The role of Doublecortin-like kinase 1 (DCLK1) in epilepsy progression

Thesis
Brown University
Providence, Rhode Island
May 26, 2024

Brendan M. McCarthy-Sinclair
B.S., University of California, Davis, 2015

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Division of Biology and Medicine at Brown University.

SIGNATURE PAGE

BROWN UNIVERSITY
THE GRADUATE SCHOOL

Name: Brendan Michael McCarthy-Sinclair

Division/Department/Program/School: Molecular Biology, Cell Biology and Biochemistry

Previous degrees: B.S. University of California, Davis, 2015

Title of thesis: "The Role of Doublecortin-Like Kinase 1 (DCLK1) in Epilepsy Progression"

Thesis approved for department by: Judy Liu

Approval of thesis recommended to Graduate Council by Committee:

Alexander Jaworski
Christopher de Graffenried
Eric Morrow
Justin Botterill

Date of Final Examination: March 8, 2024

Votes on recommending degree (to be signed by examining committee):
Aye: **Nay:**

Presiding Officer

Brendan McCarthy-Sinclair
Curriculum Vitae

EDUCATION

Brown University, Molecular Biology/Neuroscience Department
Graduation: Mar 2024
Dissertation: The role of Doublecortin-like kinase 1 (DCLK1) in epilepsy progression
Degree: Doctor of Philosophy

Program: MCB/Neuroscience

University of California, Davis, Honors Program
Graduation: May 2015

Degree: Bachelor of Science
Major: Molecular Biology and Biochemistry

GRADUATE RESEARCH EXPERIENCE

Epilepsy and Autism Laboratory – Ph.D. Student
Sept 2017 – Present
Advisor: Judy Liu, M.D., Ph.D. Affiliation: Brown University – Alpert Medical School
Quantitative Modeling of Synaptic and Neuroanatomical Changes Induced by Epilepsy in a DCLK1 Knockout Mouse

EEG:
Established multiple methodologies for surgical electroencephalographic (EEG) implantation into mice brains
Developed scoring methods and analysis pipelines for quantifying abnormal brain waveforms in EEG
Used Pinnacle and R software programs for studying multiple parameters of sleep quality and power spectral analysis

Electrophysiology:
Created novel and more effective epilepsy induction protocols in mice
Measured brain excitability ex vivo using local field potential (LFP) recordings in multiple hippocampal areas
Combined protocols for drug exposure and brain slice physiology during LFP to measure hyperexcitability in adult mouse models

Molecular Biology:
Used super-resolution/confocal live imaging and fixed imaging of tissue and cultures using antibodies, metal stains, and affinity stains
Performed western blotting of neuronal cultures and individual brain sections
Completed viral plasmid construction, viral production, and successful viral infection in primary neurons

RELEVANT WORK EXPERIENCE AND MENTORING

UC Davis Microbiome Research Laboratory – Lab Manager
Sep 2015 – May 2017
Advisor: Maria Marco, Ph.D. *Department: UCD Microbiology*

Performed all management for laboratory including maintenance, ordering, and scheduling
Conducted original research and published on the effect of lignans on the microbiome
Mentored undergraduate students and supported graduate student projects

UC Davis Parasitology Research Laboratory – Student Lab Assistant
May 2014 – Sept 2015
Advisor: Scott Dawson, Ph.D.
Department: UCD Microbiology
Designed time course experiments for examining Giardia infections in vitro
Created new protocols for luciferase assays to determine Giardia encystation rates
Published one paper and multiple posters on Giardia encystation rates and changes in vivo

Graduate Student Mentoring – Brown University
Spring 2019-2021
Jenny Lee: Taught variety of molecular biology techniques
Oversaw and advised in poster production
Helped write and submit successful applications for the Undergraduate Teaching and Research Award (UTRA) and Fulbright Scholarship
Samy Amkieh: Taught and implemented EEG analysis skills and confocal microscopy use Spring 2021- 2022

Translational Neuroscience Outreach Program (TNOP) – Brown University
May 2021 – Sept 2023
Program Head: Eric Morrow, M.D., Ph.D. Affiliation: Brown University – Alpert Medical
Taught high school students about the process of doing science and gathering data
Conducted an individual research project with a student each year using real data and performing experiments
Participated in multiple panel events on advising for college, neuroscience, and taking opportunities in research

CERTIFICATES AND TEACHING EXPERIENCE

Leadership, Organizing, and Action: Leading Change
Jan 2013 – May 2013
Advisor: Marshall Ganz, Ph.D. Affiliation: Harvard University – Kennedy School of Government
Designed and implemented a clinical program to teach nutrition at a local clinic
Met with advisors and mentors weekly to discuss progress and learn leadership training
Completed with Honors

Leadership Experience for Aspiring Physicians
Jan 2014 – Aug 2014
Affiliation: Stanford University – Stanford Medical School
Attended in-person seminars on leadership and community organizing
Formed a college group focused on medical outreach and local programs
Completed with Honors

Molecular Biology Teaching Assistant – Brown University
Spring 2018
Biochemistry: Brown teaching assistant
Instructors: Dr. Gerwald Jogl, Dr. Louis Lapierre, Dr.Alexandra Deaconescu

Lumiere Tutoring Agency – Graduate Tutor
Aug 2021 – Aug 2022

Agent: Stephen Turban

Developed teaching plans for introductory neuroscience course material
Designed review projects based on scientific area of interest
Helped write and edit literature reviews for students

PUBLICATIONS

Xu Y, Bolvig AK, **McCarthy-Sinclair B**, Marco ML, Bach Knudsen KE, Hedemann MS, Lærke HN. The role of rye bran and antibiotics on the digestion, fermentation process and short-chain fatty acid production and absorption in an intact pig model. Food Funct. 2021 Apr 7;12(7):2886-2900. doi: 10.1039/d1fo00213a.

Maybury-Lewis S, Brown AK, Yeary M, Sloutskin A, Dhakal S, **McCarthy-Sinclair B**, Juven-Gershon T, Webb AE. Changing and stable chromatin accessibility supports transcriptional overhaul during neural stem cell activation. bioRxiv 2020.01.24.918664; doi: https://doi.org/10.1101/2020.01.24.918664

Bolvig AK, Nørskov NP, Hedemann MS, Foldager L, **McCarthy-Sinclair B**, Marco ML, Lærke HN, Bach Knudsen KE. Effect of Antibiotics and Diet on Enterolactone Concentration and Metabolome Studied by Targeted and Nontargeted LC-MS Metabolomics. J Proteome Res. 2017 Jun 2;16(6):2135-2150. doi: 10.1021/acs.jproteome.6b00942.

Barash NR, Nosala C, Pham JK, McInally SG, Gourguechon S, **McCarthy-Sinclair B**, Dawson SC. *Giardia* Colonizes and Encysts in High-Density Foci in the Murine Small Intestine. mSphere. 2017 Jun 21;2(3):e00343-16. doi: 10.1128/mSphere.00343-16. PMID: 28656177; PMCID: PMC5480036.

MANUSCRIPTS IN PREPARATION

McCarthy-Sinclair B, Higashimori H, Papendorp C, Goicouria L, Liu JS. DCLK1 deletion increases severity of epileptic hallmarks in a pilocarpine model of epilepsy. In progress.

ABSTRACT PUBLICATIONS

McCarthy-Sinclair B, Liu JS. (2023, December) *DCLK1 deletion increases severity of epileptic hallmarks in a pilocarpine model of epilepsy.* Abstract accepted for poster pres. at American Epilepsy Society conference 2023.
McCarthy-Sinclair B, Liu JS. (2022, October) *DCLK1 deletion increases severity of mossy fiber sprouting and interneuron loss in a pilocarpine model of epilepsy.* Abstract accepted for poster pres. at Mechanisms of Epilepsy and Neuronal Synchronization Gordon Research Conference 2022.
McCarthy-Sinclair B, Liu JS. (2022, October) *DCLK1 deletion increases severity of mossy fiber sprouting and interneuron loss in a pilocarpine model of epilepsy.* Abstract accepted for poster pres. the Society for Neuroscience, virtual 2022.
McCarthy-Sinclair B, Liu JS. (2021, December) *DCLK1 deletion increases mossy fiber sprouting and sleep dysfunction in a pilocarpine model of epilepsy.* Abstract accepted for poster pres. at American Epilepsy Society conference 2021.

Lee J, **McCarthy-Sinclair B,** Liu JS. (2021, August) *DCLK1 overexpression increases VAMP2 vesicle translocation in Hela cells.* Abstract accepted for poster pres. at Brown University Research Symposium 2021.
McCarthy-Sinclair B, Nosala C, Dawson SC. (2015, May) *Determining the Effect of Population Density on Rates of Encystation for Giardia lamblia.* Abstract accepted for poster pres. at UCD Undergraduate Research Conference 2015.
McCarthy-Sinclair B, Nosala C, Dawson SC. (2014, April) *Determining the Effect of Population Density on Rates of Encystation for Giardia lamblia.* Abstract accepted for poster pres. at Stanford Research Conference (SRC) 2014.

ORAL PRESENTATIONS

McCarthy-Sinclair B (2023, June). *DCLK1 deletion increases severity of epileptic hallmarks in a pilocarpine model of epilepsy.* Oral presentation for Brown University Translational Neuroscience Outreach Program 2023.
McCarthy-Sinclair B (2022, June). *DCLK1 deletion increases severity of mossy fiber sprouting and interneuron loss in a pilocarpine model of epilepsy.* Oral presentation for Brown University Translational Neuroscience Outreach Program 2022.
McCarthy-Sinclair B (2021, April). *DCLK1 deletion increases mossy fiber sprouting and sleep dysfunction in a pilocarpine model of epilepsy*. Oral presentation at Brown University's Neurodevelopment Group Meeting 2021.
McCarthy-Sinclair B (2021, March). *DCLK1 deletion increases mossy fiber sprouting and sleep dysfunction in a pilocarpine model of epilepsy.* Oral presentation at Brown University's Molecular Biology Department Data Symposium 2021.
McCarthy-Sinclair B (2020, July). *DCLK1 deletion increases mossy fiber sprouting and sleep dysfunction in a pilocarpine model of epilepsy.* Oral presentation at Brown University's Neurodevelopment Group Meeting 2020.
McCarthy-Sinclair B (2020, March). *DCLK1 deletion increases mossy fiber sprouting and sleep dysfunction in a pilocarpine model of epilepsy*. Oral presentation at Brown University's Molecular Biology Department Data Symposium 2020.
McCarthy-Sinclair B (2018, May). *KLIF is expressed at the ingrowing furrow during cytokinesis in Trypanosoma brucii*. Oral presentation at Brown University's Molecular Biology Department Rotation Presentations.
McCarthy-Sinclair B (2018, March). *FOXO3 knockout increases the expression of NeuroD in neuronal stem cells*. Oral presentation at Brown University's Molecular Biology Department Rotation Presentations.
McCarthy-Sinclair B (2017, December). *CLAMP association with chromatin mediates dosage compensation in Drosophila melanogaster*. Oral presentation at Brown University's Molecular Biology Department Rotation Presentations.
McCarthy-Sinclair B (2015, May) *Determining the Effect of Population Density on Rates of Encystation for Giardia lamblia.* Oral Presentation at UCD Undergraduate Research Conference 2015.

POSTER PRESENTATIONS

McCarthy-Sinclair B, Liu JS. (2023, December) *DCLK1 deletion increases severity of epileptic hallmarks in a pilocarpine model of epilepsy.* Abstract accepted for poster pres. at American Epilepsy Society conference 2023.
McCarthy-Sinclair B, Liu JS. (2023, September) *DCLK1 deletion increases severity of epileptic hallmarks in a pilocarpine model of epilepsy.* Poster pres. at Brown University Molecular Biology Department Retreat 2023.
McCarthy-Sinclair B, Liu JS. (2022, October) *DCLK1 deletion increases severity of mossy fiber sprouting and interneuron loss in a pilocarpine model of epilepsy.* Poster pres. at Mechanisms of Epilepsy and Neuronal Synchronization Gordon Research Conference 2022.
McCarthy-Sinclair B, Liu JS. (2022, September) *DCLK1 deletion increases severity of mossy fiber sprouting and interneuron loss in a pilocarpine model of epilepsy.* Poster pres. at Brown University Molecular Biology Department Retreat 2022.
McCarthy-Sinclair B, Liu JS. (2022, October) *DCLK1 deletion increases severity of mossy fiber sprouting and interneuron loss in a pilocarpine model of epilepsy.* Poster pres. the Society for Neuroscience, virtual 2022.
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McCarthy-Sinclair B, Liu JS. (2021, September) *DCLK1 deletion increases mossy fiber sprouting and sleep dysfunction in a pilocarpine model of epilepsy.* Poster pres. at Brown University Molecular Biology Department Retreat 2021.
Lee J, **McCarthy-Sinclair B,** Liu JS. (2021, August) *DCLK1 overexpression increases VAMP2 vesicle translocation in Hela cells.* Poster pres. at Brown University Research Symposium 2021.
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McCarthy-Sinclair B, Liu JS. (2019, September) *DCLK1 deletion increases mossy fiber sprouting and sleep dysfunction in a pilocarpine model of epilepsy.* Poster pres. at Brown University Molecular Biology Department Retreat 2019.
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McCarthy-Sinclair B, Nosala C, Dawson SC. (2014, April) *Determining the Effect of Population Density on Rates of Encystation for Giardia lamblia.* Poster pres. at Stanford Research Conference (SRC) 2014.

AWARDS

Brown University Appointment COVID Extension Award: Award covers tuition and salary for 1 year
Brown University travel awards: award for conference travel and registration for the years 2021, 2022, and 2023
Deans List – Spring 2013, Spring 2014, Spring 2015, Spring 2016

TRAVEL GRANT AWARDS

McCarthy-Sinclair B, Liu JS. (2023, December) *DCLK1 deletion increases severity of epileptic hallmarks in a pilocarpine model of epilepsy.* American Epilepsy Society conference 2023, Orlando, FL.

McCarthy-Sinclair B, Liu JS. (2022, October) *DCLK1 deletion increases severity of mossy fiber sprouting and interneuron loss in a pilocarpine model of epilepsy.* Mechanisms of Epilepsy and Neuronal Synchronization Gordon Research Conference 2022, Barcelona, Spain.
McCarthy-Sinclair B, Liu JS. (2021, December) *DCLK1 deletion increases mossy fiber sprouting and sleep dysfunction in a pilocarpine model of epilepsy.* American Epilepsy Society conference 2021, Chicago, IL.

Acknowledgements

I'd like to begin by thanking my mentor throughout this whole process, **Dr. Judy Liu**. I often think how challenging this process has been and I'm grateful you have been in my corner from the very beginning and into the end. Your leadership has been so greatly enhanced by your compassion for every member of the lab and your inspiring ideas to guide our projects. I am sincerely grateful for all of the support and encouragement you provided over the years to make this project and period of my life possible and successful.

I'd also like to thank my family **John Sinclair, Cindy McCarthy, and Aislinn McCarthy-Sinclair**. What a ride huh? It seems like just yesterday we were packing up the Corolla and setting our sights for the 3000 mile trip to Providence. I have felt your support every step of the way, whether it was weekend phone calls or surprise care packages received in the mail. I'd like to think I've developed, changed, and matured throughout this process as a son, brother, and man, but one thing that has never wavered is the energy you have all provided me to drive me to success. I am forever grateful and love you all very much.

A special thanks to **Tyler Lewy**, probably the only person I would brave New York traffic to visit. I am forever grateful I did so terribly in my intro to chemistry class at SRJC that we ended up in the same organic chemistry class years later. No matter how much FIFA we play, I hope we never get good. Cheers buddy.

Additionally, I have to shout out my colleagues and friends **Carin Papendorp**, **Luis Goicouria**, **Kelvin de Leon**, **Alice Lin**, and **Steph Torres**. Thank you all for all of the support over the years. We've had so many good times together both inside and outside of the lab. Being able to come into the lab every day and knowing my friends will be there to share all of my successes and failures alongside me is an incredible gift and something I don't take for granted. I can't imagine drinking seven cups of coffee and playing word puzzle games with any other group of people.

Thank you to my thesis committee consisting of **Dr. Alex Jaworski**, **Dr. Eric Morrow**, and my committee chair **Dr. Chris de Graffenried**. You have all been critical supporters of my journey, both for giving me great advice during my project and keeping me on track to finish strong. **Dr. Justin Botterill** has also been of great help by serving as a crucial knowledge source on all things epilepsy as my outside reader and through his numerous publications that have guided my experiments for many years.

I would like to extend my gratitude to all other members of the Liu lab both past and present, especially **Dr. Haruki Higashimori**. You were a great help to me in perfecting many techniques and learning so many of the basics in fundamental electrophysiology. **Dr. Li-Jin Chew** was also always a great resource who made herself available at all times and her help was greatly appreciated. Thank you LJ!

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1 Introduction

1.1 Overview of epilepsy

While up to 10% of people will have a seizure at some point in their lives, epilepsy is a condition defined as having two or more spontaneous seizures at least 24 hours apart (Sirvin 2015). According to the WHO it affects roughly 50 million people globally and is the fourth most common neurological disorder but there remain many unknown factors as to its origin and propagation in many cases (Chakravarthy et al. 2009). However, our understanding of the disease has increased exponentially with the development of next generation diagnostic and molecular tools for identifying previously indiscernible characteristics of the disease (Scheffer et al. 2017, Zhang 2020, Reddy & Saini 2021, Trinka & Leitinger 2022, Barfuss et al. 2023, Yuan et al. 2023). The treatment of a condition that results in repeated seizures tends to focus on suppressing the seizures themselves. Patients suffering from epilepsy have access to dozens of anti-seizure medication (ASM) options but roughly one third do not have adequate seizure control (Löscher et al. 2020). These patients have few other options for the management of their symptoms and may need to resort to therapeutic resection of the epileptic focus if it can be identified and safely excised. With the heavy burden of this disease and the lack of completely effective pharmacological solutions, a better understanding of the pathology is required to develop more treatments.

Broadly, seizures are the result of an excitation/inhibition imbalance in the brain, and a lack of homeostatic regulation causing uncontrolled hypersynchronous firing. The scale of the root cause of this dysfunction can vary widely, ranging from changes in ionic conductance or number of synaptic connections to neural population level or circuit

feedback loop changes (Chakravarthy et al. 2009). Usually the brain attempts to maintain a homeostatic state of excitation and inhibition, with an asynchronous neuronal firing pattern (Chakravarthy et al. 2009). This dysregulated synchronous firing can happen in a myriad of ways, areas, and frequencies, resulting in dozens of unique seizure presentations (Table 1, adapted from Sarmast et al. 2020). The four overarching classes of epilepsies are organized by where the abnormal activity originates in the brain. These can be focal, generalized, focal and generalized, or unknown (Sarmast et al. 2020). A focal seizure has abnormal electrical activity that originates in one cerebral hemisphere and can spread to the other, while a generalized seizure occurs in both hemispheres simultaneously and may spread around to other neural networks (Fisher et al. 2018, Brodie et al. 2018, Fisher et al. 2017, Fisher et al. 2017, Sarmast et al. 2020). It is speculated generalized seizure occurs in separate hemispheres simultaneously due to issues with forces that affect the brain as a whole such as upstream thalamocortical relays becoming synchronous or problems with disinhibition systemically instead of in one specific area (Williamson et al. 2009, Lindquist et al. 2023).

Focal	Generalized	Unknown origin
Aware/Impaired awareness	Motor onset (8 subtypes)	Motor (Tonic-clonic, epileptic spasm)
Motor onset (7 subtypes)	Non-motor onset (4 subtypes)	Non-motor (Behavior arrest)
Non-motor onset (5 subtypes)		Unclassified
Focal to bilateral tonic-clonic		

Table 1. There are dozens of unique seizure signatures a patient can experience.

Seizures are divided into three subgroups based on the origin of the seizure focus. Specific seizure loci and presentation can help inform diagnostics and treatment on a more individualized level.

One of the more unique and pressing questions for a disease that revolves around a repetitive excitotoxic event is whether that event then predisposes an individual to a greater number or increased severity of seizures. This phenomenon described by the phrases “seizures beget seizures” is known as “kindling” and has been used as a concept for epilepsy modeling and a topic of interest for human biology. In animal models of kindling induction, focal electrical stimulation is applied to trigger a seizure of relatively short duration with minimal behavioral reaction. However, additional stimulations cause increases in seizure duration and behavioral severity as measured by Racine scale until a plateau is reached, implying increased susceptibility (Goddard et al. 1969, Racine et al. 1972). While some reports indicate spontaneous seizures may occur after sufficient stimulations, this effect is far from consistent and varies greatly depending on laboratory and species. This stimulation kindling model most closely resembles temporal lobe epilepsy (TLE) in animals, but other forms of epilepsy have also shown clear kindling-like characteristics without exogenous stimulation. Some rodent genetic models of absence seizures have a clear onset and distinct disease development, progressively experiencing more absence seizures per day over time (Blumenfeld et al. 2008, Makinson et al. 2017). Similarly, when evaluating the effect of duration of epilepsy, patients who have had epilepsy longer are associated with higher probabilities of an abnormal EEG (Brito et al. 2021). Conversely, long-lasting cases of TLE in humans often do not have changing symptoms either clinically or electrographically (Blume et al. 2006, Ben-Ari et al. 2008).

