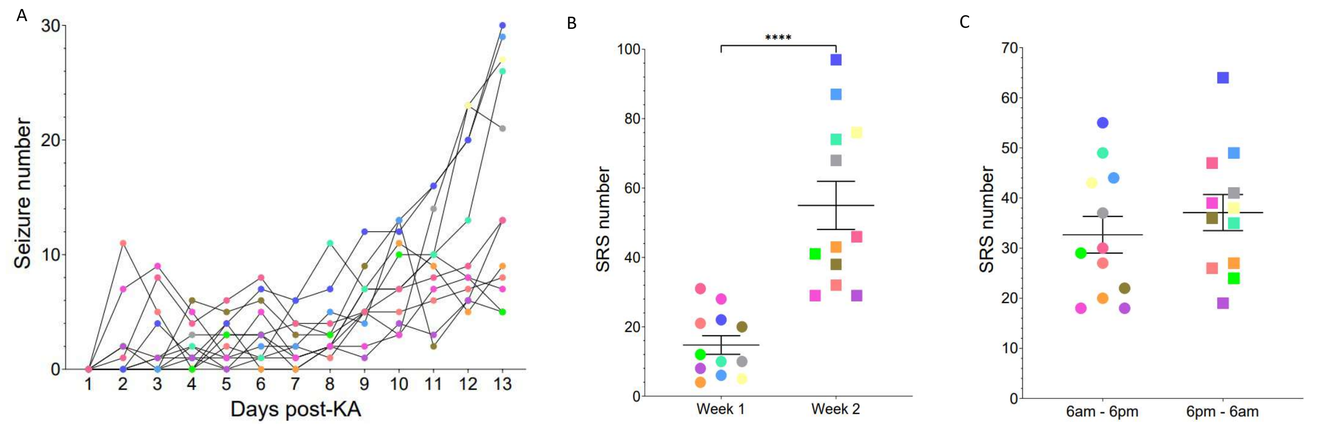
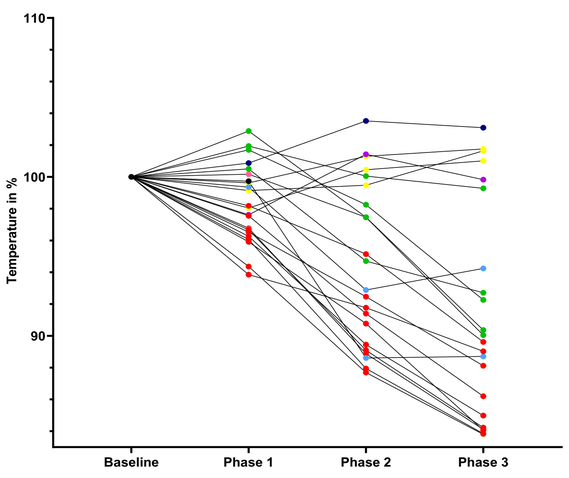


Fig. 3. Progression of SRS frequency over days (A) and weeks (B), comparison of active (6pm – 6am) versus inactive (6am – 6pm) period of the day (C). Different colours represent data from different mice.

Results

2. Body temperature changes progressing during Status Epilepticus (SE)

We observed a consistent pattern of transient body temperature decrease during status epilepticus, prior to the administration of lorazepam. This confirms that the temperature drop is not related to the mice's movements during seizures



P values (t-test):

- Baseline vs. Phase 1: p = 0.0048
- Baseline vs. Phase 2: p < 0.0001
- Baseline vs. Phase 3: p < 0.0001
- Phase 1: 1st 20 min after KA
- Phase 2: 2nd 20 min after KA
- Phase 1: 1st 20 min after LZ

Fig. 4. Progression of body temperature drop during status epilepticus across three phases. The graph shows a significant transient decrease in body temperature, with statistical analysis revealing the following p-values: Phase 1 (p=0.004), Phase 2 (p=0.001), and Phase 3 (p=0.001). These temperature drops occurred prior to the administration of lorazepam and are independent of any movement-related factors, as confirmed by the observed pattern

3. Relationship between body temperature and SRS burden and duration

All seizure events occurred within a body temperature range of 33°C to 38°C. We observed a significant inverse correlation between body temperature and SRS duration, with lower temperatures being associated with longer seizure durations, and higher temperatures correlating with shorter durations. This suggests that seizure-terminating mechanisms may be activated more quickly or efficiently at higher body temperatures.

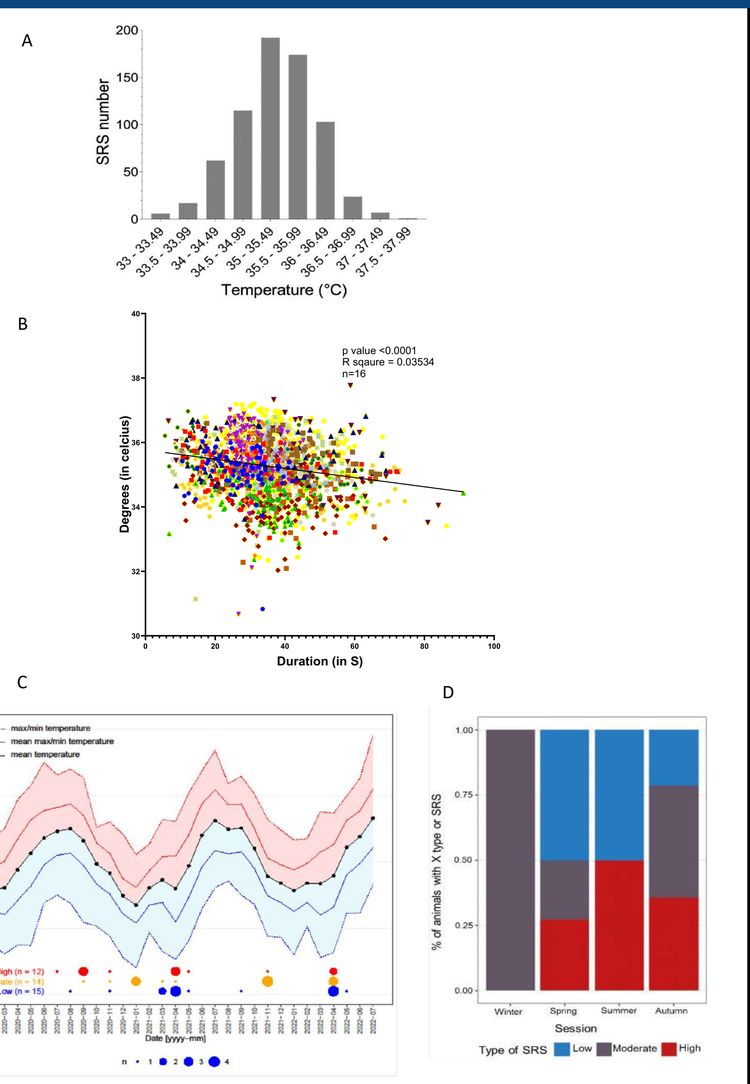


Fig. 5. Distribution of Seizure Events Based on Ictal Body Temperature and Correlation Between SRS Duration and Ictal Body Temperature: All SRS events occurred within a body temperature range of 30.5°C to 37.5°C, with a slightly left-skewed normal distribution (A). The relationship between seizure duration and body temperature revealed a significant inverse correlation (p=0.0001), with Pearson's correlation coefficient (R) = 0.03. N=16. Different colours represent data from individual mice (B). (C, D) Seasonal variability in seizure severity throughout the year is shown, with seizure frequency categorized as low (1-3 seizures per day), moderate (3-12 seizures per day), and high (above 12 seizures per day, associated with SUDEP).

4. Body temperature Changes During Pre-ictal, Intra-ictal, and Post-ictal Periods:

We observed a consistent pattern of transient body temperature increase, peaking during the seizure event and gradually returning to the pre-ictal baseline afterward. This suggests that body temperature, possibly combined with recently identified plasma-based markers[1,2], could be a useful predictor of seizure occurrence.

