Altered Neural Dynamics and Sleep Patterns in Epilepsy and the Role of Potassium Buffering

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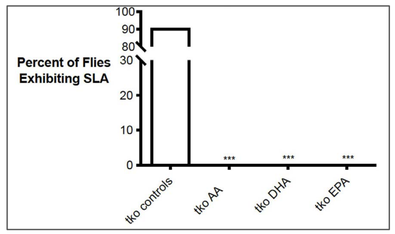
Altered Neural Dynamics in Epilepsy and the Potential of Omega Fatty Acids as a Treatment

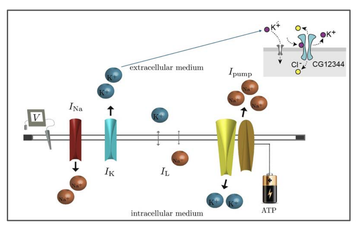
- Epilepsy is a neurological disease characterized by periods of abnormal brain activity presenting as seizures or reduced awareness
- Increased excitatory transmission, reduced inhibitory transmission, and mutations to ion channels and ion uptake can all contribute to a brain state that is prone to seizure (Banerjee and Jirsa, 2024), however there is a not a uniform event that has been characterized as being involved in all forms of epilepsy or seizure, rendering it still not completely understood
- Omega-3 and omega-6 fatty acid supplementation in diet shown to eliminate seizure-like activity in bang-sensitive mutant technical-knockout *Drosophila* after mechanical stimulation (para^{bss} flies exhibit sodium-channel mutations resulting in hyperexcitability)

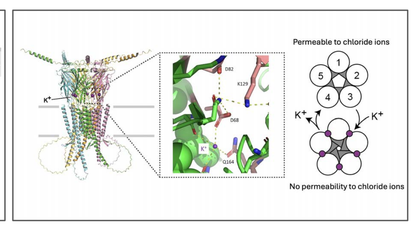


(Kuebler P., Hutson A.N., Tabuchi M., & Kuebler D.)

pH and Potassium Ion-Activated Chloride Channel Discovered in *Drosophila* Neurons and Glia

- CG12344 gene-encoded Alka channel (alkaliphile) discovered in *Drosophila* gustatory receptor neurons (GRNs)
  - High pH-gated chloride channel involved in aversive taste responses to basic food (Mi et al., 2023)
  - Initially thought to only exist in GRNs, further research elucidated its presence in the brain (both neurons and glia)



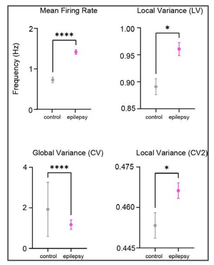


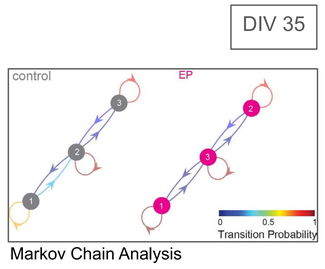
(Tabuchi, M., 2024).

- How could this channel relate to epileptogenesis?
  - Astrocytes mediate potassium ion buildup in the extracellular space, preventing hyperexcitability
    - Hyperexcitability uncouples astrocytes by alkalization of their interiors, reducing their ability to clear excess potassium, leading to excessive hyperexcitability (Onodera et al., 2021)
    - The expression of the Alka channel in neuronal glia and its role in ion movement and clearance may similarly affect excitability of neurons and induce changes that can lead to seizure activity
- Our initial interest in this channel will explore how it affects sleep dynamics, also allowing us to compare its effects to those seen in epilepsy
  - Sleep and epilepsy bidirectionally affect one another
    - Epilepsy demonstrated to be related with an increase in sleep onset latency, wake time after sleep onset, and instability of sleep stage transitions
    - Sleep deprivation shown to be related with increased seizure activity and epileptiform discharges (Lanigar and Bandyopadhyay, 2017)

Epilepsy and Healthy Patient-derived iPSCs Demonstrate Changes to Firing Patterns and Network Stability

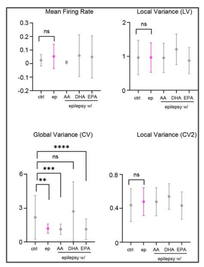
- Analysis comparing healthy- and epilepsy-derived differentiated neuron cultures reveals increased excitatory firing, increased local irregularity in spiking patterns, and changes to stable neural network states and state transitions