A lack of disease progression despite repeated seizures may be attributed to homeostatic mechanisms established in response to the disease such as increases in adult neurogenesis (Jain et al. 2019).

1.2 Causes of epilepsy

But what are the causes of epilepsy? There are many potential causative factors including genetics, structural brain abnormalities, infections, and brain injuries. Infections and brain injuries resulting in epilepsy are often more clearly causational than subtle genetic or structural aberrations. Acute seizures can occur in up to 30% of patients with a CNS infection and are a risk factor for later development of spontaneous recurrent seizures in a patient's lifetime (Beghi et al. 2010, Singhi et al. 2011, Shorvon et al. 2010, Lowenstein et al. 2009). The risk of developing epilepsy after a traumatic brain injury (TBI) correlates with the severity of the damage. The incidence of late-onset seizure over 30 years for a mild TBI is 2%; moderate TBI 4%; and severe TBI 15% (Annegers et al. 1998). These seizures occurring later in life after the incident are most likely caused by architectural changes in the brain making it more vulnerable to hyperexcitation, including neuronal loss, rewiring, and sprouting (Noebels et al. 2012).

Genetic mutations are a very common cause of epilepsy. Genetic generalized epilepsies, for example, are genetically determined and account for about one third of all epilepsy cases (Engel et al. 2001, Panayiotopoulos et al. 2005, Sarmast et al. 2020). While focal epilepsies were often traditionally thought of as the result of an environmental insult there is now a large body of evidence for significant genetic contribution. First degree relatives of focal epilepsy probands have a much higher risk of epilepsy and monozygotic twins exhibit a higher concordance compared to dizygotic twins (Kjeldsen et

al. 2003, Corey et al. 2011,Vadlamudi et al. 2014, Perucca 2018). Despite these genetic contributions, there is often overlap between the genetic and structural, further complicating epilepsy causation.

Which genes contribute to epilepsy and the extent of their contribution can be complex, but some efforts have been made to organize epilepsy-associated genes. One of these organizational efforts led to classifying 977 epilepsy-associated genes into four distinct subtypes:

- 84 genes only cause epilepsy or have syndromes with epilepsy as their core symptom
- 73 genes are related to neurodevelopmental defects resulting in gross brain abnormalities/malformations and epilepsy
- 536 “epilepsy-related” genes that cause gross physical malformations or other systemic abnormalities that also accompany epilepsy or seizure
- 284 genes are classified as “potentially” epilepsy-associated, and require further investigation into their relationship with epilepsy (Wang et al. 2017).

Although each group of genes can aid in our understanding of the multivariate nature of genetic interactions, the first subgroup most specifically resulting in epilepsy can provide insight into discrete fundamental pathways most directly causing disease. When examining those 84 genes specifically associated with epilepsy, researchers found ion channel mutations to be the most common, accounting for 28/84 genes. The second most common was a much more generalized group characterized by mutations in enzymes or modulators of enzymes, and accounted for 25/84 genes. The remaining 31 genes were spread between a half dozen diverse cellular function genes (22/84) or remain

unclassified (9/84). While this list highlights the variation of genetic contributions, the majority of the associated genes being specifically ion-channel related clearly highlights the importance of circuit balance in the pathology of the disease.

Examination of the second subgroup, genes associated with neurodevelopmental defects, can give insight into how larger scale dysfunction can result in epilepsy as more of a symptom of dysfunction. While the most prevalent subtype is rather general and includes enzymes/enzyme modulators (24/73), the second most prevalent subtype is cytoskeletal genes (15/73). These neurodevelopmental disorder gene subtypes differ substantially from epilepsy-specific genes, containing no ion channel genes and many more cytoskeletal genes. Many of these cytoskeletal genes result in dysfunction of organization or migration in the brain (e.g. lissencephaly, double cortex syndrome, Galloway-Mowat Syndrome), producing hallmark pathologies such as a smooth brain, multiple cortical layers, and microcephaly (Gleeson et al. 1998, Di Donati et al. 2017, Colin et al. 2014). Differentiation of these genes that solely affect epilepsy and those that cause disorders including epilepsy help demonstrate the spectrum of phenotypes that can be encompassed by the disease and can give us clues on origins and treatments for the condition.

How do mutations in these genes actually contribute to seizure occurrence in patients? They cause dysfunction in brain circuit homeostasis in one way or another, making the brain more prone to hyperexcitability and the causative synchronous neuronal firing. Ion channel genes, as mentioned earlier, are a common genetic contributor to seizure manifestation. For example, Dravet syndrome is one of the more common genetic causes of epilepsy and most often results from haploinsufficiency in the SCN1A gene

(Ding et al 2021). This gene encodes the alpha subunit of the voltage-gated sodium channel Nav1.1. The mechanisms behind the how this channel mutation directly causes epilepsy are very complex, but one implicated cause in mouse disease models comes from compromise of hippocampal fast-spiking inhibitory interneurons (Valassina et al. 2022). Researchers were able to upregulate the functional copy of SCN1A and improve behavioral outcomes and reduce seizure occurrence (Valassina et al. 2022). When examining the fast-spiking interneuron populations in the CA1 affected by this upregulation they found that this rescue treatment had restored the reduced maximal firing rate. They also found the rescue mice had recovered a higher number of evoked action potentials at low and high stimulation intensities compared to the haploinsufficient mice. This is a clear example of how epilepsy caused by distinct circuit homeostasis mechanisms can be rectified by attempts to reestablish functional excitation/inhibition balance, especially in models of ion channel dysfunction.

Although gene mutations affecting ion channels are commonly causative of epilepsy, there are other malformation changes in the brain causing sweeping changes resulting in epilepsy that are derived from genetic mutations. These mutations often cause migration or organizational problems in the brain during development, causing cell populations to be absent or even completely misplaced, compromising circuitry organization. Such is the case for mutations causing lissencephaly and subcortical band heterotopia. In lissencephaly, a gene mutation (usually cytoskeletal) causes an uncharacteristic smoothness of the brain. This is the result of thickened cortical gray matter absent of gyri. The abnormal cortical layering is caused by failure in neuronal migration, and this faulty migration causes widespread miswiring of circuits. Because

these circuits are aberrantly wired, the proper circuit homeostasis organization is completely disrupted, leading to abnormal circuits that may be prone to hyperexcitability and synchronous firing. Subcortical band heterotopia is similarly a disease characterized by migration defects that cause faulty organization in the brain (Deuel et al. 2006, Momen et al. 2015). Due to a mutation in DCX or LIS1, neurons meant to migrate to the cerebral cortex (the outer surface of the brain) do not complete their migration and instead form a band of cells underneath the cerebral cortex (Deuel et al. 2006). This abnormal placement of cells results in unintended circuit connections that make the brain more prone to hyperexcitability and epileptic activity because the proper excitation/inhibition balance could not be established. These migration defects and the aforementioned ion channel dysfunctions caused by gene mutations are two of the many ways epilepsy can be caused by genetics.

1.3 Temporal lobe epilepsy (TLE) is an important subset of epilepsy cases

While the seizure type and presentation can vary greatly from case to case, the most common of all epilepsy cases in adults are classified as TLEs. Despite the commonality of this epilepsy type and the development of dozens of anti-seizure medications (ASM) over the past decades, up to 75% of TLE patients are refractory to medication (Jallon et al. 1997, Yang et al. 2020). One third of all epilepsy cases have the seizure onset within the temporal lobe (Blair et al. 2012). Of these TLEs, 80% arise in the mesial temporal lobe, originating in areas such as the hippocampus, rhinal cortices, and amygdala (Asadi-Pooya et al. 2017, Bartolomei et al. 2005). Most cases are sporadic but about one-fifth of all non-lesional TLE cases possibly have a familial attribute, although penetrance is often

incomplete and inheritance patterns remain complex (Berkovic et al. 1996, Crompton et al. 2010).

The circuitry of the hippocampus is of particular interest for many groups studying TLE. The first inputs are from the pyramidal neurons of the entorhinal cortex, which mostly extend their axons into the outer and medial layers of the molecular layer surrounding the dentate gyrus (Krook-Magnuson et al. 2015). It is here that they connect to the dendrites of the granule cells, the neuronal cell type that constitutes most of granule cell layer. These granule cells extend axons called mossy fibers that wrap around the CA3 and extend to the CA2 to continue the circuit (Krook-Magnuson et al. 2015). The CA3 and CA2 then connect to the CA1, which finally connects to a different layer of the entorhinal cortex (Krook-Magnuson et al. 2015). This circuit is known as the trisynaptic circuit and has many characteristics of importance to TLE. After status epilepticus (SE), which is a prolonged seizing event, there is very visible acute cell loss throughout the hippocampus that can be visualized by FluoroJade staining (stain for degenerating/dying neurons) (Jain et al. 2019, Botterill et al. 2019). High proportions of neuronal death, especially in the CA3-CA1, are indicative of potential epilepsy from this SE later in life. There are also stark anatomical contrasts in the hippocampi of epileptic animals and patients. One of the most controversial and potentially impactful is mossy fiber sprouting (MFS).

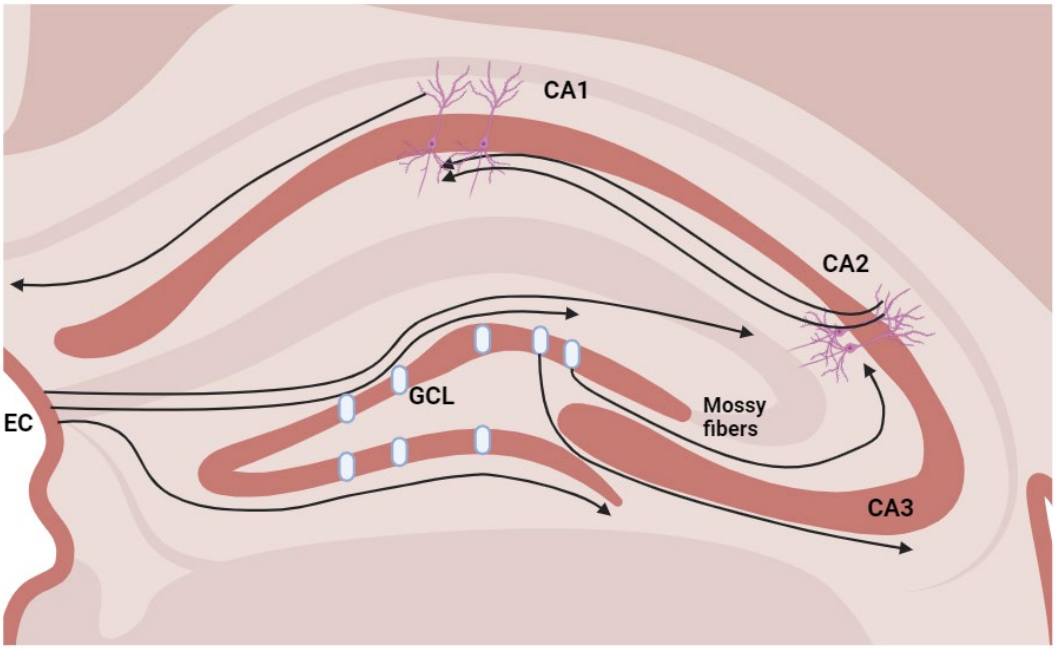

Figure 1. Circuitry of the hippocampus.

Pyramidal neurons from the entorhinal cortex synapse with the dendrites of dentate granule cells, which extend mossy fiber axons to CA3 and CA2. The circuit is continued by projections to the CA1, which complete the circuit by innervating a different layer of entorhinal cortex. EC: entorhinal cortex, GCL: Granule cell layer.

Mossy fiber sprouting is an abnormal axonal outgrowth involving the mossy fiber axons that come from the dentate granule cells. In a healthy brain, the mossy fibers extend around the CA3 and to the CA2, forming synapses that will continue the trisynaptic circuit. In epileptic animals and patients, staining of these mossy fibers reveal sprouted branches that grow the opposite way (Heinemann et al. 1992, Lothman et al. 1992). Instead of following the parent axon to connect to the downstream trisynaptic circuit structures, the mossy fibers grow back towards the granule cell layer they originated from. These mossy fibers can penetrate through the granule cell layer and synapse with the dendrites of granule cells in the molecular layer. This enables the formation of unintended

functional recurrent circuits in the molecular layer (Nadler et al. 1980, Zhang et al. 2012). Formations of unintended recurrent circuits tend to be very problematic, but there is still much controversy about whether epilepsy is worsened by more severe MFS or if MFS is simply a symptom of the disease.

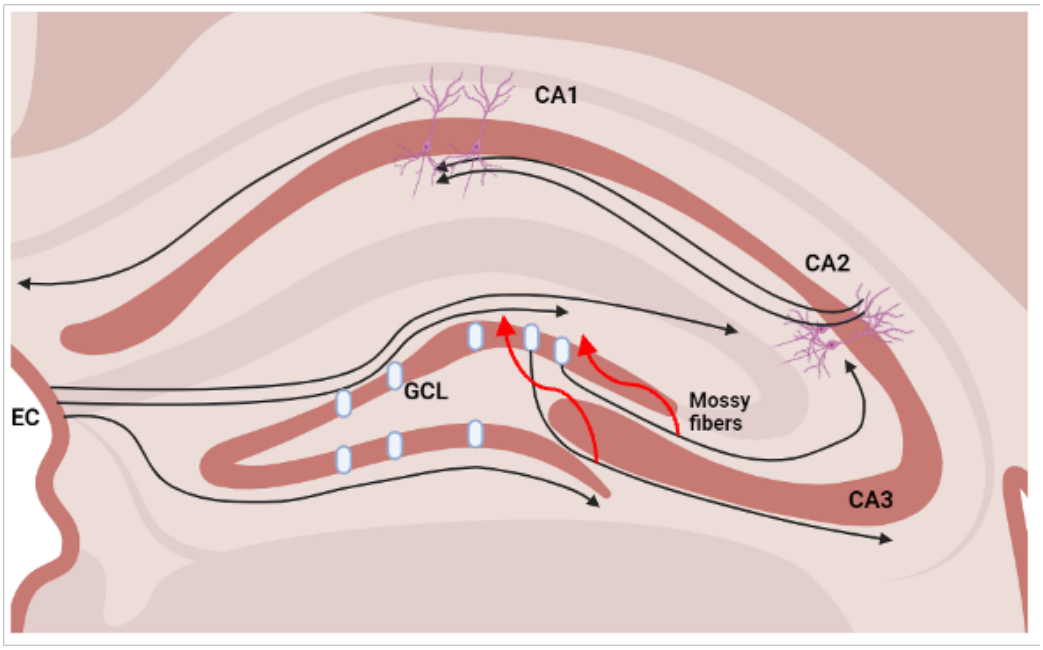

Figure 2. Mossy fiber sprouting (MFS) in the hippocampus.

In a healthy hippocampus, the mossy fibers project from the granule cells, through the hilus, and extend to the CA3 and CA2. In epileptic patients and animals, these mossy fibers can sprout axons that grow in the reverse direction (red arrows). These mossy fibers can reach the molecular layer around the dentate gyrus where the dendrites of the granule cells are located, and can form functional recurrent circuits between granule cells.

Another point to consider is the location of this recurrent excitation, as these granule cells serve as a “dentate gate” to protect downstream hippocampal substructures from excess excitation. As mentioned previously, prolonged seizing activity known as status epilepticus (SE) can trigger widespread death in the hippocampus but most visibly

in the CA3-CA1, structures directly downstream of the granule cell layer. Granule cells attempt to protect these downstream substructures through its remarkably low excitability, propagating only a fraction of the input they receive to downstream areas. In healthy tissue, granule cells exhibit not only significant feedforward and feedback inhibition but also possess unexcitable intrinsic characteristics, including a notably hyperpolarized resting membrane potential, low input resistance, and a relatively high firing threshold (Heinemann et al. 1992, Lothman et al. 1992, Acsády et al. 1998, Henze et al. 2002, Coulter 2007 et al, Yu et al. 2013). Conversely, downstream hippocampal regions are vulnerable to excitotoxicity due to the pyramidal cells ability to generate action potentials more easily and the highly prevalent intrinsic recurrent excitatory connections there (Krook-Magnuson et al. 2015). Compromise of these granule cells' excitability resistance from the recurrent networks formed by MFS is a leading hypothesis for the dentate gate theory in epilepsy.

It is currently unknown exactly how mossy fiber sprouting occurs. Some of the most compelling evidence comes from rapamycin administration in mice (Heng et al. 2013). These mice did not experience any change in seizure severity but experienced far less MFS. This would indicate that mTOR inhibition is somehow involved in the process of MFS, but how exactly it modulates that process has not been explored. There is also some evidence for a mechanism dependent on hilar neuron loss being responsible for MFS. This theory is support in various studies both correlational and directly causatively. For instance, in a rat model of temporal lobe epilepsy, the degree of mossy fiber sprouting has been observed to correspond with the decline of mossy cells, while in patients with mesial temporal lobe epilepsy, it correlates with the reduction of hilar neurons (Babb et

al. 1991, Masukawa et al. 1992, Houser et al.1990). However, it's important to note that the mere loss of mossy cells doesn't fully account for the extensive branching and long-range reverse projections to the molecular layer. Instead, it may serve as the basis for mossy fiber sprouting rather than its direct cause. This notion was underscored by a particular study utilizing a conditional transgenic mouse line expressing the diphtheria toxin receptor solely in mossy cells (Jinde et al. 2012). The study revealed significant degeneration of mossy cells throughout the hippocampus's longitudinal axis a week post-diphtheria toxin injection. Surprisingly, neither spontaneous behavioral seizures nor mossy fiber sprouting were observed five to six weeks after the toxin injection. However, an increase in GAD67 immunoreactivity, indicating axonal sprouting in GABAergic interneurons, was evident two weeks post-injection. Additionally, observations from a rodent febrile seizure model suggest that mossy fiber sprouting can occur without notable neuronal loss or aberrant neurogenesis following hyperthermia-induced seizures (Bender et al. 2003). These findings suggest that while the loss of mossy cells plays a role, additional seizure-related alterations are likely necessary to prompt mossy fiber sprouting.

What are some of the other structural hallmarks of TLE? The most common hallmark of human drug-resistant TLE is hippocampal sclerosis (36.4% of cases) followed by long-term epilepsy-associated tumors (23.6%) and focal cortical dysplasias (19.8%) (Blumcke et al. 2017). Mossy fiber sprouting (MFS), another common hallmark of TLE, is when granule cell axons grow in the wrong direction and synapse with their own dendritic fields (Houser et al. 1990, Sutula et al. 1989). There is significant controversy over whether these functional recurrent circuits contribute to maintenance and worsening of epilepsy (Buckmaster et al. 2014). One of the clearest and most significant risk factors

for onset of mTLE is the occurrence of a seizure during infancy or young childhood, especially a febrile seizure (seizure resulting from fever). In one cohort of mTLE patients requiring surgery 78% experienced a febrile seizure during childhood and 94% of those patients for whose data was available experienced febrile status epilepticus (FSE) (French et al. 1993). FSE is a more severe occurrence and is classified as a febrile seizure lasting >30 minutes or multiple febrile seizures without recovery for >30 minutes (Thurman et al. 2011). However, debate exists whether febrile seizures increase the risk of mTLE or whether there is underlying dysfunction making the brain more vulnerable to febrile seizure and mTLE. One prospective study examined acute hippocampal T2 hyperintensity (a proxy of hippocampal damage) in MRI data from children immediately following FSE to quantify the presence of acute hippocampal damage (Lewis et al. 2014). If FSE is causing significant acute damage, it could be the reason for increased TLE risk later in life. But of the 226 FSE patients studied only 22 showed evidence of acute T2 hippocampal hyperintensity, and when 14/22 follow-up MRIs were obtained, 10 exhibited hippocampal sclerosis. Of the 116 patients who originally showed no acute hyperintensity, only one exhibited hyperintensity in follow-up MRIs. These studies and many more like it have served to underscore the relationship between FSE and TLE but do little to clarify the nature of the connection (Fernandez et al. 1998).