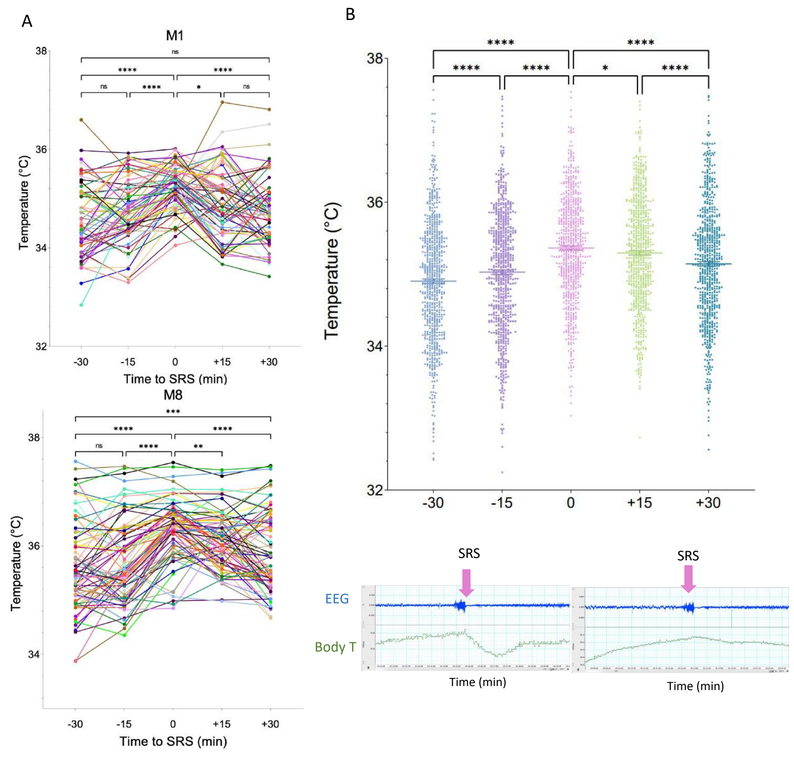


Fig. 6. Body Temperature Changes Before, During, and After Seizure Events: (A) Representative linear plots illustrate temperature changes as mice progress from 30-15 minutes before seizure onset, through the seizure event itself, and 15-30 minutes post seizure. (B) The overall pattern shows a peak in body temperature during the seizure, followed by a gradual return to near-baseline levels after the seizure resolves. Statistical analysis using repeated-measures ANOVA revealed significant differences with p*<0.05, p<0.01, p***<0.001, and p****<0.0001. N=12**

Conclusion

- **Body Temperature as a Predictive Biomarker**: Body temperature rises during seizure episodes and gradually returns to baseline in the postictal phase. This suggests that fluctuations in body temperature could serve as a potential predictive biomarker for seizure activity.
- **Inverse Correlation Between SRS Duration and Body Temperature**: There is a significant negative correlation between SRS duration and body temperature, with longer seizures associated with lower temperatures.
- **Seizure Frequency and Daily Rhythms**: In this model, mice exhibit a progressive increase in seizure frequency over time; however, no significant differences were observed in SRS rates between active and inactive periods of the day.
- **Impact of Seasonal Kainic Acid Induction**: The season in which the kainic acid model is induced may influence the severity of the subsequent SRS burden.
- These findings enhance our understanding of this model and open avenues for future research and potential therapeutic applications

Future plans

- Investigate effect of changing external temperature on SRS burden and severity
- Assess if similar trends translate to TLE patients

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Concordance between increased brain temperature and loss of hypothalamic neurons in a mouse model of acquired epilepsy

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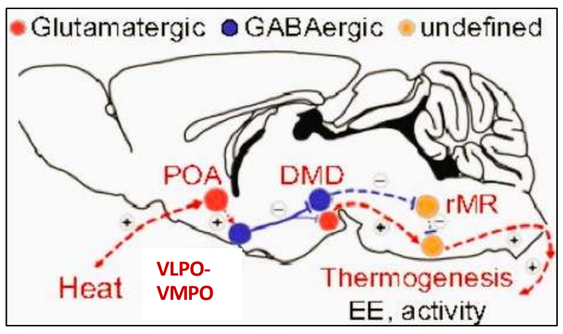
Poster # 500

Background

- Climate changes and heat waves may impact seizures and comorbidities in people with epilepsy (PWE)[1]. Elevated ambient temperature (T) may act as a precipitant of seizures in PWE but the underlying mechanisms remain unclear.
- **We hypothesize** that neuronal cell loss occurs in key hypothalamic thermoregulatory nuclei (ventromedial preoptic nucleus, *VMPO;* dorsal part of dorsomedial nucleus, *DMD*[2]) in epilepsy, leading to impaired thermoregulation. This reduces the ability of PWE to adapt their core (and brain) T to a thermal challenge with effects on seizure threshold.



The schematic drawing describes the model by *Zhao et al, 2017[1]: the activation of the VMPO-VLPO-DMD neuronal pathways reduces core T in response to a thermal challenge.* *Solid line* represents connection verified by *Zhao et al; dashed lines* represents proposed connections based on other reports.
(+) activation; (–) inhibition.

Objective

Using a mouse model of acquired epilepsy, we studied whether:

- Hypothalamic T-sensing areas such as *VMPO* and *DMD* undergo neurodegeneration in epileptic mice.
- Changes in brain T occur during epileptogenesis.

Methods

- Epilepsy was induced in adult male C57Bl/6N mice by intra-amygdala kainate injection triggering status epilepticus (SE)[3]. Sham mice were injected with saline.

- Spontaneous seizures were ECoG monitored (24/7).
- Neuronal loss in the hypothalamus was quantified by Nissl staining[3], and volume changes were assessed by MRI[4] .
- Hippocampal T was measured by proton Magnetic Resonance Spectroscopy (^{1}H MRS)-based brain thermometry (7T MRI) during epileptogenesis[5].