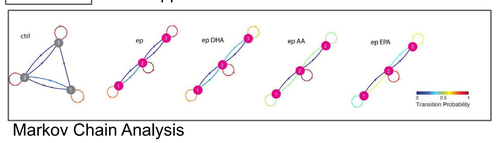


Markov Chain Analysis

Omega-3 and Omega-6 Fatty Acid Result Observed in *Drosophila* Fails to Carry Over to iPSC Model

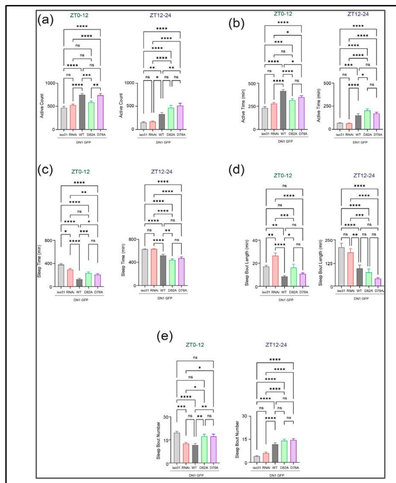


- DIV 55 does not demonstrate the same trends seen previously, and therefore fails to provide an adequate basis for a comparison which could reveal a potential therapeutic effect of omega-3 and omega-6 fatty acids
- Future experimentation should involve earlier supplementation of the FAs

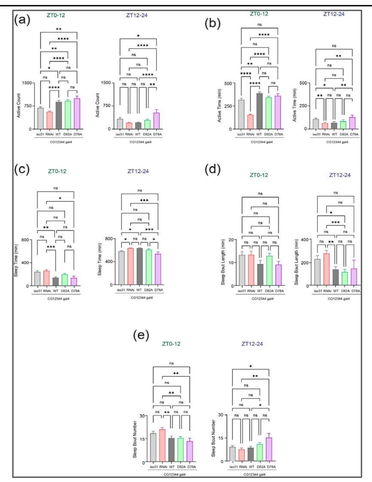


Markov Chain Analysis

CG12344 Encoded Alka Channel Revealed to Mimic Effects of Epilepsy and Play a Significant Role in Sleep



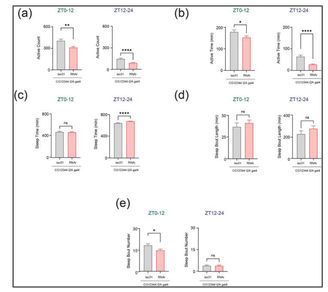
DN1 GFP



Gal4 CG12344 Driver

- GFP-labeled DN1 crossed with iso31, CG12344 RNAi, WT, D82A, and D78A show general trends of increased channel expression correlating with increased active count and time and decreased sleep time and length, with the mutations (D82A and D78A) exhibiting similar trends
- Less clear trends present with the UAS-gal4 driver system may indicate that driving overexpression prevents the system from mimicking physiological conditions

Sleep Screen Data Analysis Continued



t2A Gal4 CG12344 Driver

- The t2A gal4 driver system similarly increases expression of the CG12344 gene encoding the Alka channel
- However, this particular driver system induces a lethal phenotype, preventing all genotypes from being observed, which is why only the iso31 and RNAi genotypes are compared
- Notably, in comparison to the iso31 control genotype, RNAi, which decreases expression of the channel, decreased activity and slightly increased sleep time
- These results would indicate that Alka presence decreases sleep and increases activity, which follows the same trend observed in the DN1 GFP cross

Conclusions and Future Directions

- iPS cell model conclusions and future directions
  - The observed change in trends between DIV 35 and 55 represents a need to administer fatty acids earlier in the cell culture lifetime, and brings awareness to the fact that the cell line dynamics change overtime
  - Therefore, an optimal time period for electrophysiological analysis should be established and utilized
  - With earlier supplementation, the significant differences seen between the control and epilepsy-derived cells should remain, allowing for analysis of the potential of omega-3 and -6 fatty acids
  - More specificity regarding epileptic phenotypes should also be included
- CG12344 - Alka channel sleep screen conclusions and future directions
  - Results thus far are promising and indicate a clear role of the Alka channel in *Drosophila* sleep patterns and regularity
  - Similar to epileptic sleep phenotypes, overexpression of this channel increases activity and decreases sleep, indicating a potential route of investigation into this channel being involved in hyperexcitability of the brain
  - Future investigations should include behavioral mechanical shock response analysis to indicate if this channel is involved in seizure activity
  - This channel could serve as a new target for AEDs with positive future research

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