Multiple animal models have been developed to study TLE, each with their own strengths and weaknesses. Among the most widely used are the kainic acid model and the pilocarpine model. Both are induced models of epilepsy where a wild type animal is exposed to an epileptogenic insult (either kainic acid injection or pilocarpine injection) that causes status epilepticus (SE) (Cherian et al. 2009). Animals surviving SE will

experience a latency period without any seizures for 1-6 weeks (depending on conditions and animal model) before potentially developing spontaneous recurrent seizures (SRS) (Ahmed et al. 2020, Cavalheiro et al. 1991, Curia et al. 2008, Lévesque et al. 2013). The kainic acid can be administered either systemically or locally into the hippocampus or amygdala (Araki et al. 2002, Arabadzisz et al. 2005, Drexel et al. 2012). Each injection area results in different pathologies in the brain. Systemic kainic acid injection avoids the drawbacks of surgical procedures required for brain injection, but multiple injections may be needed depending on the mouse which may introduce severity bias (Lévesque et al. 2013). This severity bias can create a range of different epilepsy severities as mice will experience SE to different degrees, decreasing consistency and introducing unwanted variation. Pilocarpine induction of SE is usually administered systemically and tends to yield the damage and dispersion in the hippocampus that are hallmarks of mTLE (Turski et al. 1983). Additionally, although kainic acid administration does result in damage to the hippocampus, it does not consistently generate SRS in mice (Lévesque et al. 2013, Iyengar et al. 2015).

Modeling mTLE with kainic acid in rats has led to many important findings over the decades, but its inability to consistently produce SRS in mice like pilocarpine may limit its use for studying epileptic progression in some contexts. Because of this the pilocarpine model has gradually become a very popular model for examining TLE and the evolution of SRS in mice. Although systemic administration of kainic acid can induce status epilepticus in mice, it is much more rapid than pilocarpine (Levesque et al. 2016). This can make distinguishing the stages of epilepsy using the Racine scale difficult. While pilocarpine also dependably induces SE, the onset and progression are much easier to

distinguish and score using the Racine scale. Mice treated with the kainic acid induction of SE also experience increased psychological stress compared to pilocarpine, including higher degrees of anxiety and exhibiting depression-like behavior (Ratté and Lacaille 2006, Gröticke et al. 2008). Additionally, while seizures from kainic acid induced epilepsy taper off after 22-46 days, pilocarpine-induced epilepsy is more consistent over a longer timespan, even up to 120 days or even 325 days (Cavalheiro et al. 1982, Cronin and Dudek 1988, Cavalheiro et al. 1991, Mello et al. 1993). There do remain drawbacks to the pilocarpine model, chief among them being variable reactions to the drug and establishment of a consistent protocol. Concentrations used by each experimental group tend to be individually titrated and are often different between each group. Whether the concentration and conditions are effective at inducing SRS and maintaining survivability are highly dependent on age, strain, sex, and individual differences in pharmacokinetics. Additionally, some groups will omit mice that do not appear to reach a certain severity of SE, and other groups will even use these less affected mice as controls. Having a wide variation in reaction to the drug and the development of the phenotype based on this variable reaction is a significant drawback to this model and other models of induced epilepsy.

1.4 Modifiers of TLE: Doublecortin-like kinase 1

Gene mutations that cause gross brain malformations resulting in epilepsy are uncommon, but within that group of mutations cytoskeletal genes are over-represented (Wang et al. 2017). However, loss of DCLK1 is also required for dysfunction to occur in many rodent models, and DCLK1 also plays a significant role in neuronal connectivity

and wiring. To understand the functions of DCLK1 in the brain it is important to understand its genetics and protein structure.

Doublecortin-like kinase 1 (DCLK1) was originally mapped in 2001 and resides on human chromosome 13 in the 13q13.3 loci (NCBI). The gene spans a locus of roughly 362 kb, with the coding sequence of the longest transcript reaching ~2.2 kb (NCBI). It is present in various tissues including the colon and heart, but its expression is highest in the brain (NCBI). Understanding the function of DCLK1 can be complex due to its multiple splice isoforms, even when just focusing on expression in the brain (Bergoglio et al. 2021). According to the most recent NCBI data there are 20 exons and 12 unique isoforms that can be codified by the presence or absence of key domains in the full-length DCLK1 transcript (Burgess et al. 2002). The full-length transcript is a 729 amino acid protein with a distinct but simple domain assembly (Fig 1).

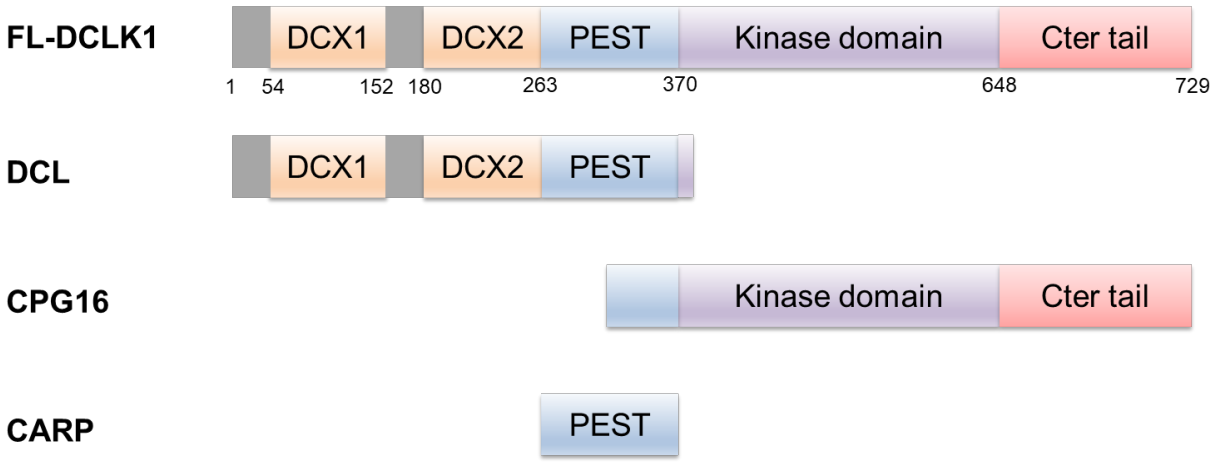

Figure 3. Domains of the DCLK1 full-length protein and architecture of splice isoforms.

DCLK1 contains the tandem doublecortin domains that bind MTs, a PEST linker region, a Ser/Thr kinase domain, and a C-terminal tail.

The N-terminal portion of DCLK1 shares high homology (~70%) with the DCX protein and contains two tandem doublecortin domains that are responsible for microtubule (MT) binding and bundling functionality (Lin et al. 2000). The C-terminal portion of the protein contains a Ser/Thr kinase domain with high homology to the Ca2+/calmodulin-dependent protein 1 (CaMKI) kinase domain but differing in its lack of the canonical calmodulin-binding site (Patel et al. 2016). Sandwiched between the doublecortin domains and the kinase domain is a 100 amino acid PEST linker region that can be a target of proteolytic cleavage and may play a role in the MT binding function of the doublecortin domains (Burgess et al. 2001, Nagamine et al. 2011).

The three splice variants are DCL (kinase domain absent), CPG16 (MT-binding domain and portion of PEST absent), and the mostly overlooked CARP (everything absent but portion of PEST) (Bergoglio et al. 2021). Western blot analysis in the late mouse embryonic stage and up to P30 have shown full-length DCLK1 to consistently be the isoform with highest expression in brain cortices (Bergoglio et al. 2021). The earliest detectable expression comes at E11, roughly the same time DCX expression begins (Deuel et al. 2006). Although DCL is one of the more commonly investigated of the alternate isoforms, its expression is always dwarfed by DCLK1 and is fractional by P7. Conversely, CPG16 shows moderate expression in the late embryonic stages before being robustly expressed into adulthood. In situ data for the isoforms in the hippocampus reveals distinct patterns between isoforms and hippocampal substructures from P0 to P30. DCLK1 is ubiquitously expressed throughout the hippocampus, CPG16 is present in the Ammon's horn, and DCL is relegated to the dentate gyrus and the CA1. High expression and specific segregation of each of these DCLK1 isoforms in the hippocampus

may suggest importance for both development and function in this structure throughout adulthood.

1.5 Cellular and molecular functions of DCLK1

DCLK1 has been a protein of interest in numerous contexts since its discovery and plays an important role in various important cellular functions in the brain. One of the most important details for understanding the relevance of DCLK1 is the functional redundancy it shares with the DCX protein. As mentioned earlier, the N-terminal portion of the DCLK1 protein contains tandem doublecortin domains that allow the protein to bind and bundle MTs and shares high homology to DCX. This may allow for survival in DCX knockout mice, as a DCLK1-DCX double knockout shows significant embryonic and perinatal lethality (Deuel et al. 2006). DCX loss in humans, while not embryonically lethal, does result in significant developmental defects such as lissencephaly, double cortex syndrome, a high prevalence of epilepsy, and shortened lifespan (Kaur et al. 2015, des Portes et al. 1998, Gleeson et al. 1998, Bahi-Buisson et al. 2013, Deuel et al. 2006). However, DCX knockout mice were found to have a much milder phenotype, experiencing disrupted hippocampal organization and reduced male lifespan but maintaining normal neocortical layering (Corbo et al. 2002). Conversely, DCLK1 knockout alone does not result in severe developmental defects and does not appear to cause epilepsy, although it's considered a gene of interest in some mental health disorders (Deuel et al. 2006, Håvik et al. 2012, Wu et al. 2012, le Hellard et al. 2009). This redundancy has led many researchers to investigate the roles of both proteins simultaneously using double knockout models.

While it is common to use the double knockout to investigate DCLK1 function, many studies have found individual contributions of the protein outside of direct DCX involvement. Many of these functions relate to its effect on other MT-associated proteins (MAPs) and cytoskeletal structures. When it was first discovered, DCLK1 was found to stimulate MT polymerization at physiological concentrations and bundling when overexpressed (Lin et al. 2000). Further studies exploring this relationship reveal that the removal of the kinase domain actually increased binding to MTs, and that DCLK1 autophosphorylates its C-terminal tail region to reduce its kinase activity and increase MT binding affinity (Patel et al. 2016, Shang et al. 2003, Agulto et al. 2021). Ablation of the C-terminal tail negates all MT binding of the protein, which can be rescued by mutating phosphosites in the doublecortin domains, indicating MT affinity is dependent on their lack of phosphorylation (Agulto et al. 2021). This domain assembly may act as an internal control for the protein's affinity for MTs, despite the phosphorylations leaving the gross protein conformation unchanged.

The localization of DCLK1 is tied closely to its functionality. In the developing neuron, DCLK1 is expressed in the soma and throughout the processes (Shin et al. 2013). However, although expression is widespread throughout the neuron, the DCLK1 localization is more intense in the tips of the processes. Once the neuron has matured more, e.g. by DIV25, this intensity is uniform throughout the neuron and doesn't localize preferentially at the tips (Shin et al. 2013). Tendency for DCLK1 to localize at the tips of these growing processes is likely related to its function in the growth of these processes as it is highly expressed in the growth cones of axons and dendrites (Liu et al. 2012, Shin et al. 2013). To examine this a group use shRNAs to reduce DCLK1 protein levels and

examine the effect on numerous parameters of dendritic development in DIV4 hippocampal neurons in vitro (Shin et al. 2013). They found that the total dendrite length of these knockdown neurons were significantly reduced and it could be rescued by overexpression of a DCLK1-GFP plasmid by DIV9. shRNA knockdown also decreased the dendritic complexity of the neurons, with fewer intersections occurring between dendrites.

Interestingly, while dendritic length and complexity were significantly decreased by shRNA knockdown of DCLK1, this decrease was not completely mirrored in axonal length decrease. The mean total axon length for control neurons was ~1250 um, while the mean total axon lengths for the two tested DCLK1 shRNAs were ~950 um and ~750 um at DIV5. Although these decreases appear sizable they were not statistically significant using a Tukey's test and counting ~30 cells for each group. Other groups have similarly investigated the effect of DCX and/or DCLK1 knockout on axon length using different methods and different neuronal ages. One group examined axon length in rat superior cervical ganglion neurons after DCX/DCLK1 double knockdown using siRNA at DIV4 (Jean et al. 2012). They found that a double knockdown significantly reduced the length of these axons, although their methodology for neuronal culture is somewhat irregular. The researchers plated the neurons and allowed growth until DIV3 before they dissociated and replated the neurons, noting that these neurons under low or medium density could attach but could not actually extend axons. The researchers used a treatment of laminin the following day to stimulate axon growth and measured axon length 2-3 hours later. These axonal lengths were very different compared to the previously

discussed DIV5 neurons, with mean length of the control neurons only reaching ~160 um and double knockdown neurons reaching ~140 um.

A different group followed a more standard protocol to examine axon growth and benefited from using control neurons, DCX RNAi knockdown neurons, DCLK1 knockout neurons, and DCX knockdown/DCLK1 knockout treatment groups (Deuel et al. 2006). Using these different combinations allowed the researchers to examine both the individual contributions to axon elongation from DCX and DCLK1 and the combinatorial contribution from loss of both proteins. At DIV5-6, the axons of control neurons reached a mean length of 977 um, while the axons of DCX knockdown neurons were significantly shorter with a mean length of 702 um. Axon lengths from DCLK1 knockout neurons very closely mirror the DCX knockdown axon lengths, with a mean length of 745 um. Finally, DCX was knocked down in DCLK1 knockout neurons to test how loss of both proteins affected axon length. This generated the most striking difference, with most axons not reaching even 200 um in length and the mean of the axon length being 230 um. These results help demonstrate just how strongly compensatory these two proteins are. Loss of either causes a phenotypic defect of a very similar magnitude, while loss of both severely compounds this defect. Unfortunately, although there are four groups that could be compared, it appears the researchers did not opt to use Tukey's test to compare all of the groups. They instead used two student's t-tests to compare control vs DCX RNAi and to compare DCLK1 knockout vs DCLK1 knockout/DCX RNAi. Because of this there was never statistical analysis carried out to compare DCLK1 knockout neurons and control neurons, leaving us unsure if loss of DCLK1 alone can significantly contribute to axon elongation defects from this set of experiments.

To probe which domains of DCLK1 might be responsible for increasing dendritic length and complexity, they began by removing the kinase domain. Expression of this RNAi-resistant kinase-dead mutant did not alter dendritic length or complexity, indicating that the mechanism responsibly is likely not dependent on kinase activity. The researchers then replaced the microtubule binding domains of DCLK1 with the MAP2 microtubule binding domain. While MAP2 does localize to dendrites and this chimeric DCLK1-MAP2 construct was able to bind and bundle microtubules in non-neuronal cell types, neurons with this chimeric protein suffered decreases in dendrite length and complexity similar to the DCLK1 knockdown neurons. This result underscores the importance of DCLK1's microtubule binding specificity, as the tandem doublecortin domains likely allow DCLK1 to bind either different microtubules or bind to microtubules in a different manner. While MAP2 and DCLK1 both prefer to bind to tyrosinated (more dynamic, less stable) microtubules, it is possible that replacing DCLK1's binding sites causes DCLK1 to bind too irregularly to perform its function (Lipka et al. 2016). The change in MT binding domain might also make it more difficult for DCLK1 to accumulate at the tip of the dendrite, which could impede its function (Lipka et al. 2016).

While the authors of the Shin et al. study make some very interesting observations, there are some troubling aspects related to their observations related to DCLK1 and its microtubule association promoting dendritic growth. In their experiments they transfect with a DCLK1-GFP repeatedly to visualize DCLK1 at the dendritic tip and to observe the localization throughout the neuron. However, they also comment on the bundling activity they observe at the dendritic growth cone in the DCLK1-GFP neurons and postulate that this bundling could be part of the mechanism by which DCLK1 helps to extend dendrites

and affect dendrite morphology. The authors fail to mention anywhere about how overexpression of microtubule-associated proteins can cause bundling to occur, even if those proteins do not bundle microtubules at physiological concentrations. This is despite both previous observations in their own work that do not appear to show significant bundling by antibody staining of neurons or based on previous in vitro work done on DCX, which contains the same microtubule binding domains (Gleeson et al. 1999). Based on these oversights it is important that we treat possible mechanisms that rely on protein overexpression with due skepticism, as they often do not reflect the behavior of the protein at physiological concentrations.

While examining other areas of distinct DCLK1 expression, researchers found it associated with a biochemically purified PSD fraction possibly corresponding to dendritic spines (Jordan et al. 2004, Yoshimura et al. 2004, Phillips et al. 2004). Double labeling of PSD-95 and DCLK1 in hippocampal neurons showed DCLK1 was enriched in the PSD-95 rich dendritic spine compartments, possibly indicating a functional role in dendritic spines (Shin et al. 2013). To explore this relationship these researchers overexpressed DCLK1-GFP using adenoviruses in neuronal culture and found that while PSD-95 immunoreactivity was reduced, it was not due to a decrease in the protein amount. They found that this increase in DCLK1 expression was actually modifying the presynaptic structures alongside the dendritic spines. Overexpression caused a decrease in VGLUT1-positive presynaptic puncta and a decrease in dendritic spines. This decrease in dendritic spines was accompanied by a decrease specifically in mushroom bodies. When DCLK1 was knocked down using shRNAs, dendritic spine number and volume were both increased, and the ratio of mushroom bodies was increased as well. These observations

taken together are evidence that excitatory synaptic development is diminished by DCLK1.

DCLK1 uses both its kinase domain and MT binding domains to modulate cellular functions that utilize the cytoskeleton. Researchers have found MAP7D1 is responsible for complete callosal axon elongation made possible after it is phosphorylated by DCLK1 (Koizumi et al. 2016). DCLK1 is also necessary for mediating the KIF1-dependent trafficking of presynaptic vesicles and dense core vesicles into dendrites through its association with MTs (Lipka 2016 et al, Liu et al. 2012). Certain actin-associated pathways, such as dendritic spine growth and maturation, have been shown to require DCLK1 binding at specific motifs (Murphy et al. 2023). Additionally, DCLK1 affects multiple other important neuronal functions with mechanisms that have not been explicitly attributed to its association with microtubules. Most notably, DCLK1 enables growth cone formation, growth cone turning, and dendrite length and complexity (Fu et al. 2013, Shin et al. 2013, Nawabi et al. 2015). Research continues to show new and evolving understanding of the role of DCLK1 in many fundamental processes in the brain.

1.6 Why study DCLK1 in temporal lobe epilepsy?

While the connection between DCX, neural development, and epilepsy is well-established, the connection between DCLK1 and epilepsy is more complex. Loss of DCX results in gross morphological defects in humans, including lissencephaly and subcortical band heterotopia, which are likely the cause of the dysfunction. While loss of DCX in mice results in a far less severe morphological difference and no isocortical defects (attributed to compensation by DCLK1), there are still significant hippocampal abnormalities and reports of sporadic spontaneous seizures (Nosten-Bertrand et al. 2008, Lapray et al.

2010). Loss of DCLK1 alone in both human and mouse models also does not appear to cause gross morphological changes, although some report anatomical differences in the corpus callosum (Koizumi et al. 2006). However, mice with DCLK1 knocked out alone do not appear to experience seizures, and require the tandem knockout of DCX for the appearance of the most severe phenotype (Deuel et al. 2006).

Despite this lack of seizure activity in the DCLK1 knockout mouse, there is additional evidence it may still play a role in epilepsy even outside of its compensation for DCX. One study examined changes in gene expression in epileptogenic tissue from epileptic human patients experiencing tuberous sclerosis or focal cortical dysplasia (Li et al. 2017). DCLK1 expression was significantly higher in epileptogenic tissue in both sets of patients, almost double compared to control tissue. This is reminiscent of early findings from 1999, where a research group identified an mRNA transcript acutely elevated in the dentate gyrus, CA1, and CA2 of rats after kainate induced seizures (Vreugdenhil et al. 1999). That transcript actually coded for CARP, one of the aforementioned splice variants of DCLK1. When CARP was artificially overexpressed in mice, researchers found a decrease in CA3/CA1 excitability (Schenk et al. 2010).