Results

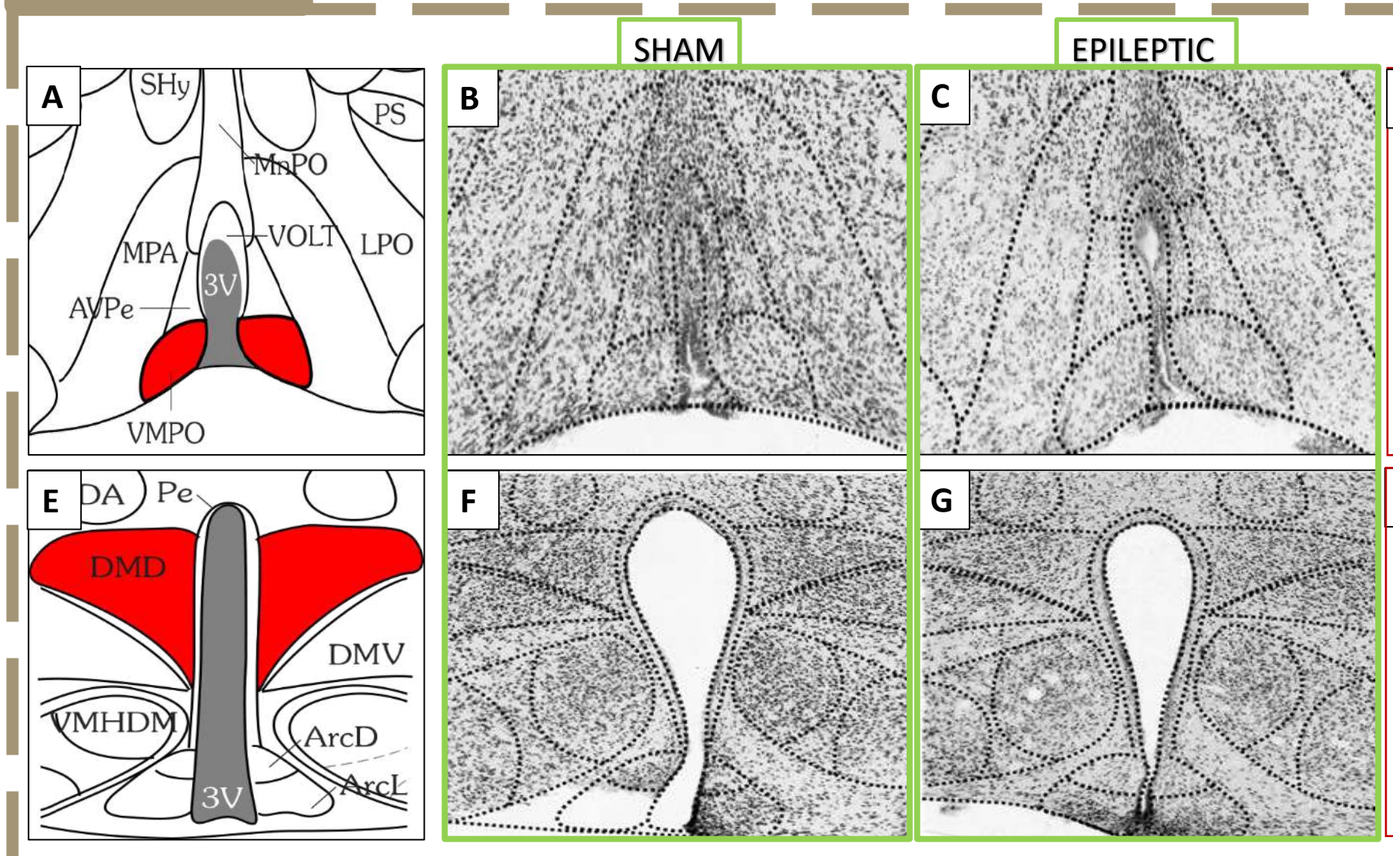
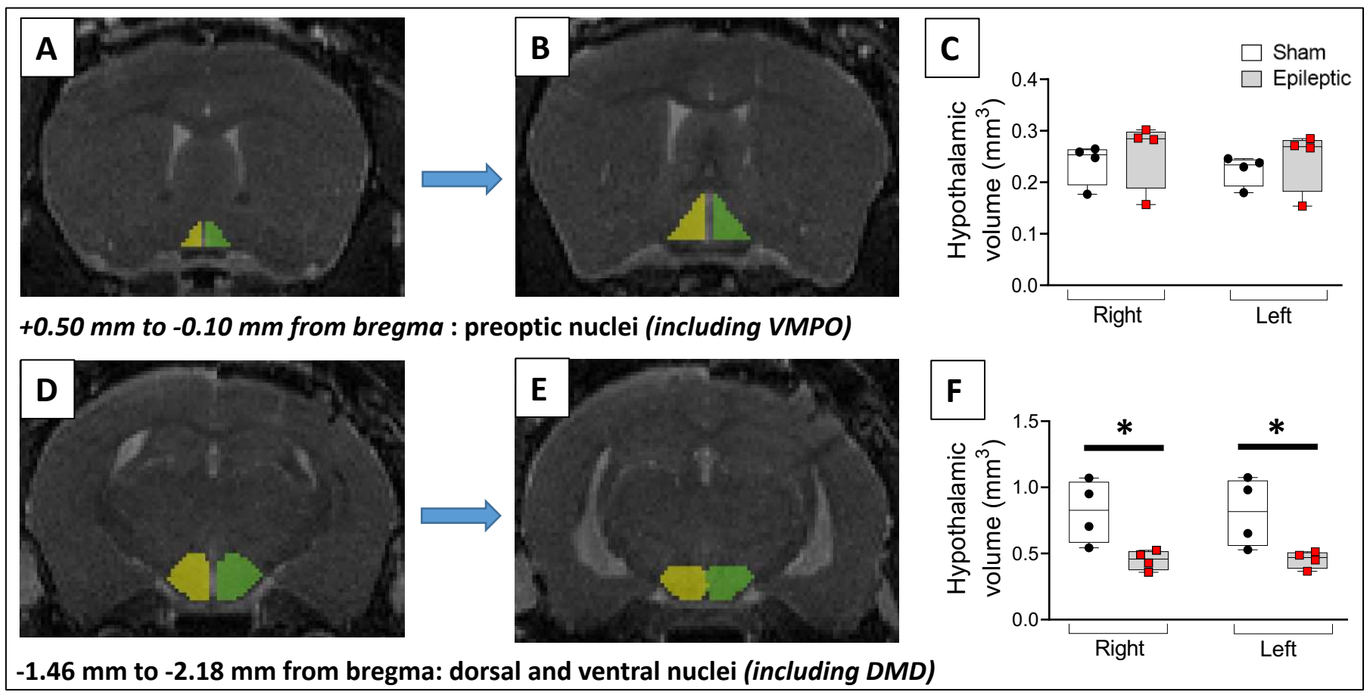


Figure 1. Neuronal loss in *VMPO* and *DMD nuclei in epileptic mice*

Panels *A,E* depict the location of *VMPO* and *DMD* in hypothalamic slices (*+0.38* and *-1.82 mm from bregma,* respectively). **Panels *B,C (VMPO)* and *F,G (DMD)*** display representative Nissl-stained slices from sham and epileptic mice. **Panels *D (VMPO) and H (DMD)*** show neuronal cell density in the hemisphere ipsi- (R,right) and contralateral (L,left) to kainate-injected amygdala.

Data show a *bilateral* neuronal loss in both hypothalamic nuclei: *15.8* ± 0.4% decrease in VMPO and 18.2 ± 0.8% decrease in DMD in epileptic vs sham mice (n=9-10/group; *p<0.05;**p<0.01 vs sham by *unpaired t-test).*



+0.50 mm to -0.10 mm from bregma* : preoptic nuclei *(including VMPO)

-1.46 mm to -2.18 mm from bregma: dorsal and ventral nuclei *(including DMD)*

Figure 2. Quantification of hypothalamic volume by 7T MRI.

A 3D rapid acquisition with relaxation enhancement (RARE) T2-weighted sequence was performed to assess anatomic changes. Brain region volume was quantified by selecting manually the region of interest (ROI*; highlighted by yellow and green colors*).

Panels A,B and panels ***D,E*** depict the levels of hypothalamic sections analyzed in epileptic and sham mice (n=4/group). ***Panels C,F*** show the respective quantification of the volume.

Data show a reduction in the volume of the hypothalamic region encompassing *DMD* in epileptic vs sham mice (F; p<0.05 *by two-tailed t-test*)**. *MRI analysis was not sensitive enough to detect cell density changes in VMPO (C).***

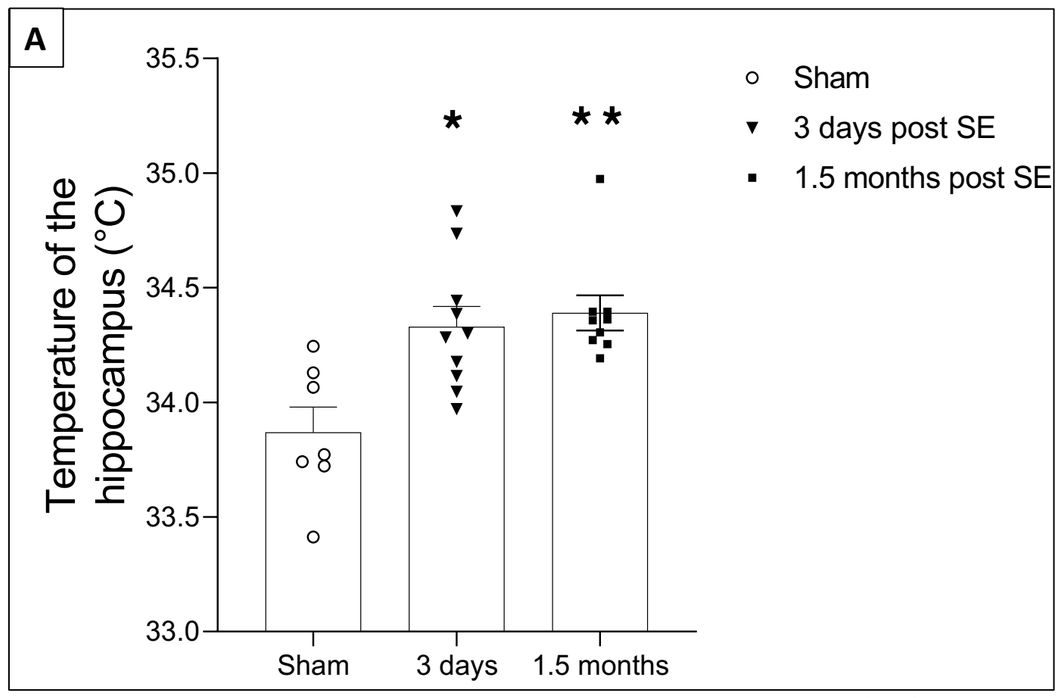


Figure 3. Hippocampal brain temperature measurement by ^{1}H-MRS during epilepsy development.