How DCLK1 could affect epilepsy in an activity-dependent manner could be related to splice variant changes that are a result of activation. One research group used dual bioinformatics approaches, ANOSVA (ANalysis Of Splicing VAriation) and FIRMA (Finding Isoforms using Robust Multichip Analysis) to identify acute splice variant changes in response to kainate induced seizures (Denkena et al. 2020). Mice were given either a vehicle control or given kainic acid to induce status epilepticus and hippocampal samples were collected either 1 hour, 4 hours, or 8 hours after injection. DCLK1 splice

variants were among the most highly upregulated of the genes analyzed using the chip analysis, showing the most upregulation 1 hour and 4 hours after seizure onset. The DCLK1 exon showing the clearest upregulation in response to seizure was exon 9, responsible for a transcript encoding the beginning amino acids of the kinase domain of DCLK1. These researchers then consulted two additional resources: RNA sequencing databases from murine hippocampus after seizures and dissociated primary neuronal cultures after neuronal firing was activated using potassium-induced depolarization. These additional databases also show that DCLK1 exons 7-9 are upregulated in response to activity, strong evidence that there is a connection between these exon upregulations and excitation. Exons 7 and 8 encode transcripts responsible for most of the PEST linker region, connecting the microtubule-binding doublecortin domains to the kinase domain in the protein. This activity-dependent change agrees with in vitro experiments that show a potassium-induced excitation of primary hippocampal neurons triggers similar splicing changes (Quesnel-Vallières et al. 2016). While it is unclear how this increase is occurring or what its function is, there is one report that examined changes in microRNAs in the dentate gyrus of epileptic rats (Zhang et al. 2017). The microRNA most consistently downregulated in epileptic rats was Rno-miR-187-3p, which only targets a handful of genes, one of them being DCLK1. Conversely, researchers found that DCLK1 expression was significantly downregulated in a mouse model with overactive calcium channels and dysregulated Ca2+ response that caused seizure-like events (Majewski et al. 2019).

Although multiple lines of research have shown DCLK1 expression changing in response to epilepsy, there have been no studies that have explored that further. Significant phenotypic differences in a DCX knockout mouse require loss of DCLK1, yet

DCLK1 has been completely neglected concerning studies examining its effect in epileptic animals. This is all compounded by the fact that DCLK1 is highly expressed in every segment of the hippocampal circuit, contributes significantly to synaptic connectivity, and is important for numerous aspects of neuronal development. Considering TLE is a disease involving dysfunctional circuit remodeling in the hippocampus, the role of DCLK1 in this process requires further inquiry.

2 Materials and Methods

2.1 Animals and mouse model

Mice were housed in split cages with no more than 4 animals per cage and adhered to all Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) facility guidelines. Care guidelines and procedures strictly adhered to National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee at Brown University (protocol 20-10-002). DCLK1 floxed mice were obtained from The Jackson Laboratory (strain #: 013170). All founder mice were backcrossed at least three times to The Jackson Laboratory C57BL/6J (strain: #000664) background.

Experimental mice were genotyped using a polymerase chain reaction (PCR). The WT forward primer (5'-CAGGACACAGATGGGGAACT-3') was used with the mutant forward primer (5'-CTTCCCACTGATATGTTCATTC-3') and a common reverse primer (5'-AGTGAGATGGTTTACAGGCAAG-3'). The thermocycler conditions used were: 95C for 3 min before 34 cycles of 95C for 30 sec, 62C for 45 sec, then 72C for 1 min with a final extension at 72C lasting 5 min. PCR products were run on a 1.5% agarose gel and imaged. The wild type fragment amplifies at 382 bp while the mutant fragment is 480 bp. DCLK1 f/f mice were crossed with a female ZP3 cre mouse from The Jackson Laboratory (strain #: 003651) to acquire females with +/- offspring which were then crossed again to acquire full knockout progeny. Loss of gene expression was verified using western blot.

2.2 Pilocarpine induction of epilepsy

Mice were handled 1 day prior to pilocarpine induction to ease stress and improve survival during SE. Pilocarpine concentration was either 335 mg/kg at ~ZT6 for mortality and Western blot experiments or 250 mg/kg at ~ZT10 for all other experiments. 8-10 week old mice were given 22.5 mg/ml ethosuximide, 0.3 mg/ml methylscopalamine, and 0.3 mg/ml terbutaline hemisulfate 30 min prior to pilocarpine injection. After pilocarpine injection mice were monitored for 30 min to ensure successful SE induction by continuous behavior at Racine score >2.5. Racine score is a scoring system to grade a seizure's severity from 0-5 (Racine 1972). 0 is no abnormal activity, 1 is mouth and facial movements, 2 is head nodding, 3 is forelimb clonus, 4 is rearing, 5 is rearing and falling. After 2 hrs of continuous SE mice were given 10 mg/kg diazepam and 1 ml Ringer's solution. Mice were placed back in original cages with heating pads and access to hydrating diet gel. Mice were monitored for 3 days afterwards and additional hydration with Ringer's solution was administered as needed.

2.3 Electroencephalography depth electrode implantation

4 month old mice were deeply anesthetized using continuous flow isoflurane and given buprenorphine (0.5 mg/ml, 0.1 mg/kg) prior to surgery. Mice were placed in a stereotaxic frame on a heating pad for the duration of the surgery. While under anesthesia the head was shaved, sterilized with povidone and ethanol, and lidocaine was injected subcutaneously under the scalp at target incision area. The area was sterilized again with ethanol and betadine before incisions revealing the scalp were made. The exposed skull was then sterilized with hydrogen peroxide before application of 1 drop of iBond. After UV hardening for 40 secs, a sharpie was used to denote drill points for the three leads. First a marking point was established 2 mm posterior to the bregma on the midline. Then two

drill points were then established by marking the points 2 mm to the left and right of that marking point. The hippocampal and cortical drill points were established as those 2 mm to the left and right of the point 2 mm posterior of the bregma. The cerebellar drill point was located 1 mm posterior of the lambda along the same line as the midline. Holes were then drilled at those three spots. A headset (853-43-006-20-001000, Sager electronics) with wire leads soldered on with lengths 0.3 cm (hippocampus), 0.1 cm (cortex), and 0.3 cm (cerebellum) were then inserted through the drill sites. The EMG leads were 1 cm and inserted to make contact with the muscles of the upper back. Dental cement was then mixed and applied to seal and solidify the headset on the skull. Super glue was used to stabilize the edges of the dental cement. After removal from the frame mice were placed on a heating pad for at least 30 min to recover and regain ambulation before returning to a cage. Sirenia software was used to collect and analyze EEG data. Sirenia sleep was used to calculate power spectral analysis data and sleep/wake bouts and duration.

2.4 Sleep architecture quantification

Sleeping data was collected starting at 7 am of the second day of recording and lasting 24 hours for all forms of EEG analysis. Sleep and wake cycles were determined based on hippocampal and cortical EEG, traces on the EMG, and visual inspection from video recording. A change in the sleep or wake cycle lasting less than 30 seconds was excluded from analyses that quantified bouts of sleep. Characteristics of wakefulness and sleep such as bout number, average duration, and total duration were quantified using Sirenia Sleep Pro. This was done by labeling all sleeping and waking periods and using default software settings to average and total duration. Bout number was also calculated by the

software using default settings and is a count of the discrete numbers of sleeping and waking periods. Researchers were blinded to the genotype and treatment of the mice.

2.5 Immunohistochemistry

Brain acquisition and preparation

Mice were given a lethal dose of pentobarbitol and loss of consciousness was validated by toe pinching and a blink reaction to a paintbrush. The absence of a blink reaction is a measure of corneal reflex, used to confirm a loss of consciousness or death. Mice were then transcardially perfused with 30ml cold PBS followed by 30ml of cold 4% paraformaldehyde (wt/vol) at pH 7.4. Brains were then stored in 4% PFA for 48 hours before being stored in 30% sucrose until saturated for cryoprotection. To slice the brain we used a Micro HM 430 freezing microtome (Thermo Scientific) and cut sections 30um in thickness. Slices were then stored in an anti-freezing solution at -20C until antibody staining.

Staining

Slices stored in anti-freezing solution were rinsed in PBS before staining. Slices were then blocked and permeabilized in a pH 7.4 PBS solution containing 0.05% Triton-X100 and 10% goat serum for 1 hr in a 24 well plate. Antibodies were then applied in specified dilutions in primary solution (antibody, 5% goat serum, PBS pH 7.4). Slices incubated in primary solution overnight at 4C. The next day the primary solution was removed and slices were washed for 10 min in pH 7.4 PBS for ten minutes 3 times. After washing the secondary antibody solution was applied, consisting of PBS pH 7.4 and a 1:500 dilution of secondary antibody. Slices were incubated in secondary antibody solution for 2 hrs in the dark. Secondary antibody solution was then removed and replaced with a 1:2000

solution of Hoechst 33342 solution (Invitrogen, H3570). This incubated for 10 minutes before being removed and washed three times with PBS pH 7.4. Slices were then placed on slides and mounted with Fluoromount-G (Cat#0100-01, Southern Biotech, Birmingham, AL). Mountant was allowed to harden at least 48 hours before imaging. Coverslips were sealed using a clear nail polish. Samples were then stored at 4C. Slides were imaged using an Olympus FV3000 confocal microscope and processed using ImageJ.

2.6 Timm staining

We utilized the FD Rapid TimmStain Kit (FD NeuroTechnologies) for perfusant and visualization of mossy fibers. Instructions for perfusing were followed as provided in the kit using the provided perfusant. Brains were sliced on a freezing microtome at -30 C and cut at a 30 micron thickness. Subbed slides were used for mounting the slices for staining, all other steps for staining and washing were done as outlined in the instructional manual. Slides images were captured at 4X, 10X, or 20X on a Nikon Ti2-E fluorescence microscope. Mossy fiber sprouting severity was scored from 0-5 as described in Cavazos et al. 1991. The scoring system is as follows: 0, no granules; 1, sparse granules in the supragranular region; 2, increasingly numerous granules in the supragranular region, less patchy with a more continuous distribution; 3, prominent granules in the supragranular region with occasional confluent patches; 4, prominent granules within the supragranular region forming a confluent dense band; 5, confluent dense band of granules extending into the inner molecular layer.

2.7 Immunocytochemistry

Primary neurons grown on coverslips were fixed by immersion in 4% Paraformaldehyde with PBS pH 7.4 at 37C for 10 min to preserve MT structure. Cells were then washed three times with room temperature PBS pH 7.4. We permeabilized the cells by immersing them in a 0.1% Triton X-100 mixture for 10 min. They were then washed again in PBS three times. Cells were then incubated in a blocking solution containing: 10% goat serum and 0.1% Triton X-100 in PBS for 30 minutes. Cells were then incubated with a primary antibody solution for 2h comprised of: 5% goat serum, 0.1%, and the specified concentration of primary antibodies in PBS. After this incubation cells were incubated for ten minutes in a 1:2000 hoechst 33342 solution (Invitrogen, H3570) in PBS for 10 min before being washed two times in PBS for 10 min. Coverslips were then mounted onto slides using Fluoromount and allowed to harden overnight. Coverslips were imaged using the Deltavison OMX (General Electric) using a 0.014 optical oil (General Electric).

2.8 Antibodies

Immunochemistry

The following antibodies were used for staining brains: DCLK1 (Abcam, ab31704, 1:500), Parvalbumin (#P3088, Sigma Aldrich,1:400), Somatostatin (Peninsula labs, T-4103.0050, 1:, 1:500), Calretinin (Swant, CG1, 1:600). Primary cells were stained with: DCLK1 (ab31704, 1:500), Znt3 (SYSY, 197 004, 1:1000), Calbindin (Swant, CB38, 1:500), VGAT (Synaptic systems 131 004, 1:1000), Prox1 (Novus, 30045, 1:500). Western blots were stained with: DCLK1 (ab109029, 1:2500), GAPDH (Merck Millipore, ABS16, 1:2500), DCX (Merck, AB2253, 1:2500). All secondary antibodies were Alexa fluors acquired from Invitrogen at 1:5000.

2.9 Western blot

Brain acquisition and preparation

Mice were cervically dislocated and decapitated with scissors. The whole intact brain was removed with forceps and surgical scissors. The brain was then separated into different sections (hippocampus, cortex) using a razor blade. Brain sections were put on dry ice before being put into storage at -80C. For western blotting preparation brains were homogenized in a dounce homogenizer with 1% protease inhibitor in RIPA buffer. Brain tissue samples were centrifuged for 15 min at 4°C at 13,200 RPM. Protein was quantified using a BCA analysis. Samples were mixed with Laemmli buffer and run at roughly 30 ug of protein per sample. Samples were run for approximately 1hr at 100V on an NuPAGE 4%-12% SDS-polyacrylamide gels (Invitrogen) and transferred onto a PVDF membrane. Membranes were then blocked for an hour before being incubated with the primary antibodies in blocking buffer overnight. Membranes were washed 3x with TBST for 5 min each before secondary antibody in blocking buffer was introduced for 1h. This was then washed off again 3x with TBST for 5 min each before being imaged on a Li-Cor Odyssey Clx Infrared Fluorescent Western Scanning / Imaging System. All proteins were normalized to a loading control mentioned (e.g. GAPDH).

2.10 Dissociated primary hippocampal neuronal culture

Hippocampal neurons were acquired and plated as previously described (Ouyang et al. 2013). Cells were seeded at a density of 3.0×10^5/ml into Neurobasal medium with 2% 114B27 and 1% Glutamax supplementation. Cultured neurons were fixed using 4% PFA at DIV 10.

2.11 FluoroJade-B staining

Neuronal degeneration was visualized three days post pilocarpine-induced SE using the gold standard anionic fluorescein derivative FluoroJade B (Millipore Sigma 32160702). Brains were sliced as previously mentioned from the Immunochemistry section and mounted on gelatin-coated slides. Slices were stained with Fluoro-jade B solution according to previously published work (Botterill et al. 2019).

2.12 Local field potential recording

Mice were briefly anesthetized with isoflurane before cervical dislocation and decapitation. The skull and brain were immediately immersed in ice cold Sucrose-ACSF (90 nM sucrose, 80 mM NaCl, 24mM NaHCO3, 10 mM glucose, 3.5 mM KCl, 1.25 mM NaH2PO4 monobasic, 5 mM MgCl2 hexahydrate, 1 mM CaCl2) with a constant supply of oxygen being bubbled into solution. Osmolarity of solutions was measured using the model 3320 Osmometer (Advanced Instruments Inc). Brains were sliced on a Leica VT1200S vibratome at 0.16 mm/s, 1.00 mm vibration, at a 300 um thickness using Double-edge blades (Ted Pella Inc, #121-6). Slices were allowed to rest 45 mins in room temperature ACSF (130 mM NaCl, 24 mM NaHCO3, 10 mM glucose, 3.5 mM KCl, 1.25 mM NaH2PO4 monobasic, 2 mM MgCl2, 2 mMCaCl2) with a constant supply of oxygen being bubbled in before recording. The recording chamber is by Scientific Systems Design Inc, using a Peri-star Pro pump (World Precision Instruments) that flows oxygenated ACSF over slices at 3 ml per min. The slices were visualized in the chamber using an AmScope LED-144 illumination microscope. Glass pipettes for stimulation and recording were drawn using a P-1000 Flaming/Brown micropipette puller (Sutter Instrument) with 1 MΩ resistance. The Digitimer Neurolog System model NL905 was

used as an amplifier. Power for stimulations was provided by a Constant Current Isolated Stimulator (Digitimer Ltd) through an A-In NL100 electrode, and for series of currents applied the current was increased manually by 20 uA for each step. Each step was stimulated and recorded twice and the average was taken for each power.

pClamp was the software used for stimulating and recording LFP. The number of population spikes was recorded as the measured number of spikes at the highest current injection (320 uA) in the slice. For measuring population spike threshold the lowest current injection that elicited a population spike was used. Clampfit 11.2 was used to measure the initial slope of the fEPSP. The initial slope of the fEPSP was measured as the slope of the line made by two points, one at the center of the trough and one at the peak of the fEPSP.

2.13 Epileptiform activity identification in sleep and wake

For each mouse sleep was evaluated for establishment of baseline sleeping brain activity, based on prolonged periods of sleep time with no interruption. This baseline value included amplitude maxima that fell within a normal range of baseline brain activity. When identifying spikes, we looked for activity with characteristic sharp rise and decline wave patterns that had amplitudes ~2 times greater than the baseline brain patterns for sleep. Usually these discharges were much greater than this threshold and easily identifiable as a contrast from a quiet sleeping background. Mice were video recorded 24 hours a day so any spikes were double checked by visual observation and EMG recording to make sure no movement was confounding EEG activity. Seizure events while awake were determined by rhythmic >3 Hz deflections continuously repeated for at least 5 seconds with accompanying behavioral reaction of Racine 3 or higher.

2.14 Statistical analysis

The criterion for significance is always set at $p < 0.05$. All graphical data is expressed as the mean with the SEM. All statistical tests used (student's t test, ANOVA/Tukey's test, Fisher's exact test) were performed in Prism (Graphpad). Trends are occasionally designated when groups may appear somewhat different but significance may be hampered by sample size or variance. The only post-hoc test used (Tukey's test) is corrected for multiple comparisons inherently. Quantification or observation of any kind for all treatment groups were blinded whenever possible.

3 Results

3.1 DCLK1 expression is increased by pilocarpine-induced SE in mice

There have been several studies reporting an increase in DCLK1 or its transcript variants in epileptic brains or tissue. These studies have been done using human tissue or using rats and their acute response to a kainate-induced seizure (Vreugdenhil et al. 1999, Li et al. 2017, Denkena et al. 2020). While useful, no studies up to this point have investigated whether the expression of full-length DCLK1 changes in a mouse model of epilepsy, either acutely or over a greater span of time. To answer this question we took two month old mice and induced SE using 335 mg/kg of pilocarpine hydrochloride. These mice hippocampi were then collected either one day, seven days, or 60 days post SE. We then performed Western blot analysis on these brains to and compared them to control mice to determine whether DCLK1 expression was acutely or latently increased. We found that expression did not significantly change one day or seven days post injection, but was greatly elevated 60 days post injection (Figures 4A and 4B). This increase agrees with literature showing increases in DCLK1 in epileptic humans (Li et al. 2017). Based on these observations it appears that DCLK1 expression increases during chronic epilepsy.

3.2 DCX expression is unchanged by pilocarpine-induced SE in mice

Many of the experiments investigating DCLK1 functionality have been coupled with DCX because of their compensatory mechanisms. DCX is also a marker for newborn neurons, especially in the dentate gyrus, a site where neurogenesis commonly occurs (Spampanato et al. 2012). Adult neurogenesis can be stimulated by seizure, increasing the number of newborn granule cells up to two weeks after SE in rats (Parent et al. 1997).

To see whether DCX expression is increased concomitantly with DCLK1 expression after pilocarpine treatment, we performed a Western blot analysis on the same pilocarpine-treated animals. We found DCX expression was not significantly changed during any time period after SE (Figures 4C and 4D). This is helpful for understanding any changes resulting from pilocarpine-induced epilepsy are unlikely to be attributable to increases in DCX, and that the response to pilocarpine is specific to DCLK1.

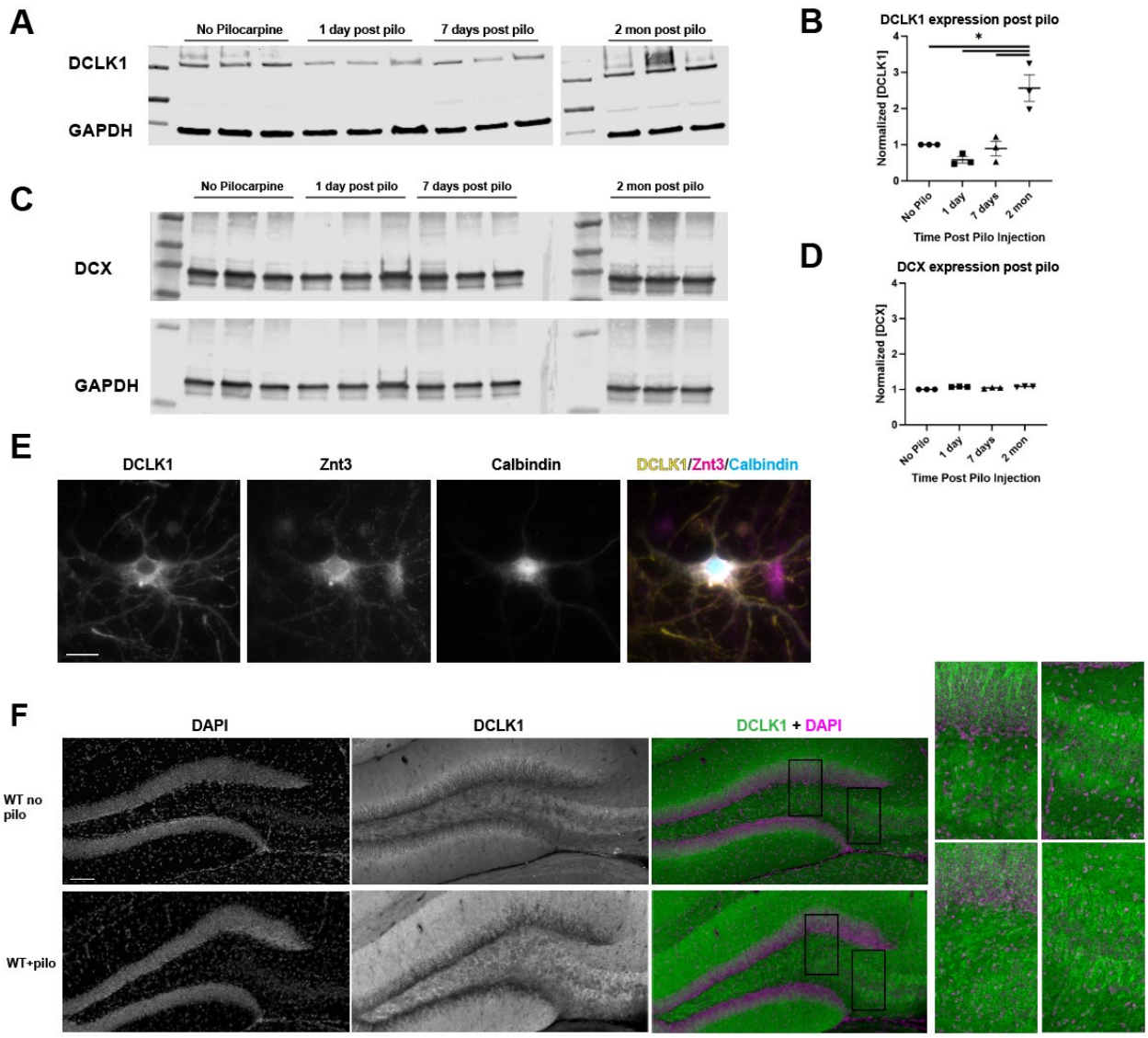

Figure 4. DCLK1 is expressed throughout the hippocampus and its expression is increased in epileptic mice.