Panel A depicts the rise in hippocampal T in SE mice before epilepsy onset (*3 days*: 34.3 ± 0.3°C, n=10) and during chronic epilepsy (*1.5 months*: 34.4 ± 0.2°C, n=9) vs sham mice (33.9 ± 0.3°C, n=7). *p<0.05, **p<0.01vs sham by Kruskall-Wallis followed by Dunn's test.

Data show that hippocampal T increases during epileptogenesis and this increase persists after the clinical onset of epilepsy.

Conclusions

Neuronal loss occurs in hypothalamic thermoregulatory nuclei in epileptic mice and is associated with an increased hippocampal temperature during epileptogenesis. We propose that cell loss impairs the thermoregulatory function of the VMPO-DMD pathways. This impairment, if occurring in PWE, may disrupt temperature homeostasis during thermal challenges thus promoting seizure precipitation. MRI analysis of hypothalamic nuclei may help to identify changes which predict patient's susceptibility to ambient T challenges.

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Effect of High-Temperature on Absence Epilepsy Seizures in Rats

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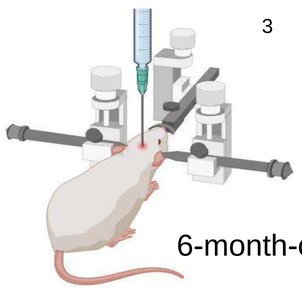
INTRODUCTION

Most epilepsies are likely to be aggravated by climate change, especially heatwaves.[1,2] Absence epilepsy seizures could be affected during extreme weather events like **heatwaves** regarding the possible effects of heat on the brain physiology, and emotional stress occurring during and after those events.

This study's aim is to examine the effects of acute high-temperature exposure on both anxiety levels and characteristics of Spike-and-Wave-Discharges (SWDs) on electroencephalography (EEG) recordings.

METHOD



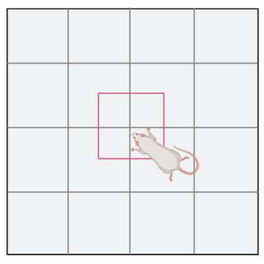
3

Implantation of EEG electrodes to Genetic Absence Epilepsy Rats from Strasbourg (GAERS) with stereotaxic surgery (Stoelting Model 51600, Stoelting Co., Illinois, USA)

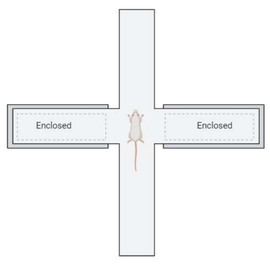
6-month-old male rats (n=5)

Basal measurements for anxiety and SWDs_Room-temperature

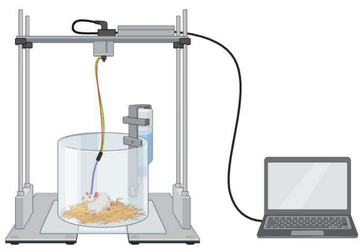
GAERS underwent testing for anxiety-like behaviors in locomotor activity (LMA) (AMS 9701, Commat Ltd., Ankara, Turkey) and elevated plus maze (EPM) tests.



LMA Test
(5 minutes)
(09.00-10.00)



EPM Test
(5 minutes)
(09.00-10.00)



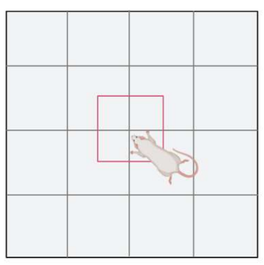
Basal EEG recording (PowerLab 8S System running Chart v.7, ADI, Oxfordshire, U.K.) (10.00-13.00)

Body temperature measurement with rectal thermometer (Brannan 290512, Cumbria, U.K.)

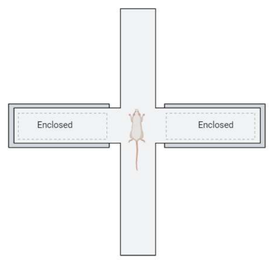
Measurements for anxiety and SWDs_High-temperature

Day 1

High-temperature exposure with infrared-heater (1 hour)

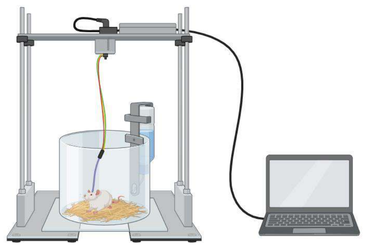


LMA Test
(5 minutes)
(09.00-10.00)



EPM Test
(5 minutes)
(09.00-10.00)

Day 2



EEG recording (10.00-13.00)

Body temperature measurement with rectal thermometer

- 10.00-11.00: basal EEG recording
- 11.00-12.00: infrared-heater on
- 12.00-13.00: infrared-heater off

Analysis

One-way ANOVA test for SWDs' characteristics
Unpaired t-test for findings of behavioral tests
GraphPad Prism 10.1.1(270)

Significance level: $p<0.05$

RESULTS

- Mean body temperature (37.8°C) measured in room temperature (20-25°C), increased to 39.0°C after high-temperature exposure (34-35°C).
- No significant change was found in LMA test results following acute high-temperature exposure compared to room-temperature (Figure 1). In the EPM, time spent in open-field decreased , whereas time spent in closed-field increased following high-temperature exposure (Figure 2).
- Number and cumulative duration of SWDs significantly changed following acute high-temperature exposure compared to basal EEG recordings (Figure 3).

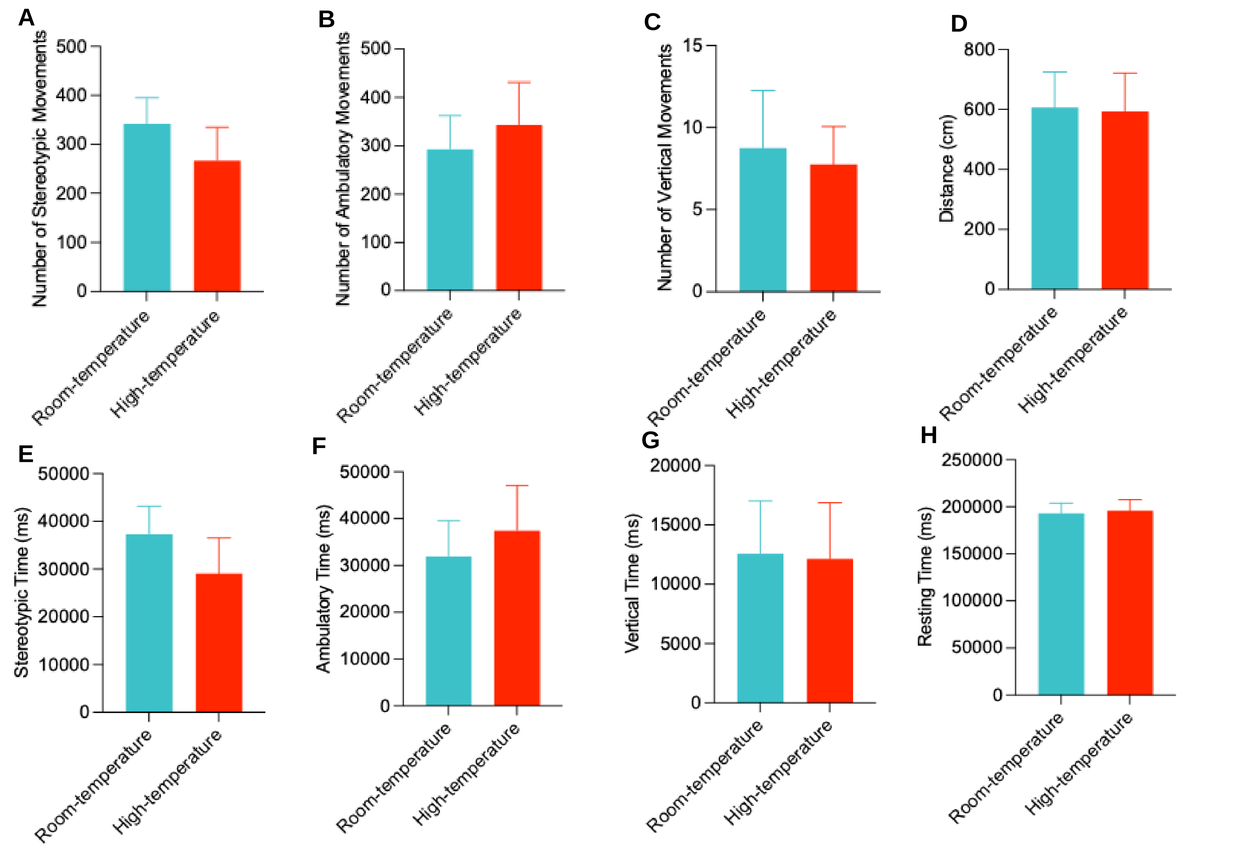


Figure 1: LMA test results for GAERS during room-temperature vs high-temperature; **(A)** Number of stereotypic movements; **(B)** Number of ambulatory movements; **(C)** Number of vertical movements; **(D)** Distance travelled; **(E)** Time spent during stereotypic movement; **(F)** Time spent during ambulatory movement; **(G)** Time spent during vertical movement; **(H)** Time spent during resting.

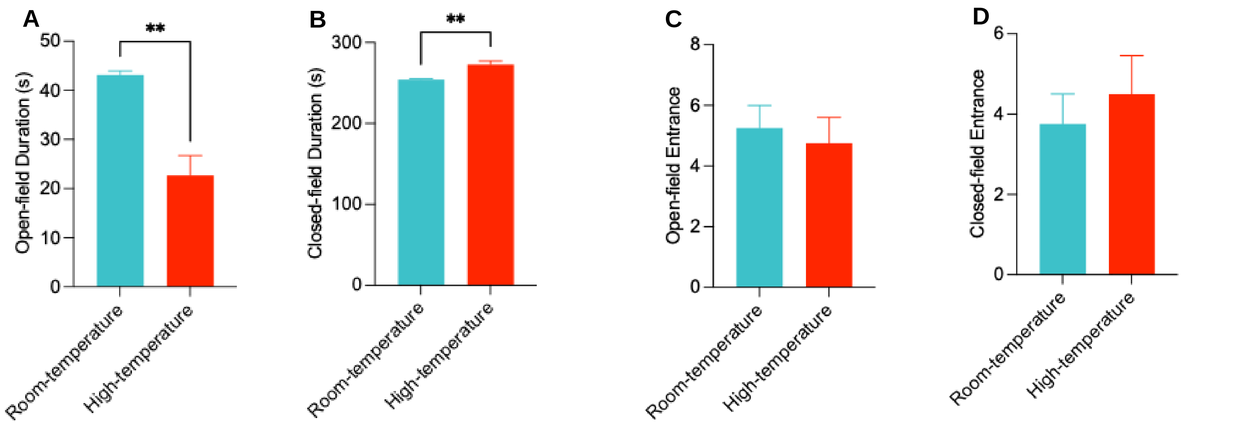


Figure 2:. **(A)** Time spent in open-field; **(B)** Time spent in closed-field; **(C)** Number of entrances to the open-field; **(D)** Number of entrances to the closed-field.

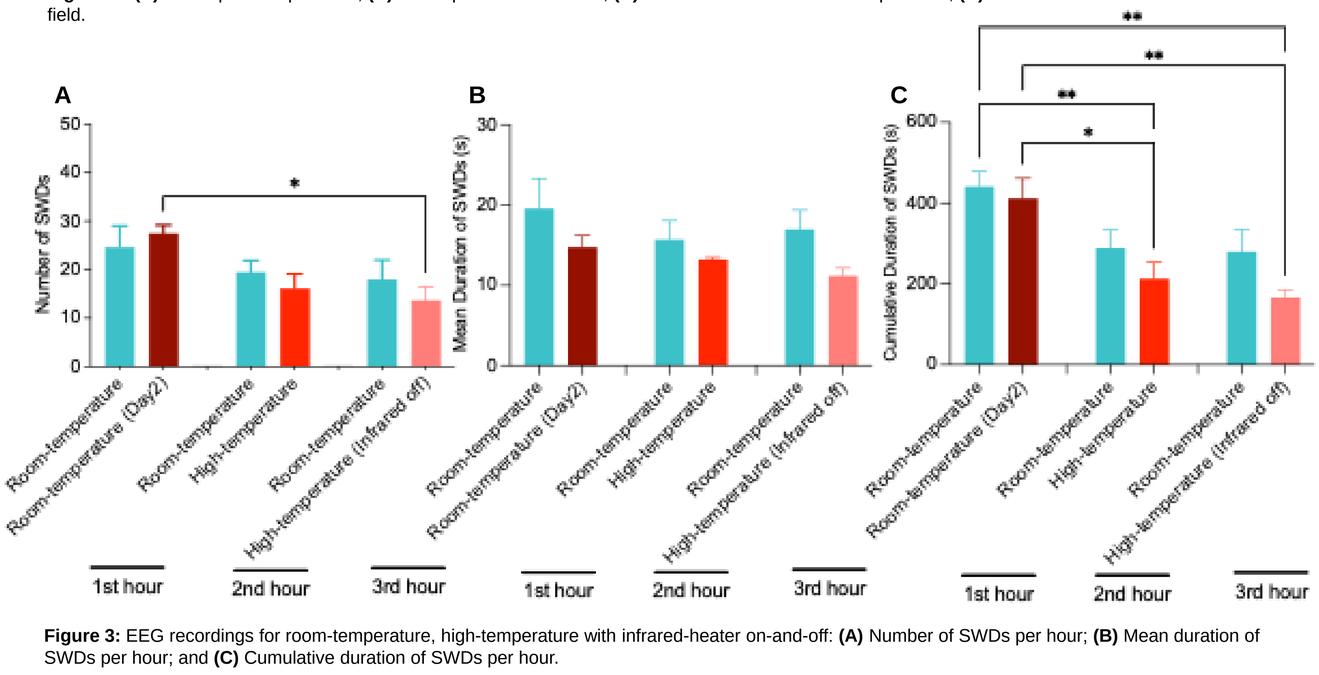


Figure 3: EEG recordings for room-temperature, high-temperature with infrared-heater on-and-off: **(A)** Number of SWDs per hour; **(B)** Mean duration of SWDs per hour; and **(C)** Cumulative duration of SWDs per hour.