***A*,** DCLK1 expression in the hippocampus without pilocarpine injection and expression 1 day, 7 days, and 2 months after pilocarpine injection in WT mice. All mice were 2 months old at time of injection; n=3 for each group of mice. ***B*,** Western analysis of DCLK1 protein concentration 1 day, 7 days, and 2 months after pilocarpine injection reveals an increase 2 months after injection. DCLK1 expression was normalized to GAPDH expression, and treated groups were normalized to the WT expression set at one. Groups were analyzed with a one-way ANOVA (*$p < 0.05$, asterisk over the bar). ***C*,** DCX expression in the hippocampus from the same mice as figure **(A)**. ***D*,** Western analysis of DCX protein concentration 1 day, 7 days, and 2

months after pilocarpine injection shows no change in DCX protein level. DCX expression was normalized to GAPDH expression, and treated groups were normalized to the WT expression set at one. Groups were analyzed with a one-way ANOVA (*$p < 0.05$, asterisk over the bar). ***E*,** DIV10 hippocampal neurons with granule neuron markers show robust DCLK1 expression. Scale bar: 10 um. ***F*,** DCLK1 expression appears to be slightly higher in the hilus and adjacent CA3 in WT mice 2 months after pilocarpine injection. Scale bar: 100um.

3.3 DCLK1 is expressed in immature granule cells

The first region of the hippocampus that receives input from the entorhinal cortex is the middle and outer layers of the molecular layer, where they synapse with the dendrites of the granule cell layer. This array of cells is mostly comprised of granule cell neurons, a class of neuron characterized by its infrequency in propagating electrical signals downstream to the rest of the hippocampus, serving as a “gate” of sorts (Krook-Magnuson et al. 2015). This lack of electrical signal propagation is critical for the health of these downstream hippocampal substructures, as they are vulnerable to excess electrical signal and require full functionality of this gating function. These granule cells are also one of the few neuronal cell types that participate in adult neurogenesis, with substantial consequences on epilepsy progression (Jain et al. 2019). To identify whether DCLK1 is expressed in these immature granule neurons we plated dissociated mouse hippocampal neuron cultures and performed immunostaining at DIV10. By costaining with multiple markers we identified granule neurons and found robust expression of DCLK1 (Figure 4E). This would indicate that DCLK1 is strongly expressed in the dentate granule cells of the hippocampus.

3.4 DCLK1 expression may be increased in early trisynaptic circuit

Based on Western blot analysis we found that DCLK1 expression in the hippocampus is significantly increased in epileptic animals. To determine where this expression is occurring we stained the hippocampus for DCLK1 and compared wild type mice with mice injected with pilocarpine two months prior (Figure 4F). Both groups of mice exhibit strong DCLK1 expression throughout the hippocampus, clearly illuminated in processes in the hilus, extending apically and basally around the CA3, and extending to the CA2 and CA1.

There appears to be slightly increased expression of these processes in the hilus and around the CA3 of the animals that received pilocarpine two months prior. Interestingly, these processes in the hilus do not appear to be the contribution of solely mossy fibers, as Znt3 staining of mossy fibers causes complete saturation of the hilus where individual processes are unable to even be distinguished. Such is the case for Timm staining of mossy fibers as well. This would indicate that chronic epilepsy is causing upregulation in the initial segments of the trisynaptic circuit.

3.5 Zona pellucida 3 (ZP3) *cre* is effective for embryonic DCLK1 deletion

Previous experiments have shown that DCLK1 deletion early in mouse development does not adversely reduce mouse viability or survival and would still capture any potential developmental defects (Deuel et al. 2006). This allows for technical advantages in an embryonic knockout breeding scheme while still allowing for evaluation of a full knockout and any potential developmental defects that may contain altered brain circuitry. To knock out DCLK1 embryonically we utilized a *cre* recombinase regulated by the mouse Zona pellucida 3 (Zp3) promoter to recombinantly remove a floxed region in DCLK1 exon 3 (de Vries et al. 2000, Koizumi et al. 2006). We then performed western blot analysis to detect presence of DCLK1 in wild type, heterozygotic deletion, and full deletion hippocampus and cortex (Figure 5). We found high expression of DCLK1 in wild type mice, diminished expression in heterozygous knockouts, and absence of expression in full knockout mice.

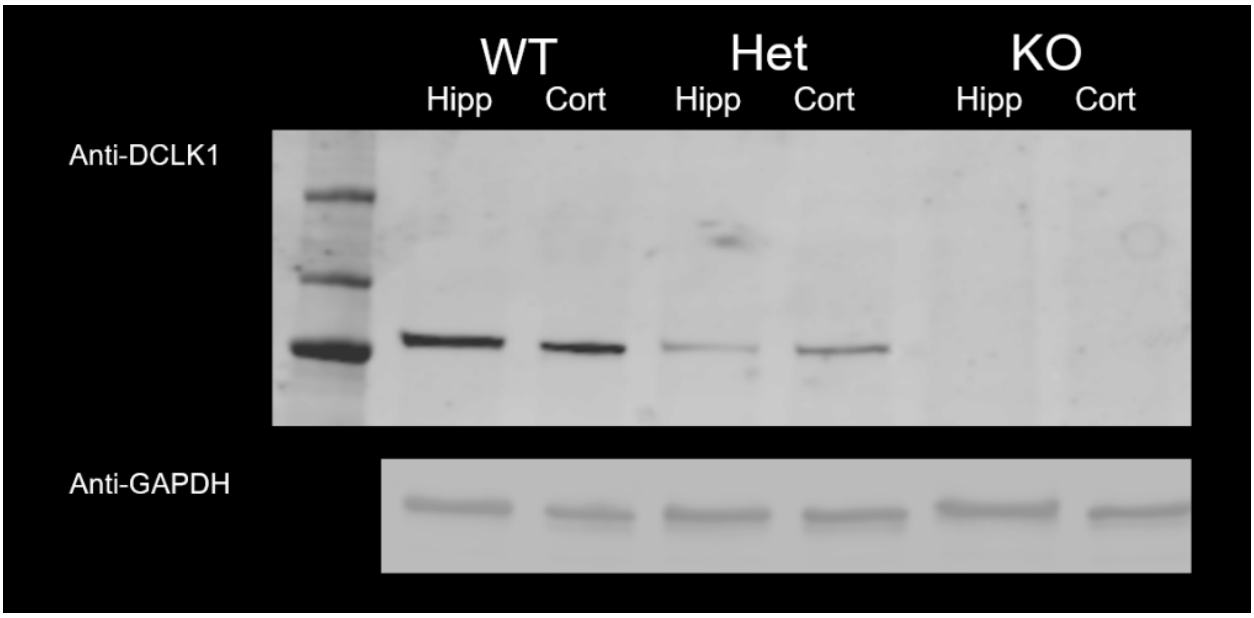

Figure 5. Zp3 regulated *cre* recombinase effectively ablates DCLK1 expression.

Western blot analysis of adult mouse brain lysates from both the hippocampus and cortex reveals Zp3-cre effectively deletes DCLK1 with mice containing the floxed alleles.

3.6 DCLK1 knockout mice have lower survival rates from pilocarpine-induced SE

To understand the role of DCLK1 in an induced epilepsy model we injected 2 month old mice with 335 mg/kg pilocarpine at ZT6 to trigger status epilepticus (SE) with the hope the mice would later develop spontaneously recurrent seizures (SRS) (Figure 6A). In wild type mice this yielded roughly a 50+% survival rate (Figure 6B). However, injecting knockout mice using the same treatment resulted in a much lower survival rate, with only ~10% of animals surviving SE. This high lethality for epilepsy induction is too high of a technical hurdle for efficient generation of epileptic DCLK1 knockout mice. To circumvent this we lowered the pilocarpine concentration to 250 mg/kg and injected the animals at ZT10, a time of day where SE is less severe and less lethal in other mouse models of epilepsy (Zhang et al. 2021). This increased the survival rate for DCLK1 knockout mice to a level likely severe enough to generate SRS while still allowing for enough survivability for sample size requirements (Figure 6B). If loss of DCLK1 was a factor for SE induction it would be useful to know how the seizure threshold is affected. To test for changes in seizure threshold we took mice of each genotype with and without pilocarpine and tested the latency to spiking activity using an implanted EEG after injection of pentylenetetrazole (PTZ), a GABA-A receptor antagonist that triggers seizures (Shimada et al. 2018, Dhir et al. 2012, Watanabe et al. 2013). Although there was a trend for DCLK1 knockout mice post pilocarpine injection, overall there was no significant difference between groups (Figure 6C). These results indicate that DCLK1 knockout mice have a similar seizure vulnerability to wild type mice but are more susceptible to death from status epilepticus.

3.7 DCLK1 knockout mice have more FluoroJade+ cells after SE

Quantifying damage to a hippocampus can be challenging, but one clear way to visualize acute neuronal death is using the FluoroJade stain to identify degenerating neurons (Jain et al. 2019, Botterill et al. 2019). We injected wild type and knockout mice with 250 mg/kg pilocarpine and perfused them three days later before staining. To quantify the number of degenerating neurons in the hippocampus we first attempted a fluorescence intensity quantification but highly variable amounts of background fluorescence reduced the reliability of this method for our samples. We then resorted to cell counting for each region of the hippocampus, combining CA3, CA2, and CA1 to avoid ambiguity in where the CA2 starts and ends. Unsurprisingly the granule cell layer had very little cell death, while the hilus had more FJ+ cells (Figure 6D). This is likely due to the granule cell layer being mostly composed of the excitation-resistant granule cells. However, DCLK1 knockout animals had more FJ+ cells in their CA1-CA3 and greater total FJ+ cells overall in the hippocampus (Figure 6E-6G). This greater number of FJ+ cells would indicate that the DCLK1 knockout mice are more vulnerable to pilocarpine-induced neuronal death.

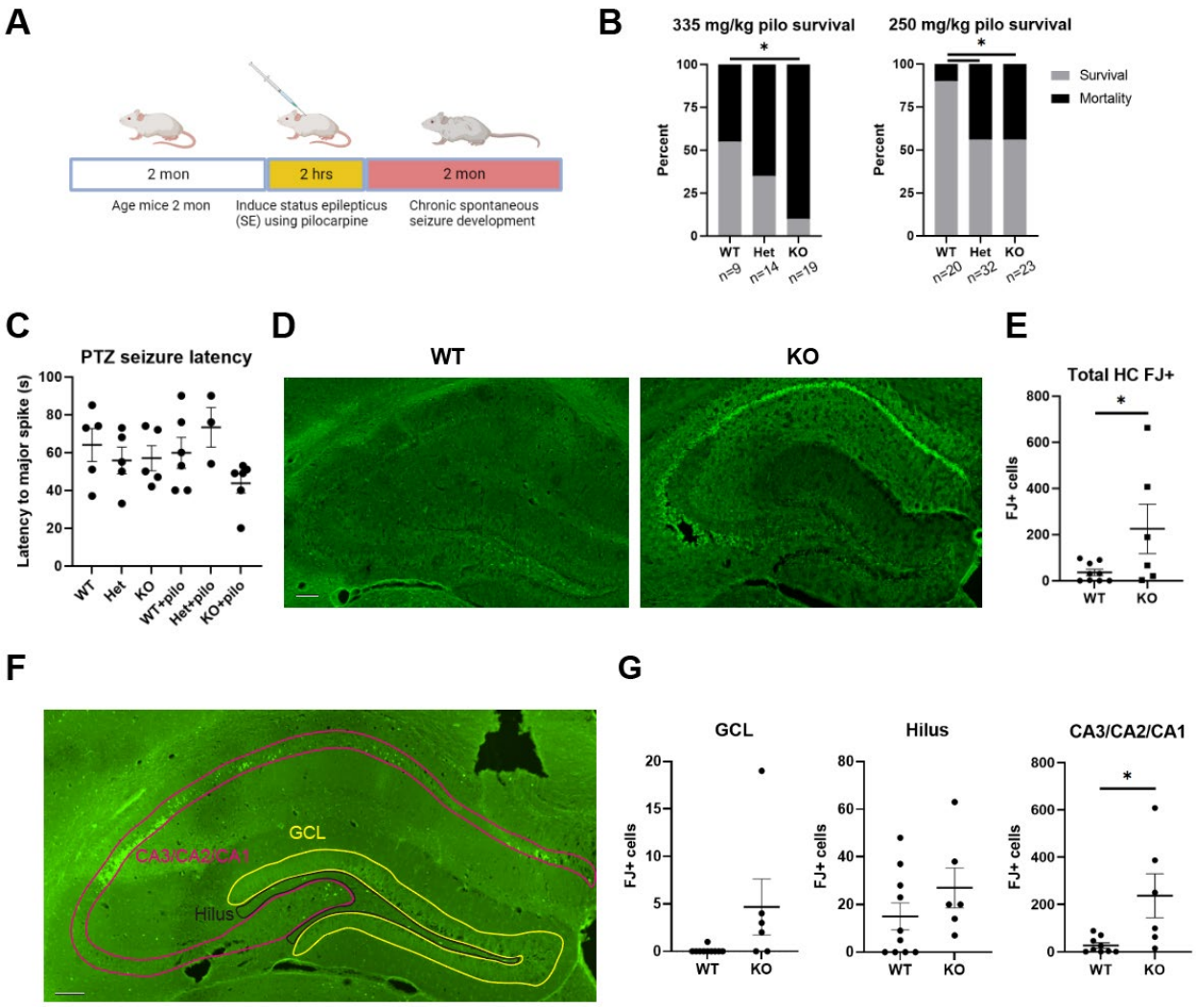

Figure 6. DCLK1 knockout mice are more susceptible to death and hippocampal damage from pilocarpine administration.

***A*,** Schematic illustrating the pilocarpine administration protocol for inducing epilepsy. ***B*,** Knocking out DCLK1 negatively affects the survival chance of pilocarpine administration at multiple concentrations. Percent survival between genotypes at 335 mg/kg of pilocarpine at ZT6 and at 250 mg/kg of pilocarpine at ZT10; analyzed by Fisher's exact test between groups (*$p < 0.05$, asterisk over the bar). For 335 mg/kg: WT n=9, Het n=14, KO n=19. For 250 mg/kg: WT n=20, Het n=32, KO n=23. ***C*,** The latency to seizure after PTZ injection in 4 mon old mice is not significantly different between genotypes or treatment groups. For WT n=5, Het n=5, KO n=5, WT + pilocarpine n= 6, Het + pilocarpine n=3, KO + pilocarpine n=6. ***D*,** Fluorojade staining of the hippocampus of two month old wild type and knockout mice three days after 250 mg/kg pilocarpine injection; scale bar is 100 um. ***E*,** Knockout mice have more fluorojade positive cells

throughout the hippocampus (GC, hilus, and CA3/CA1) than wild type mice three days after a 250mg/kg pilocarpine injection by student's *t* test (*p < 0.05, asterisk over the bar). WT n=9 and KO n=6 ***F*,** Defining the boundaries for individual cell counts of Fluorojade positive cells in the Granule Cell Layer (GCL), Hilus, and CA3/CA1. Scale bar is 100 um. ***G*,** Counts for FJ positive cells in each region of the hippocampus shows insignificant trends in the GCL and hilus and a significant increase in the knockouts in the CA3/CA1 by student's *t* test (*p < 0.05, asterisk over the bar). GCL: WT n=10, KO n=6; Hilus: WT n=10, KO n=6; CA3/CA1: WT n=10, KO n=6.

3.8 DCLK1 knockout mice post pilocarpine have fewer hippocampal PV+ neurons

In both patients and animal models of epilepsy, inhibitory interneurons are often reduced (Rosen et al. 1998, Houser et al. 1986, de Lanerolle et al. 1989, Marco et al. 1996, André et al. 2001). They help represent the critical inhibition portion of the excitation/inhibition imbalance often found in epilepsy. If there was a change in the number of these interneurons in the hippocampus of DCLK1 knockout mice, it could help explain changes in excitation/inhibition imbalance that might affect the phenotype. We stained the hippocampus of two month old wild type and DCLK1 knockout mice for Parvalbumin (PV), a marker of a major inhibitory interneuron subtype. We found no difference in the PV+ cell number in the hippocampus between wild type and DCLK1 knockout mice (Figure 4A, 4C, and 4D). To see if this remained true in epileptic mice, we stained for PV in the hippocampus of wild type and DCLK1 knockout mice two months after both groups received pilocarpine (Figure 7B-7D). We found pilocarpine-treated DCLK1 knockout mice had fewer PV+ cells in the hippocampus than pilocarpine-treated wild type mice. DCLK1 may have a protective effect on these interneurons during status or a worse epilepsy phenotype from lack of DCLK1 could be responsible for the loss.

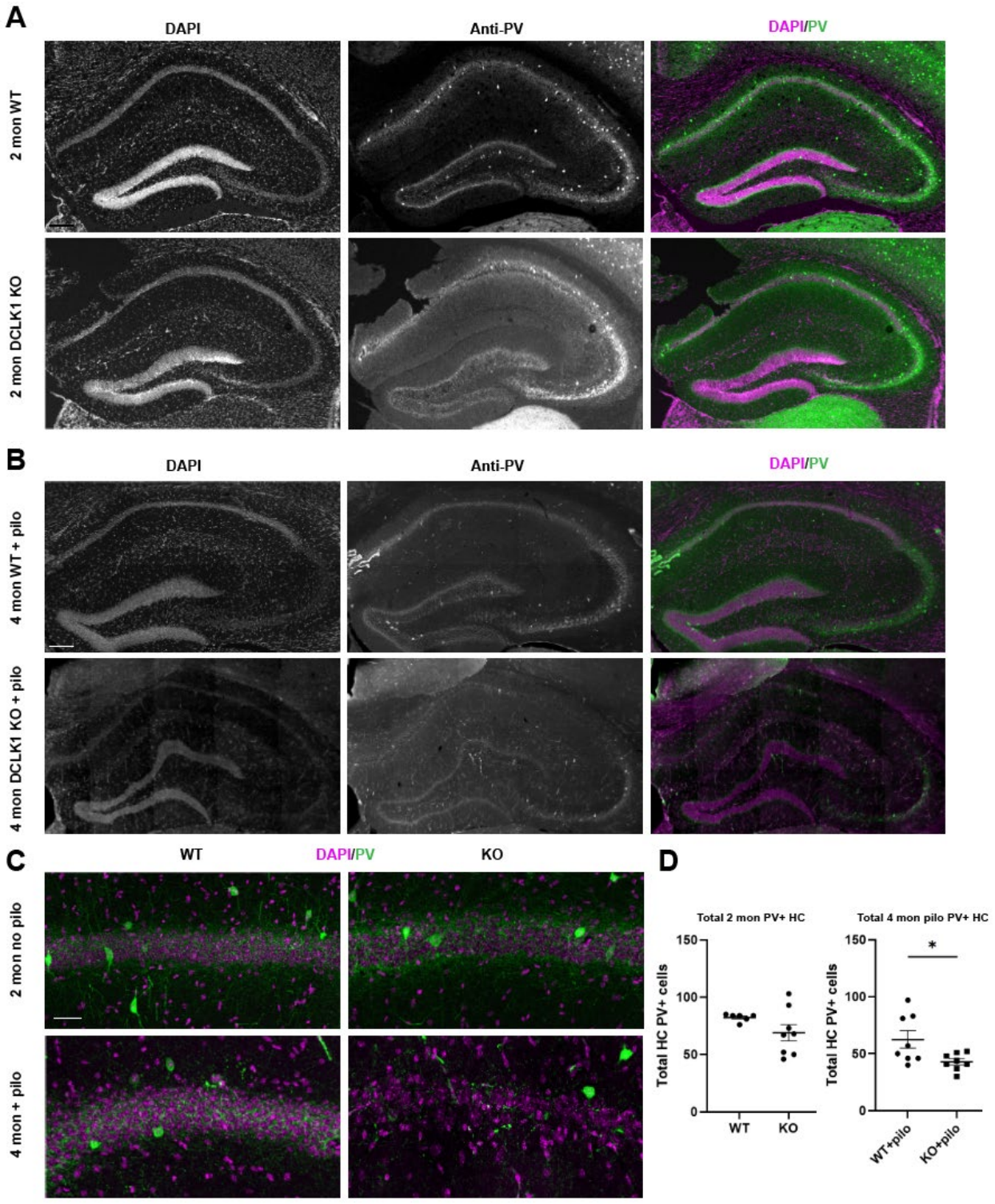

Figure 7. Pilocarpine-treated DCLK1 knockout animals have reduced numbers of PV positive cells compared to pilocarpine-treated wild type animals.