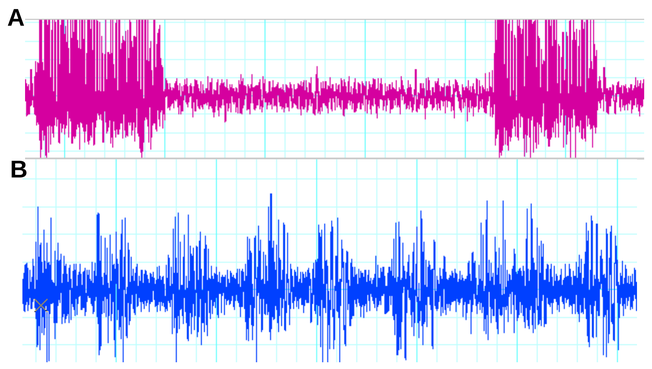


Figure 4: One minute section from **(A)** basal EEG recording of rat; and **(B)** EEG recording prior to its death.

Heat Stress Symptoms in One of the Rats

- One of the rats started to show heat stress symptoms after exposed to high-temperature for 30 minutes on Day 1 before undergoing for behavioral tests. Therefore, infrared-heater was turned off immediately and EEG recording of that rat was done (Figure 4). In 25 minutes, the number of SWDs reached 127 (baseline, 65 SWDs/1 hour) and the rat sadly died.

CONCLUSION

Our preliminary results show that absence seizures may be affected from high-temperature and vulnerability to high-temperature and response to heat stress may differ between absence epilepsy rats. Further studies with higher number of animals investigating SWDs with spectral analysis and oxidative stress markers in the brain may provide important information for the sensitivity of this genetic epilepsy model to high-temperature.

References:

[1]Gulcebi MI, Bartolini E, Lee O, et al. Climate change and epilepsy: Insights from clinical and basic science studies. *Epilepsy Behav*. 2021;116:107791. doi:10.1016/j.yebeh.2021.107791

[2]Sisodiya SM, Gulcebi MI, Fortunato F, et al. Climate change and disorders of the nervous system. *Lancet Neurol*. 2024;23(6):636-648. doi:10.1016/S1474-4422(24)00087-5

[3]All illustrations are created with BioRender.com

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15th European Epilepsy Congress
7-11 September 2024 | Rome, Italy

The changing climate, heat stress and the brain: implications for epilepsy

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Background

- Climate change is leading to chronic global warming and increased frequency of extreme weather events[1].
- Climate change's impact on human health is substantial and cannot be overlooked[2,3].
- For individuals with epilepsy, a warming world may directly precipitate seizures and worsen related triggers, such as fevers, stress, and sleep deprivation[4].

Aims

1. Identify genetic variants and genes associated with temperature-sensitive disease phenotypes or protein stability/function.
2. Identify diseases with increased vulnerability to rising global temperatures and extreme weather events.
3. Elucidate the molecular response of astrocytes to elevated temperatures.

Methods

i. Literature search to identify genetic variants linked to temperature sensitive disease phenotypes or protein stability/function.

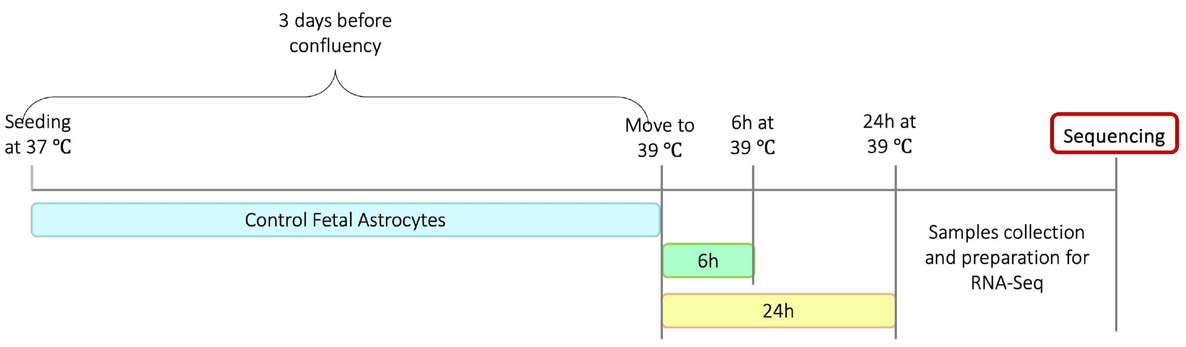

Figure 1: Primary foetal astrocytes were grown at elevated temperatures and then sent for RNA-sequencing (RNA-Seq). Control cultures were grown at 37°C for 24 hours, while exposure cultures were grown at 39°C for 6 hours and 39°C for 24 hours.

Conclusions

- Several sodium channels can harbour variants that make them sensitive to temperature changes.
- Elevated temperature induces significant transcriptomic changes in astrocytes.
- Neurological diseases including epilepsy may be more vulnerable to elevated temperatures.
- Further investigation is needed to understand how temperature changes can impact brain molecular networks.

References

1. Stott P. *CLIMATE CHANGE. How climate change affects extreme weather events.* Science. 2016 Jun 24;352(6293):1517-8. doi: 10.1126/science.aaf7271. Epub 2016 Jun 23. PMID: 27339968.
2. Romanello M et al. *The 2023 report of the Lancet Countdown on health and climate change: the imperative for a health-centred response in a world facing irreversible harms*. Lancet. 2023 Dec 16;402(10419):2346-2394. doi: 10.1016/S0140-6736(23)01859-7. Epub 2023 Nov 14. PMID: 37977174.
3. Sisodiya SM et al. *Climate change and disorders of the nervous system.* Lancet Neurol. 2024 Jun;23(6):636-648. doi: 10.1016/S1474-4422(24)00087-5. PMID: 38760101.
4. Gulcebi MI et al. *Climate change and epilepsy: Insights from clinical and basic science studies*. Epilepsy Behav. 2021 Mar;116:107791. doi: 10.1016/j.yebeh.2021.107791. Epub 2021 Feb 10. PMID: 33578223; PMCID: PMC9386889.

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Results

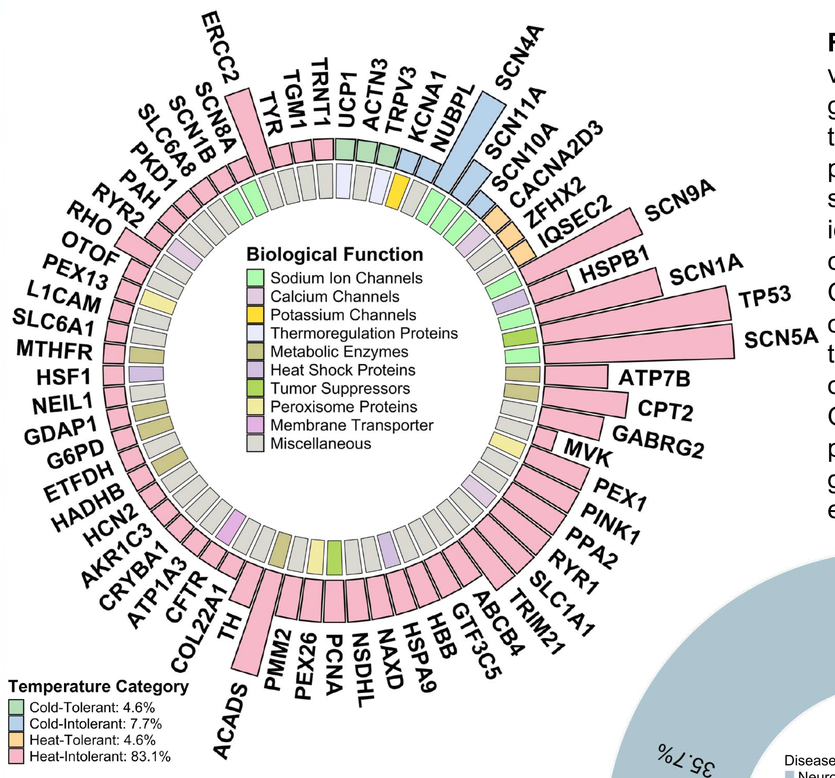

Figure 2: 141 genetic variants distributed across 65 genes associated with temperature-sensitive disease phenotypes or protein stability/function were identified. The genes were classified as Cold-Intolerant, Cold-Tolerant, Heat-Tolerant, or Heat-Intolerant based on the functional characterisation of the variants in each gene. Outer bar length is proportional to the number of genetic variants identified in each gene.

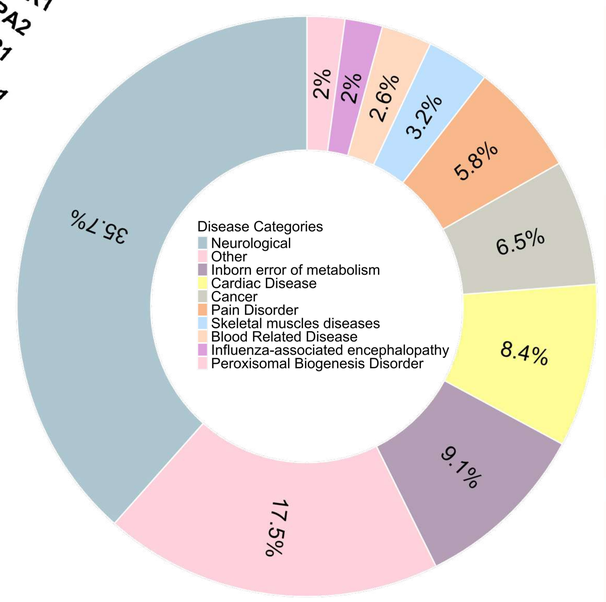

Figure 3: Disease categories associated with the identified genetic variants.

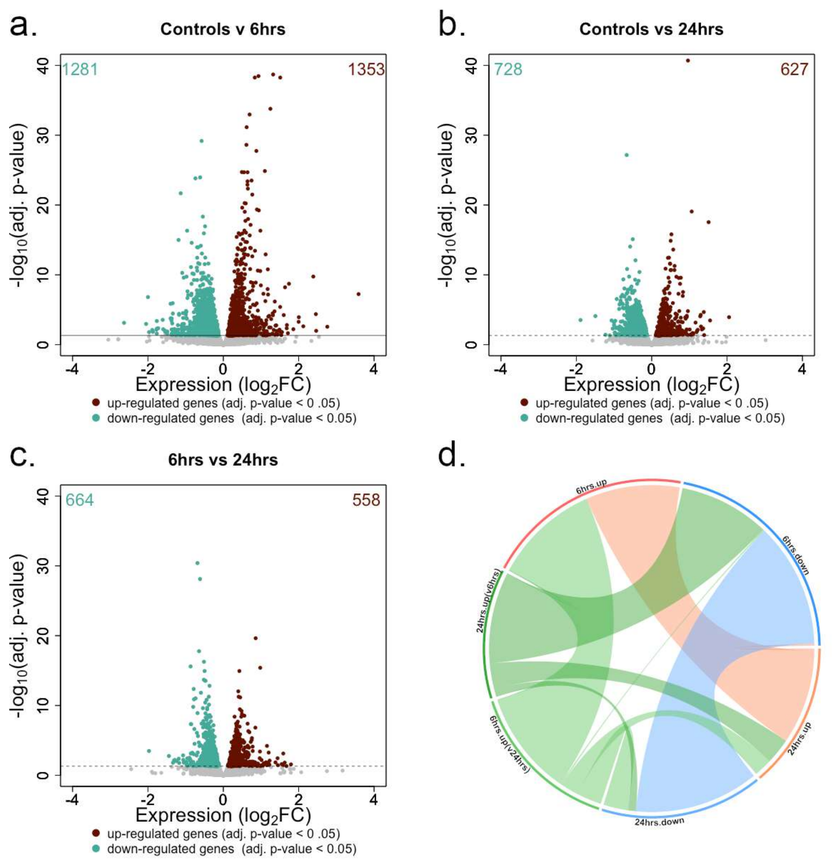

Figure 4: RNA-seq analysis of astrocytes exposed to elevated temperatures (39°C for 6 and 24 hrs).
(a-c) Volcano plots showing differentially expressed genes.
(d) Plot highlighting shared differentially expressed genes between comparisons.

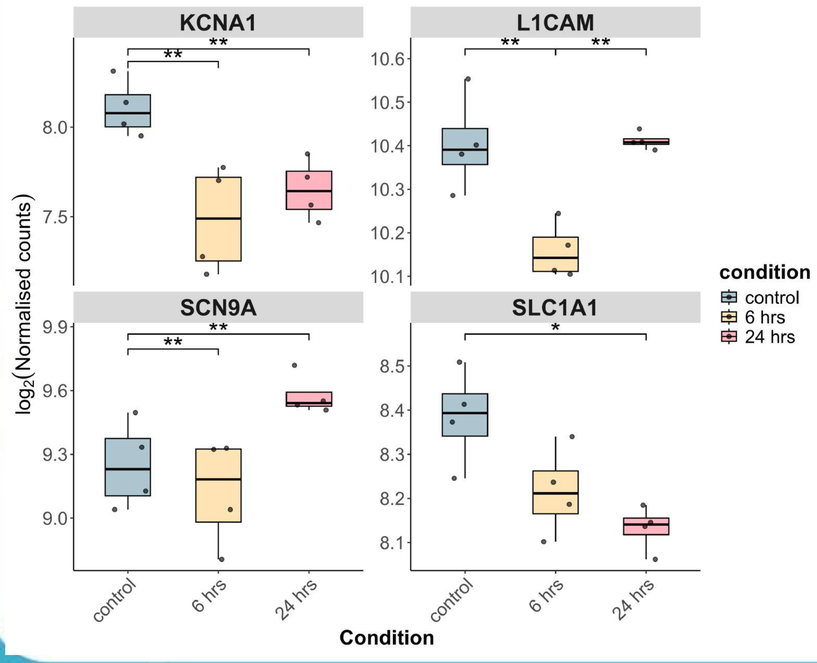

Figure 5: Genes with temperature-related genetic variants linked to seizure phenotypes that were differentially expressed in astrocytes after exposure to elevated temperatures.

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HIGH TEMPERATURE AFFECTS mRNA EXPRESSION OF HEPATIC METABOLISM ENZYMES: AN *IN VITRO* STUDY WITH CARBAMAZEPINE

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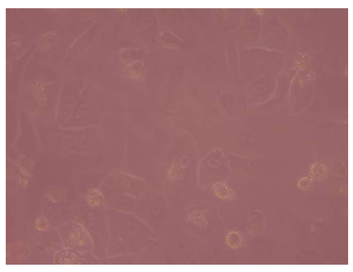
INTRODUCTION

- Seizure control in people with epilepsy could be adversely affected during extreme weather events due to potential effects of extreme temperatures on pharmacokinetics of antiseizure medications (ASMs), for example through altered hepatic metabolism of ASMs.[1]
- Carbamazepine is a well-known inducer of CYP3A, CYP2C and UGT enzymes that leads to significant drug-drug interactions.[2]
- This *in vitro* study aimed to examine the effects of high temperature on mRNA expressions of hepatic cytochrome-P450 (CYP) metabolism enzymes exposed to carbamazepine.

METHODS



Light microscope images of HepG2 cells (200X).

- HepG2 cells were seeded in 6-well plates (50.000 cells per well) and carbamazepine (10µg/mL/42.32 µM), was added to three of the wells (Figure 1).
- mRNA expressions of CYP enyzmes were examined in groups exposed to 37°C or 40°C temperature in an incubator (NUAIRE Us Autoflow CO_2 Water-Jacketed Incubator NU-4750) for 3 hours or 24 hours in two distinct experiments conducted in a triple design.
- mRNA isolation was performed from cells and the obtained mRNA were translated into cDNA (First Strand Kit™, Qiagen) and real-time PCR was performed (Rotorgene Real-Time PCR Detection System, Qiagen).
- Fold changes in the mRNA levels of CYP3A4, CYP2C9 and CYP2C19 enzymes were calculated by RT^2 method.
- Differences in the mRNA expression of genes coding CYP enzymes were calculated for each group by normalizing with GAPDH gene.
- Groups were compared by one-way ANOVA and post-hoc Tukey test (GraphPad Prism 8.4.2). The threshold for significance was set at $p<0.05$.