***A*,** PV antibody staining of 2 month old mouse hippocampi reveals no change in positive cells in knockout mice compared to wild type mice. Scale bar is 100 um. ***B*,** PV antibody staining of 4 month old mouse hippocampi (two months post pilocarpine injection) reveals a decrease in positive cells in the pilocarpine-treated DCLK1 knockout mice. Scale bar is 100 um.. ***C*,** Higher power magnification of PV antibody staining of 4 month old mouse CA1 shows a decrease in positive cells in the pilocarpine-treated DCLK1 knockout compared to the pilocarpine-treated wild type. Scale bar is 30 um. ***D*,** PV positive cell counts show fewer cells in pilocarpine-treated DCLK1 knockout animals compared to pilocarpine-treated wild type animals by student's *t* test (*$p < 0.05$, asterisk over the bar); n=6 for WT, n=8 DCLK1 knockout, n=8 for pilocarpine-treated wild type and n=8 for pilocarpine-treated DCLK1 knockout animals.

3.9 DCLK1 knockout mice post pilocarpine have fewer hippocampal SST+ neurons

Somatostatin (SST) positive interneurons are the other major subtype of GABAergic inhibitory interneuron found in the brain. To gauge if there were any differences with this subtype we again stained the hippocampus of 2 month old wild type and DCLK1 knockout mice, this time for SST. We found that there was no significant difference in SST+ cell number between these groups (Figure 8A, 8C, and 8D). When we compared wild type mice and DCLK1 mice two months after both groups had received a pilocarpine injection, we found that DCLK1 knockout mice had fewer SST+ cells in the hippocampus (Figure 8B-8D). Based on this immunostaining it appears that DCLK1 knockout mice are being affected by pilocarpine more than wild type mice and this is being reflected in greater losses of both interneuron sub types.

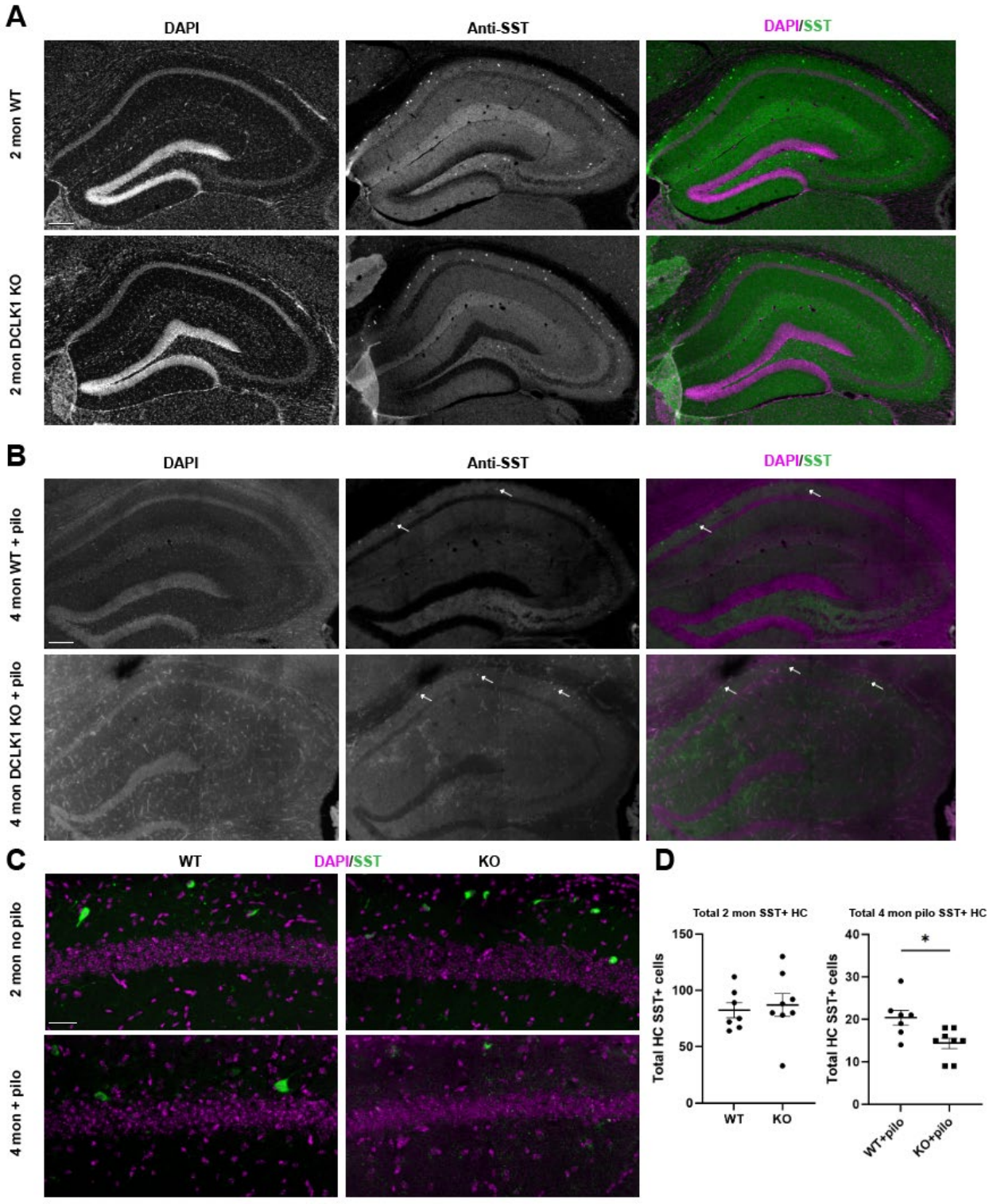

Figure 8. Pilocarpine-treated DCLK1 knockout mice have reduced numbers of SST positive cells compared to pilocarpine-treated wild type mice.

***A*,** SST antibody staining of 2 month old mouse hippocampi shows no change in SST positive cells in knockout mice compared to wild type mice. Scale bar set at 100 um. ***B*,** SST antibody staining of 4 month old mouse hippocampi (two months after pilocarpine injection) shows a decrease in positive cells in pilocarpine-treated DCLK1 knockout mice. Arrows indicate examples of SST positive cells; scale bar is 100 um. ***C*,** Higher power magnification of SST antibody staining of 4 mon old mouse CA1 segment shows a decrease in SST positive cells in the pilocarpine-treated DCLK1 knockout compared to pilocarpine-treated wild type. Scale bar is 30 um. ***D*,** SST positive cell counts show fewer positive cells in pilocarpine-treated DCLK1 knockout animals compared to the pilocarpine-treated wild type animals by student's *t* test (*$p < 0.05$, asterisk over the bar); n=7 for WT, n=8 DCLK1 knockout, n=7 for pilocarpine-treated wild type and n=8 for pilocarpine-treated DCLK1 knockout animals.

3.10 DCLK1 knockout mice post pilocarpine have more Calretinin+ cells

When studying the DCX knockout mouse, many reported few changes without deleting DCLK1 as well. However, in one of these studies researchers did find DCX knockout mice that experienced seizures and significant perturbations in EEG (Nosten-Bertrand et al. 2008, Lapray et al. 2010). One of the hallmark changes in these epileptic mice was an increase in Calretinin+ cells, a change also found in other genetic models of epilepsy, and observed in the kainic acid model (Rüttimann et al. 2004, Nosten-Bertrand et al. 2008, Jessberger et al. 2005). To measure this we took wild type and DCLK1 knockout mice injected with pilocarpine two months prior and stained the hippocampus for Calretinin (Figure 9). We counted Calretinin positive cells near the subgranular zone (SGZ), which is where newly generated granule cells reside. We found that pilocarpine-treated DCLK1 knockout mice had significantly more Calretinin positive cells in this zone than their pilocarpine-treated wild type counterparts, possibly as a result of a more severe epileptic phenotype.

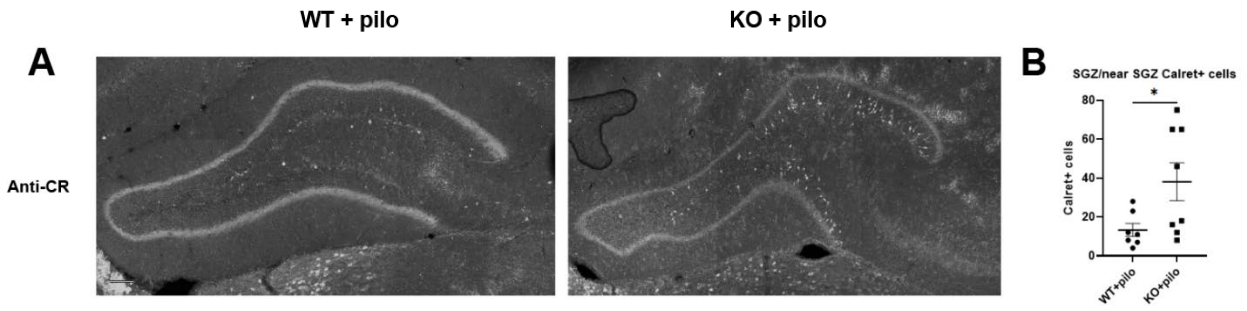

Figure 9. Pilocarpine-treated DCLK1 knockout mice have increased numbers of Calretinin positive cells compared to pilocarpine-treated wild type mice.

***A*,** CR antibody staining of 4 month old mouse hippocampi (two months after pilocarpine injection) shows an increase in positive cells in pilocarpine-treated DCLK1 knockout mice. Scale bar is 100 um. ***B*,** CR positive cell counts show more CR positive cells in pilocarpine-treated DCLK1 knockout animals compared to the pilocarpine-treated wild type animals by student's *t* test (*$p < 0.05$, asterisk over the bar); n=7 for pilocarpine-treated wild type and n=8 for pilocarpine-treated DCLK1 knockout animals.

3.11 DCLK1 knockout mice exhibit increased mossy fiber sprouting post pilocarpine

Mossy fiber sprouting (MFS) is a complex anatomical change that takes place in the hippocampus of many TLE patients and animal models (Nadler et al. 1980, Sutula et al. 1989). After an epileptic insult the axons of granule cells may sprout axon branches that, instead of growing towards the CA3/CA2, grow the opposite way towards their own dendritic arbors. Here they can form functional recurrent circuits, and many questions have been raised about how this aberrant recurrent circuit formation may affect epilepsy development and progression (Danzer et al. 2017). To determine if MFS is affected by DCLK1 knockout we performed Timm stains on each genotype with and without pilocarpine (Figure 10). None of the untreated mice of any genotype showed any significant MFS. When we stained the pilocarpine-treated animals, we found that knockout mice did exhibit significant MFS, and significantly more than any other group. Based on these observations it appears DCLK1 suppresses epilepsy-induced mossy fiber sprouting.

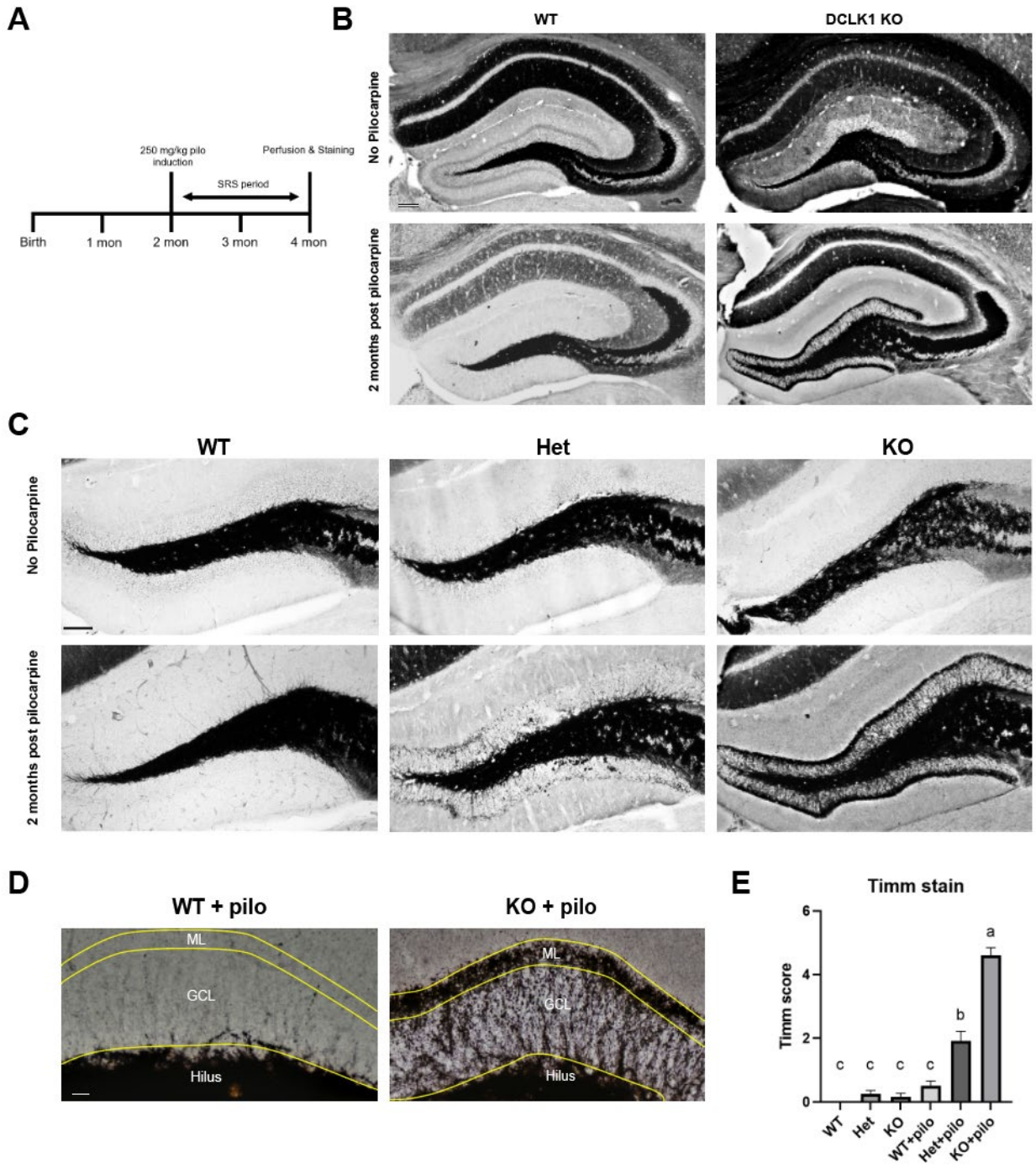

Figure 10. Pilocarpine-treated DCLK1 knockout animals experience more severe MFS than pilocarpine-treated wild type animals.

***A*,** Experimental timeline for pilocarpine induction, epilepsy onset, and sacrifice for staining mossy fibers via Timm stain. ***B*,** Hippocampal brains experiencing MFS show a highly similar pattern of Timm staining to brains without MFS, with the exception being the characteristic darkening of the granule cell layer and

molecular layer. Scale bar set at 100 um. ***C*****,** Timm staining of 4 month old hippocampal slices of varying genotypes and with or without pilocarpine injection 2 mon prior. Scale bar is set at 100 um. ***D*****,** Higher magnification images of Timm stained hippocampal slices show minimal MFS in pilocarpine-treated wild type animals and increased MFS in pilocarpine-treated DCLK1 knockout animals. The molecular layer (ML), granule cell layer (GCL), and hilus are delineated. Scale bar is 50 um. ***E*****,** Timm staining scores were assigned to each genotype with and without pilocarpine injection. Scores range from 0-5 based on the Cavazos et al. (1991) scoring criteria. DCLK1 knockout mice with pilocarpine had the most severe MFS compared to all other genotypes and treatments. DCLK1 heterozygous mice with pilocarpine had the second most sprouting, less than the full knockouts but more than the rest of groups. All other groups had a sprouting amount not significantly different from one another. Significance was determined by Tukey's test ($p < 0.05$, letters correspond to groups not significantly different from each other). Each hemisphere of the brain was scored for each mouse. For WT n=2, Het n=3, KO n=3, WT + pilocarpine n= 4, Het + pilocarpine n=3, KO + pilocarpine n=4.

3.12 DCLK1 knockout mice with pilocarpine experience increased epileptiform discharge number during sleep

EEG recordings can be useful for identifying electrical abnormalities both during sleep and wakefulness that are not apparent in the mouse's behavior (Figure 11A and 11B). Particularly useful is the analysis of epileptiform activity during sleep that does not elicit any behavioral reaction. These synchronous discharges can take place in isolation or can appear in long trains of repeated discharges that never stimulate wakefulness in the animal. To determine the extent to which DCLK1 knockout and pilocarpine-treated mice experienced these epileptiform discharges during sleep, we quantified the number of discharges over a 24 hour period for each group. The wild type and knockout mice rarely if ever experienced any activity during sleep that could be construed as epileptiform (Figure 11C). Pilocarpine-treated wild type mice experienced increased epileptiform discharges compared to untreated animals, but rarely had a series of discharges. Pilocarpine-treated DCLK1 knockout animals had the most epileptiform discharges during sleep and had the highest number of series (Figure 11C-11G). This hyperexcitability was not reflected in the recording of local field potentials for any of the treatment groups in multiple regions of the hippocampus (Figure S1A). This also did not translate to changes in population spike threshold or number of population spikes triggered by maximal current injection (Figure S1B). These experiments show that DCLK1 knockout mice with pilocarpine show the greatest amount of epileptiform activity compared to all the other treatment groups, indicating DCLK1 may play role in epileptiform suppression.

3.13 DCLK1 knockout mice with pilocarpine experience increased seizure occurrence

The best indicator of changes in epilepsy is the number of seizures a patient or animal experiences. To examine changes in the number of seizures we took pilocarpine-treated wild type and DCLK1 knockout mice and performed EEG surgery and placed them in observation chambers. We then quantified the number of motor seizures these mice experienced over the course of 24 hours (Figure 11I-K). We found that the pilocarpine-treated wild type mice rarely experienced seizures, with only 2/8 experiencing a seizure during the observation window. However, 5/7 of the pilocarpine-treated DCLK1 knockout mice experienced a seizure, and some experienced multiple seizures during this time. Altogether the pilocarpine-treated DCLK1 knockout mice experienced more seizures than the pilocarpine-treated wild type mice. This would indicate that DCLK1 may be responsible for reducing seizure occurrence in epileptic animals.