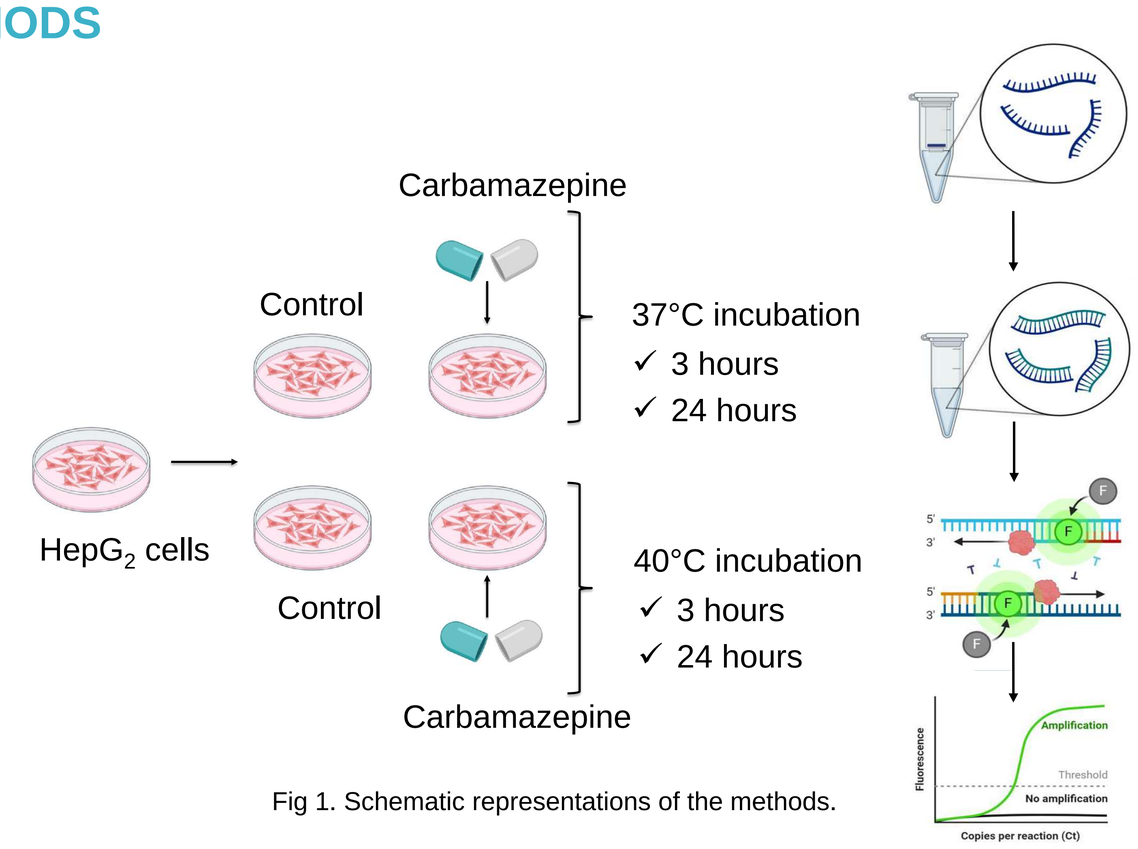


Fig 1. Schematic representations of the methods.

RESULTS

- CYP3A4 mRNA expression on exposure to 40°C temperature for 3 hours increased significantly compared to the groups exposed to 37°C temperature for 3 hours and exposed to 37°C for 24 hours ($p=0.0003$*** and $p=0.0281$* respectively, Figure 2A). CYP3A4 mRNA expression on exposure to 40°C temperature for 24 hours increased significantly compared to the group exposed to 37°C temperature for 3 hours ($p=0.0196$).
- CYP2C9 mRNA expression on exposure to 40°C temperature for 3 hours increased significantly compared to the group exposed to 37°C temperature for 3 hours ($p=0.0244$) whereas this increase did not last for 24 hours($p=0.0310$, Figure 2B).
- No significant change was detected for CYP2C19 mRNA expression on exposure to 37°C or 40°C temperatures for 3 or 24 hours (Figure 2C).

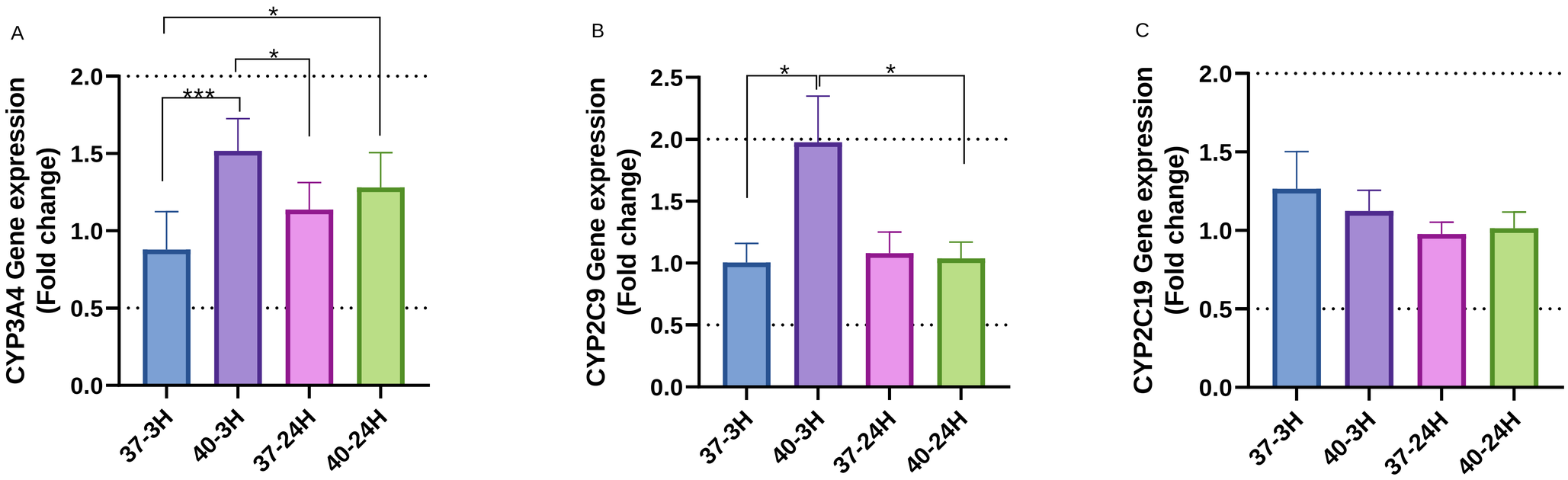


Fig 2. Gene expression (Fold change) for A. CYP3A4; B. CYP2C9; C. CYP2C19

CONCLUSIONS

- These preliminary findings show that acute and chronic exposure to high temperature may trigger carbamazepine-induced drug-drug interactions by increasing mRNA expression of CYP enzymes (especially CYP3A4).
- Further studies are urgently needed to explore the effect of high temperature on drug concentrations and their clinical importance for epileptic patients.

References:

1. Gulcebi MI, Bartolini E, Lee O, Lisgaras CP, Onat F, Mifsud J, Striano P, Vezzani A, Hildebrand MS, Jimenez-Jimenez D, Junck L, Lewis-Smith D, Scheffer IE, Thijs RD, Zuberi SM, Blenkinsop S, Fowler HJ, Foley A; Epilepsy Climate Change Consortium; Sisodiya SM. Climate change and epilepsy: Insights from clinical and basic science studies. Epilepsy Behav. 2021 Mar;116:107791. doi: 10.1016/j.yebeh.2021.107791. Epub 2021 Feb 10. PMID: 33578223; PMCID: PMC9386889.
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