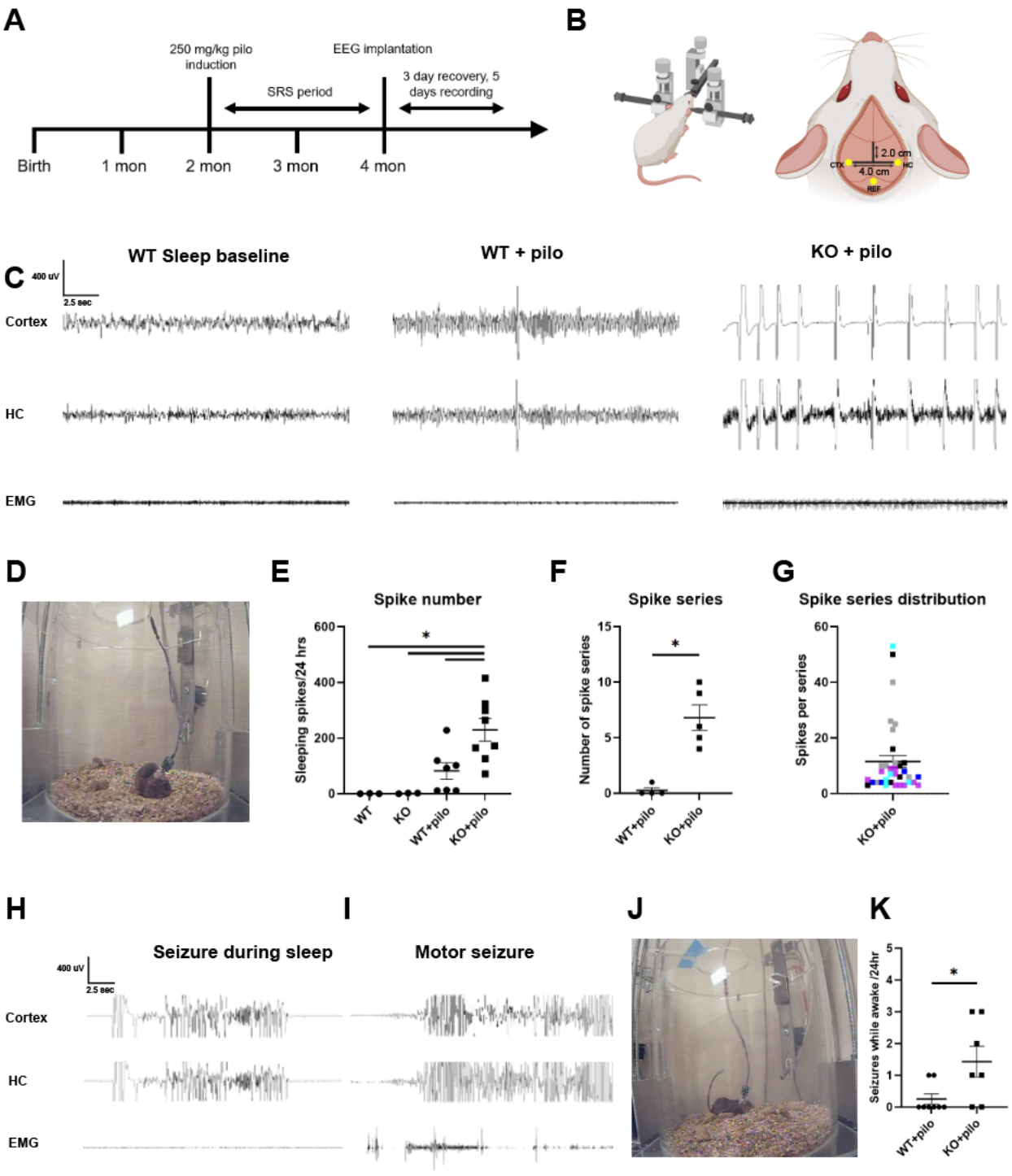

Figure 11. DCLK1 knockout animals with pilocarpine experience greater numbers of epileptic sleeping spikes and seizure events while awake than wild type mice with pilocarpine and either genotype without pilocarpine.

***A*,** Schematic representation of experimental procedure for EEG/video recording of a mouse with pilocarpine-induced epilepsy. ***B*,** For implantation of depth electrodes mice were heavily sedated with isoflurane and placed in a stereotaxic headset. Holes were drilled in the skull for electrode insertion corresponding to the hippocampus (HC), cortex (CTX), and reference in the cerebellum (REF). ***C*,** Representative sleep baseline EEG recordings for four month old wild type mice, wild type mice with pilocarpine, and knockout mice with pilocarpine. Wild type mice without pilocarpine and knockout mice without pilocarpine have similar baselines (knockout representative image not shown). The top row corresponds to traces from the cortex, the middle row is the hippocampus, and the bottom row is the EMG trace monitoring muscular response. ***D*,** Frame captured from the video monitoring of EEG recorded mice. ***E*,** Epileptiform discharges as spikes during sleep are increased in knockout mice with pilocarpine compared to other genotypes with and without pilocarpine by Tukey's test (*$p < 0.05$, asterisk over the bar). Wild type n=3, DCLK1 knockout n=3, wild type with pilocarpine n=7, knockout with pilocarpine n=8. ***F*,** Examining series of spikes during sleep shows there are more series in knockout animals with pilocarpine compared to wild type animals with pilocarpine by a student's *t* test (*$p < 0.05$, asterisk over the bar); n=4 for pilocarpine-treated wild type and n=5 for pilocarpine-treated DCLK1 knockout mice. ***G*,** A graphical depiction of the sleeping spike series distribution from the five pilocarpine-treated DCLK1 knockout mice. Each color represents a different mouse. ***H*,** A representative trace of a significant sleep event in a pilocarpine-treated knockout mouse. High amplitude and frequency waveforms occur without any EMG output as the mouse remains asleep. ***I*,** A representative trace from the beginning of a convulsive seizure as the mouse awakens from sleep. ***J*,** A frame captured from the convulsive seizure in (G). ***K*,** Motor seizures in mice during waking periods were more frequent in DCLK1 knockout mice with pilocarpine compared to wild type mice with pilocarpine by a student's *t* test (*$p < 0.05$, asterisk over the bar); n=8 for pilocarpine-treated wild type and n=7 for pilocarpine-treated DCLK1 knockout mice.

3.14 DCLK1 knockout mice show altered numbers of sleep bouts

Sleep is often significantly affected by epileptic activity (Roliz et al. 2022). To determine the extent to which this is true in DCLK1 knockout mice, we measured numerous sleep parameters in pilocarpine-treated and untreated wild type and DCLK1 knockout mice. We began by quantifying the number of sleeping and waking bouts in these mice over 24 hrs and found that DCLK1 knockout mice without pilocarpine had significantly fewer waking bouts than wild type animals that received pilocarpine and fewer sleeping bouts than both pilocarpine-treated groups (Figure 12A). While significant, this result may be confounded by the presence of one wild type animal that received pilocarpine and experienced 40+ separate sleep and wake bouts. Of the 33 animals, ~48% (16/33) experienced 10-19 wake bouts, ~48% (16/33) experienced 20-29 wake bouts, 0% experienced 30-39 wake bouts, and ~3% (1/33) experienced 40-49 wake bouts. Of these same 33 animals, ~52% (17/33) experienced 10-19 sleep bouts, ~45% (15/33) experienced 20-29 sleep bouts, 0% experienced 30-39 sleep bouts, and 3% (1/33) experienced 40-49 sleep bouts. It appears that DCLK1 knockout animals may be experiencing fewer waking bouts and are experiencing some sleep disturbances even before pilocarpine administration. Based on these observations, DCLK1 may be responsible for a mouse's sleep stability, although the data supporting this conclusion is tenuous.

3.15 DCLK1 knockout mice show higher average waking bout lengths

We next quantified the average length of each bout of sleep and wakefulness (Figure 12B). Although there was no difference in the average length of each sleeping bout in any group, the untreated DCLK1 knockout animals appeared to experience longer bouts of

wakefulness than their pilocarpine-treated wild type and DCLK1 knockout counterparts. While both of the pilocarpine-treated groups appear to have unchanged sleep bout lengths, their average wake length appears to be reduced, with the pilocarpine-treated knockout mice exhibiting the shortest average waking length. This data indicates loss of DCLK1 may cause increases in wakefulness lengths, although a lack of difference from control mice casts some doubts on the magnitude of this change.

3.16 Pilocarpine-treated DCLK1 knockout mice show altered total sleep and wake lengths

To determine the extent that DCLK1 knockout and pilocarpine injection affect the total amount of sleep a mouse experiences, we quantified the total amount of sleep and wakefulness these mice experienced over 24 hours (Figure 12C). We found that only pilocarpine-treated DCLK1 knockout mice experienced differences. These mice had significantly more total time spent sleeping and had the least time spent awake while the other groups remained unchanged. This indicates that the DCLK1 knockout mice with pilocarpine are experiencing significant sleep/wake length time abnormalities, possibly as a result of increased epileptiform activity.

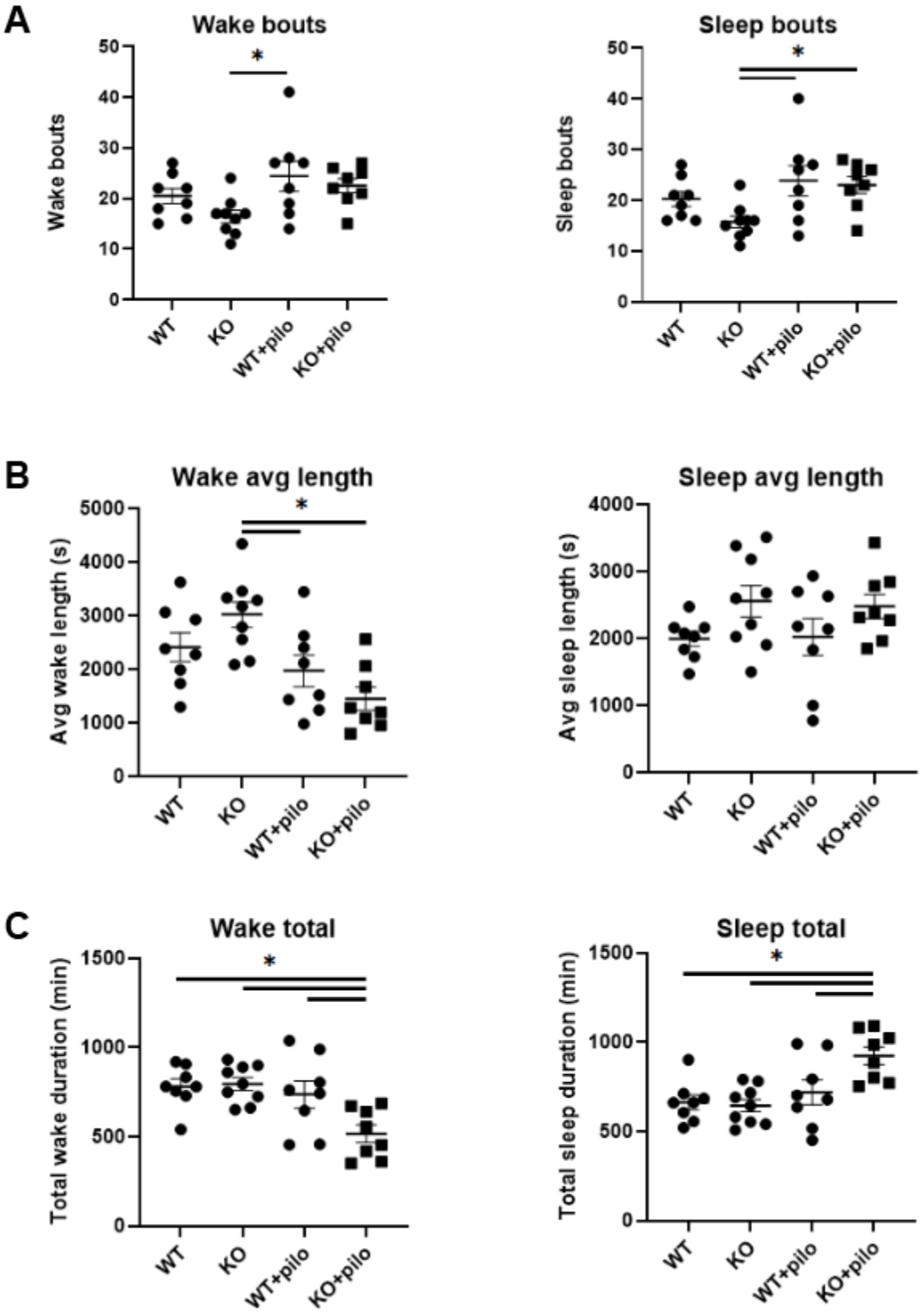

Figure 12. Pilocarpine and DCLK1 knockout affect numerous parameters of the sleep/wake cycle..

***A*,** The number of bouts of sleep and wakefulness in 4 month old wild type and DCLK1 knockout mice with and without pilocarpine injection. DCLK1 knockout mice experience fewer waking bouts than wild type pilocarpine-treated mice. DCLK1 knockout mice also experience fewer sleeping bouts than both pilocarpine-treated groups. For WT n=8, KO n=9, WT + pilocarpine n= 8, KO + pilocarpine n=8. ***B*,** There was no difference in the average sleeping bout length in any treatment groups. Untreated DCLK1 knockout mice had a greater average waking bout length compared to the pilocarpine-treated groups. Pilocarpine-treated DCLK1 knockout mice had a decreased average waking bout length compared to untreated wild type mice as well but the difference was not significant ($p = .06$). Same sample size as previously mentioned. ***C*,** Pilocarpine-treated DCLK1 knockout mice had a significantly greater total sleep duration and significantly lower wake duration than all other treatment groups. Same sample size as previously mentioned.

Supplementary data

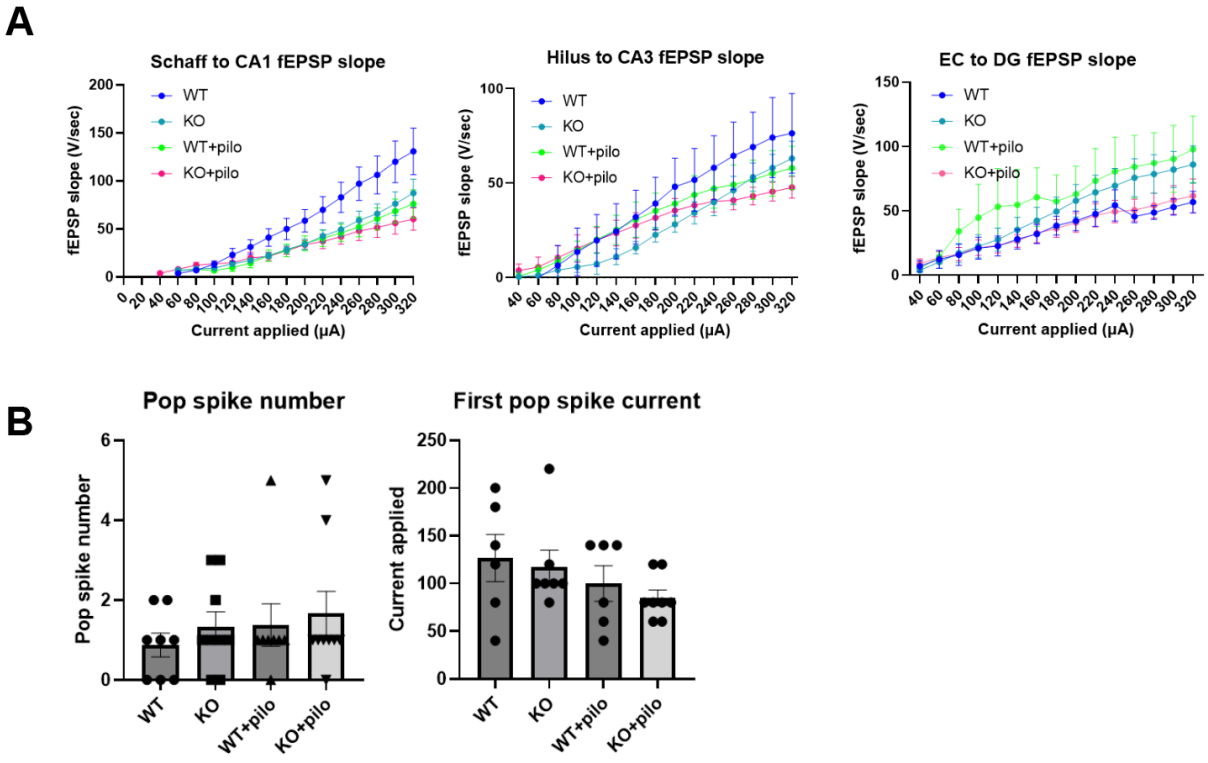

Figure S1. LFP experimentation reveals no differences in excitability between any genotype/treatment..

***A*,** The initial slope of the fEPSP from recording stimulated local field potentials. The Schaffer collaterals were stimulated and recorded at the CA1, the hilus was stimulated and recorded at the CA3, and the entorhinal cortex was stimulated and recorded at the DG. There were no significant differences for any of the genotypes/treatments by two-way RM-ANOVA. For WT n=8, KO n=9, WT + pilocarpine n= 8, KO + pilocarpine n=8. ***B*,** There was no difference in population spike number at max stimulation or the current needed to stimulate the first population spike between any treatment groups. Population spikes recorded from CA1 after stimulations in the Schaffer collaterals. Significance was measured by Tukey's test (*p < 0.05). For WT n=8, KO n=9, WT + pilocarpine n= 8, KO + pilocarpine n=9.

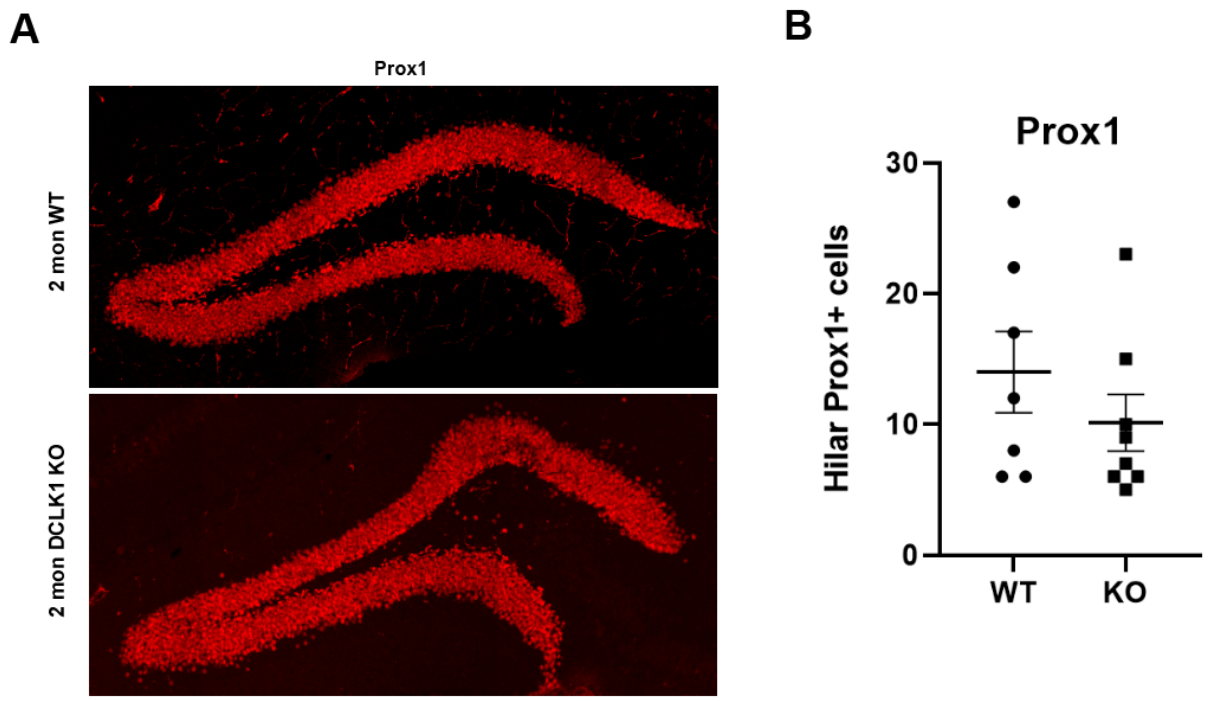

Figure S2. Prox1 staining reveals no change in hilar ectopic granule cells between 2 month old DCLK1 knockout mice and 2 month control mice.

***A*,** Prox1 staining of the hilus and granule cell layer of 2 month old DCLK1 knockout mice and 2 month old control mice. ***B*,** There was no difference in Prox1+ cells in the hilus, indicating ectopic granule cell localization, between 2 month old DCLK1 knockout mice and 2 month old control mice. Significance was measured using student's *t* test (*p < 0.05, asterisk over the bar); n=7 for control mice and n=8 for DCLK1 knockout animals.

4 Discussion

4.1 Significance of increased DCLK1 expression in the hippocampus of epileptic mice

How does epilepsy affect the expression of critical proteins in the mouse brain? In humans we can examine the difference between epileptic brain tissue and healthy brain tissue harvested through therapeutic resections of epileptic foci. If these proteins play a role in epilepsy specifically the expression pattern may even be consistent through epileptic tissue derived from different patient disorders. This is exactly what has been found for DCLK1 through expression profiling of epileptic tissue between patients suffering from focal cortical dysplasia, tuberous sclerosis, and childhood cortical dysplasia (CCD), (Li et al. 2017, Qin et al. 2017). However, how change from pilocarpine-induced SE is reflected in mice both acutely and chronically is an important unanswered question.

We found that our epileptic mouse model closely resembles human patients with respect to increases in DCLK1 expression. While DCLK1 and DCX are highly functionally redundant and compensatory in nature, we found that only DCLK1 expression is altered after mice are experiencing SRS (two months post pilocarpine injection). This lack of change in DCX expression is interesting as it is often used as a marker for neurogenesis and there have been numerous studies examining increases in neurogenesis in the context of epilepsy (Parent et al. 1997, Gray et al. 1998, Jessberger et al. 2005). The methodology for these neurogenesis studies varies greatly, with most using visual quantification methods to identify BrdU+ cells, some using DCX+, and a variety of rat, mouse, kainic acid, and pilocarpine models being used. Many of these studies agree that status epilepticus does stimulate neurogenesis but the variety of methodologies have yielded conflicting results on when it begins, how long it lasts, and the degree to which it

is stimulated. Our data agrees with the majority of groups that show no change to DCX expression in the hippocampus either acutely (1 day post injection) or longer post injection (2 mon), but some groups do report changes in DCX+ cells around the 9 day mark in rats (Parent et al. 1997, Gray et al. 1998, Jessberger et al. 2005). This is despite findings that the number of Nestin+ progenitor cells does not appear to change, but this is also in a kainic acid rat model (Jessberger et al. 2005). Nevertheless, even with any change in DCX expression there is very little evidence that these changes would extend meaningfully past even the latency period of SE (Parent et al. 1997, Gray et al. 1998).

The increase in DCLK1 expression as a result of prolonged epilepsy is an important finding. It corroborates the human data and more indirect findings in animal models that DCLK1 may be involved in epileptogenesis. One possible mechanism for this increase could be the increase in BDNF in the hippocampus of epileptic animals and humans (Ikegaya et al. 1999, Binder et al. 2001, Koyama et al. 2004). BDNF treatment in the hippocampus has been shown to upregulate expression of DCLK1 (le Hellard et al. 2009). BDNF has a complex relationship with epilepsy, with evidence pointing to negative and positive effects on epileptogenesis depending on expression level, expression location, and downstream effects (Wang et al. 2021). Similarly, the DCLK1 expression increase may be an element in the brain's response to epilepsy, used to help remodel or respond to excitotoxic damage. Previous groups have shown that overexpression of DCLK1 or its isoforms can suppress glutamatergic synaptic transmission and decrease hippocampal circuit excitability (Shin et al. 2013, Schenk et al. 2010). How accurately these exogenous overexpression models reflect the brain's increase in DCLK1

expression is not known, but it may give clues on how overexpression could be compensatory in an epilepsy.

While we primarily examine changes in DCLK1 in the hippocampus, there are other areas that could impact epilepsy that also have some limited DCLK1 expression. DCLK1 direct expression data outside of the hippocampus and cortex is quite limited. Direct western blot confirmation has been found for expression in cortex, hippocampus, and cerebellum (Shin et al. 2013). There is indirect RNA-Seq evidence for slightly weaker expression in other brain areas including the amygdala and much weaker expression in other areas like the thalamus based on data collection in ProteinAtlas. The thalamus is particularly interesting, as there is a wealth of evidence that thalamocortical dysfunction can contribute to epileptic activity and therapeutic regulation of this area can improve seizure control (Lindquist et al 2023). We know DCLK1 expression is increased in epileptic mice and humans, possibly as an activity-dependent mechanism. If this mechanism is neuroprotective in some way and this expression increase also occurs in the thalamus, it could be responsible for helping to contribute to seizure control. Loss of DCLK1 in the thalamus could thus be a cause for more severe epilepsy, but confirming evidence for any such pathway remains preliminary. DCLK1 expression and localization in the hippocampus has been the object of far greater study and thus lends itself to greater exploration.

To understand how DCLK1 may modulate the circuitry of the hippocampus, it is important to understand how it is expressed in the most important regions. The dentate gyrus is a critical gate for determining how much electrical activity the downstream hippocampal structures are exposed to, and granule cells are characterized by intrinsic

and extrinsic properties that make them unlikely to fire (Heinemann et al. 1992, Lothman et al. 1992, Acsády et al. 1998, Henze et al. 2002, Coulter 2007 et al, Yu et al. 2013). We found DCLK1 to be strongly expressed in these developing granule cells using granule-specific markers, in agreement with less direct data attempting to characterize isoform localization in tissue via in-situ hybridization (Bergoglio et al. 2021). This is important because DCLK1 expression may play a role in activity-dependent effects that involve granule cells, and compromise or change to these cells may result in dysfunction of their gating ability. When we look at wild type mice and compare them to mice that experienced SE from pilocarpine 2 months earlier, it appears that the processes coming from the granule cells are more highly enriched in DCLK1. These processes clearly penetrate the hilus and extend to the basal and apical regions of the CA3. However, it is likely that these processes are not solely mossy fibers (axons of the granule cells). Timm staining and Znt3 antibody staining both effectively target these mossy fibers which are remarkably rich in zinc transporters, allowing their path through the hilus and to the CA3/CA2 to be easily visualized (Chi et al. 2008). In these visualizations (also notably visible in Figure 10B) mossy fibers are so densely packed in the hilus that they form a solid layer, and it's practically impossible to identify individual processes. However, the processes wrapping around the CA3 richly express DCLK1 and they occupy the same area, possibly indicating a more sporadic DCLK1 expression closer to the end of the axon. It is also possible the processes come directly from the entorhinal cortex (EC), as the CA3 receives input from the EC both directly and indirectly through the granule cells (Amaral et al. 1989).

4.2 Implications of enhanced vulnerability from pilocarpine-induced SE and acute hippocampal degeneration in DCLK1 knockout mice

With the knowledge that DCLK1 expression is modulated by activity and chronic epilepsy, we generated a germline DCLK1 knockout mouse. However, 335 mg/kg pilocarpine induction of SE used to generate epileptic mice had an unexpected effect in these DCLK1 knockout mice. We found that while wild type pilocarpine-treated mice survived at ~55%, DCLK1$^{+/-}$ mice survived ~35% of the time, and DCLK1$^{-/-}$ mice only 10% of the time (Figure 6B). This increase in mortality in knockout mice rendered our current epilepsy induction protocol unusable. To increase survival we modified our protocol to include pre injection handling (1 day before), a lower dose of pilocarpine (250 mg/kg), and injection time at ZT10 instead of ZT6 (based on circadian mortality results) (Zhang et al. 2021). This did increase survival while still inflicting significant damage on the hippocampus. This increase in vulnerability to pilocarpine-induced SE may be indicative of a significant phenotypic difference between DCLK1 knockout mice and wild type mice even before epilepsy. It is possible that the difference in survival at 250 mg/kg could indicate DCLK1 knockout mice may be more likely to experience SRS and a more severe epilepsy phenotype than WT animals. While previous studies have shown alterations in synaptic structure, certain process elongation factors, and subtle changes in neuroanatomy, there have not been any significant functional changes in the mouse as a result of these differences (Koizumi et al. 2017, Murphy et al. 2023, Shin et al. 2013, Koizumi et al. 2006). There was also no change in the susceptibility to PTZ by any genotype or treatment at 4 months old. Development of epilepsy or significant alterations to the health of the mouse without any pharmacological intervention has required co-deletion of DCX.

This increase in SE vulnerability could be due to a variety of factors that are structural, specifically related to the synaptic connectivity in the brain, or activity outside

the CNS. There is still significant interconnection between these two ideas. Human epilepsy from mutated cytoskeletal proteins, such as those seen in DCX, LIS1, and TUBA1A, appear to stem from significant patterning/migration defects that cause significant brain malformations and thus appear to be the main driver of synaptic dysfunction (des Portes et al. 1998, Gleeson et al. 1998, Reiner et al. 1993, Keays et al. 2007). Conversely there exists more isolated cell autonomous mechanisms such as channelopathies, compromise of synaptic connections, and irregular growth of critical neuronal structures (Menezes et al. 2020, Bernard et al. 2012, Xu et al. 2014). While germline loss of DCLK1 can cause some minor malformations, these changes have insofar not meaningfully changed the health of the animal, and whether these defects occur may be dependent on when deletion occurs (Koizumi et al. 2006, Murphy et al. 2023). Germline recombination using conditional EIIa-Cre and removal of exon 3 results in defects to formation of the corpus callosum and anterior commisure, but germline recombination removing exons 9-11 showed no gross morphological changes at all (Koizumi et al. 2006, Deuel et al. 2006). When DCLK1 was deleted postnatally at P10-P13 using the Nex1 promoter to only target pyramidal neurons there were no observable changes in axon tracts, dendritic arborization, or cortical lamination (Murphy et al. 2023). These observations underscore the importance of the temporal regulation of DCLK1 and serve as potential examples for how developmental abnormalities may occur. Considering we utilized an unconditional germline recombination removing exon 3, it is possible certain structural abnormalities are present that could contribute to dysfunction resulting in vulnerability to SE from pilocarpine.

The increase in FJ staining we see in DCLK1 knockout mice is indicative of more damage acutely from SE within the hippocampus as a whole but mostly in the Ammon's Horn. Acute damage from pilocarpine-induced SE has been robustly visualized in previous studies to quantify hippocampal damage (Jain et al. 2019, Scholl et al. 2013, do Nascimento et al. 2012, Botterill et al. 2019). An increase in acute hippocampal damage from SE has previously been attributed to a variety of different factors. However, these factors' commonality appear to stem from a change in the E/I balance of granule cells. Researchers found that a decrease in adult neurogenesis increased hippocampal degeneration from SE and hypothesized that this difference came from the inhibitory input (Jain et al. 2019). They propose that there is a loss of inhibition because these adult-born GCs innervate DG GABAergic interneurons and increase the drive toward inhibition of more mature GCs. A separate study examined the effect of mossy cells, a glutamatergic subpopulation of neurons in the hippocampus bordering the hilus that both innervate DG inhibitory interneurons and the GCs themselves (Botterill et al. 2019). Inactivation of these mossy cells prior to SE appeared to decrease SE-induced damage by preventing the strengthening of MC-GC synapses, which more than compensated for the loss of MC-driven DG interneuron stimulation. Contrarily, a different group found activation of mossy cells optogenetically could reduce the severity of seizures in chronically epileptic mice (Bui et al. 2018). Curiously this same group also found that while direct GC inhibition in chronically epileptic mice reduced seizure duration it did not reduce convulsive seizure occurrence, even though direct GC excitation reliably invokes behavioral seizures (Krook-Magnuson et al. 2015).

4.3 Impact of PV+ and SST+ loss in epileptic DCLK1 knockout mice

These complicated circuitry dynamics even within the same cell type and same disease reflect the complexity of plasticity and synaptic architecture in response to insult both acutely and chronically. The vulnerability to acute damage in the DCLK1 knockout mouse likely has foundations in a similar DG E/I imbalance, but this imbalance does not appear to be caused by loss of PV+ or SST+ hippocampal inhibitory interneurons at the time of injection (Figures 7 and 8). It may be that there is a subtle defect in the synaptic connection responsible for stimulating inhibitory neurons when DCLK1 is not present, as loss of DCLK1 significantly reduces dendritic complexity and dendritic length (Shin et al. 2013). Compromising the inhibition of dentate granule cells before SE induction could be responsible for the acute increase in hippocampal damage and may prime the hippocampus for sclerosis and worsening of the epileptic phenotype. A more severe phenotype may be responsible for the SE-induced changes we see in the DCLK1 knockout mice with respect to inhibitory interneuron number. Many previous studies have shown reductions in the inhibitory interneuron populations in various epileptic models including rat, monkey, and human samples. One group has shown that silencing of even one inhibitory neuron population in the hippocampus is sufficient for induction of SRS in mice (Drexel et al. 2017).

Because PV+ and SST+ cell numbers are unchanged in the DCLK1 knockout before seizure induction, the decrease in pilocarpine-treated DCLK1 knockout mice is likely the result of this epileptogenesis. This result must be compared against the pilocarpine-treated wild type mice that did not experience such a severe loss even though they received the same pilocarpine-induced SE treatment. Both SST+ and PV+ interneurons showed significant decreases, so a more generalized dysfunction may be

more probable than a specific relationship between one cell type and the disease phenotype. One possible explanation for the decrease in pilocarpine-treated DCLK1 knockout mice could be the result of a more severe reaction to SE, as exemplified by the increased mortality and neurodegeneration by FJ staining. These cells may have perished from the acute excitotoxicity in greater numbers similarly to the greater loss apparent in the pyramidal neurons. Another explanation could be that the interneuron population was able to survive the acute excitotoxicity to the same extent as the wild type pilocarpine-treated animals but development of SRS and the resulting chronic damage caused the loss. Even if the initial loss of inhibitory interneurons was minimal, this may contribute to a vicious cycle as the remaining inhibitory interneurons must now survive through a more hyperexcitable circuit, and with more death the cycle worsens.

4.4 DCLK1 and mossy fiber sprouting

Our results showing increases in MFS from pilocarpine induction agree with the body of literature that allow for sufficient time post injection for sprouting to occur. However, an additional increase from loss of DCLK1 had not previously been reported. One hypothesis before experimentation could predict that loss of DCLK1 might actually inhibit MFS due to a lack of axon elongation. Previous studies have shown that loss of DCLK1 has prevented complete callosal axon elongation and one study showed a shortening of hippocampal axon elongation at DIV 5 although this change was not statistically significant (Koizumi et al. 2017, Shin et al. 2013). There are a few possible mechanisms that could be responsible for the increase in MFS in pilocarpine-treated knockout mice. It is important to note that because there didn’t appear to be any sprouting in the untreated knockout mice that the mechanism responsible for the increase is highly activity

dependent. This is an important detail, as there are some genetic models where gene knockout is sufficient to induce MFS without the induction of SE (Li et al. 2022, Shibata et al. 2013).

One mechanism that could possibly be responsible for activity-dependent increases in MFS in the DCLK1 knockout mice is a dysfunction in axon guidance. There are many examples of axon guidance affecting MFS, some with and some without an activity-dependent mechanism. One of the more compelling cases results from work done in hippocampal slice culture when researchers found that netrin-1 attracts MF when DCC is the main receptor in the MF growth cone, but repels MF when the receptor Unc5A is also present at the surface (Muramatsu et al. 2010). In control medium the MF are attracted to the CA3 but inducing excitability via K+ caused Unc5a translocation to the surface of the growth cone and repelled MFs back toward the granule cell layer, initiating MFS. This is relevant as DCLK1 expression is intimately connected to netrin sensing and axon guidance. In young hippocampal neurons loss of DCX and DCLK1 results in ablation of netrin sensing and the subsequent growth cone turning (Fu et al. 2013). The authors tested DCC and Unc5A expression and found no changes between WT and DCX/DCLK1 knockout mice, but the cells were permeabilized prior to antibody staining, meaning cytoplasmic expression was measured, not surface expression. Muramatsu et al. emphasize the importance of the difference, as Unc5A cytoplasmic expression doesn't change in response to excitability, but Unc5A surface expression turns from absent to abundant, causing repulsion. It is possible that DCLK1 interacts with these receptors at the growth cone to increase proper guidance to the CA3, and loss of DCLK1 exacerbates the dysfunctional guidance and results in increased MFS.

Another distinct but related mechanism again involves possible axon guidance dysfunction but as a more direct result of changes in cytoskeletal architecture at the growth cone. One research group studying Sema3F targets found that knocking down the receptor Neuropilin-2 (Npn-2) caused spontaneous MFS, and inducing SE with pilocarpine worsened this MFS further (Li et al. 2022). This Npn-2 loss was found to increase MFS by ablating the phosphorylation of Collapsin response mediator protein 2 (CRMP2), a regulator of growth cone collapse (Kimura et al. 2005, Niwa et al. 2017) . Phosphorylation of CRMP2 causes it to lose its affinity to microtubules and actin, which prevents its contribution to axonal outgrowth (Tan et al. 2015). Loss of this phosphorylation caused increased CRMP2 microtubule and actin affinity and increased axonal outgrowth, causing increased MFS. Importantly, CRMP2 is specifically phosphorylated by CDK5 and GSK3 at the growth cone to trigger cytoskeletal dissociation and growth cone collapse (Uchida et al. 2005, Cole et al. 2006).

When we look at other important cytoskeletal actors at the growth cone of developing neurons, we find that DCX/DCLK1 knockout similarly causes significant actin and microtubule disorganization, especially at the developing growth cone (Fu et al. 2013). Selective mutagenesis of specific residues on DCX reveals that similarly to CRMP2, phosphorylation of S297 is the driver for its dissociation from microtubules and disorganization of actin resulting in growth cone malformations (Fu et al. 2013, Tanaka et al. 2004). Fascinatingly, this phosphorylation is mediated by CDK5 and GSK3, the same kinases that regulate CRMP2, and in silico analysis predicts binding sites on DCLK1 for CDK5 and GSK3 as well (Carli et al. 2023, Tanaka et al. 2004, Schaar et al. 2004, Moselhi et al. 2019, Graham et al. 2004). Additionally, recent findings have shown DCLK1 binding

specifically to Neuron-glia related protein (NrCAM), one component of a heterotrimeric receptor that also includes Npn-2 and is responsive to Sema3F, the same complex responsible for CRMP2 modulation (Murphy et al. 2023). They found DCLK1 specifically binds to the conserved FIGQY sequence on the cytoplasmic domain of NrCAM, the recruitment site for spectrin-actin adaptor Ankyrin (Bennett et al. 2009).

Based on this shared localization, regulation, and previous studies directly showing defects in axon guidance, it seems very possible that loss of DCLK1 in epileptic animals may result in dysregulation at the growth cone and result in increased MFS. A possible model involving an axon guidance mechanism could be the result of a change in netrin sensing from loss of DCLK1 in epileptic animals. Previous work has used hippocampal neurons implanted on to hippocampal slices to interrogate variables affecting the direction of individual axonal growth on these slices (Muramatsu et al. 2010). Researchers took a hippocampal slice and cultured it organotypically before implanting neurons and measuring how many axons grew in the correct direction in response to activity and changes in chemoreception. If DCLK1 helps to decrease MFS in an epileptic brain, activity induction on these implanted neurons should still trigger some MFS and aberrant growth towards the granule cell layer. However, loss of DCLK1 could worsen this phenotype, causing more neurons to project sprouted MFs. If DCLK1 is responsible for affecting receptor presence on the tip of the growth cone, it could possibly be responsible for lowering the presence of Unc5A on the surface of the growth cone. When electrical activity is induced, DCLK1 may help to keep Unc5A from being increasingly translocated to the surface, which would result in less repulsion from netrin. This lack of repulsion and

increase in attraction could be responsible for more mossy fibers growing the correct way to the CA3 instead of being repelled toward the granule cell layer.

4.5 DCLK1 and epileptiform activity

In this study we examined numerous EEG characteristics in pilocarpine-treated and untreated DCLK1 mice and found some striking differences. We documented significant increases in interictal epileptiform discharges in the pilocarpine-treated DCLK1 knockout compared to all other groups and rarely saw any spike patterns in untreated wild type or DCLK1 knockout mice. This was coupled with increases in motor seizure occurrence in the pilocarpine-treated DCLK1 knockout mice compared with the pilocarpine-treated wild type mice. This may indicate that loss of DCLK1 increases hyperexcitability in epileptic brains. There are a few potential explanations for this.

The first may be the result of inhibitory interneuron loss. As mentioned in previous sections, the E/I balance in the hippocampus is tightly regulated but often dysfunctional in cases of TLE. We have shown that while DCLK1 knockout mice have comparable interneuron populations in the hippocampus compared to wild type mice, pilocarpine administration decreases the interneuron population more severely in DCLK1 knockout mice. Loss of even one inhibitory interneuron subtype can be sufficient for hyperexcitability, SRS, and MFS (Cobos et al. 2005, Drexel et al. 2017). When examining epileptic tissue gathered from human patients and animal models, researchers have consistently found fewer of these specific inhibitory subpopulations (Spreafico et al. 1998, Thom et al. 2004, Wittner et al. 2005, Williamson et al. 1999, Austin et al. 2004). Reintroduction of GABAergic interneurons into the brain via implantation has shown promising results by ameliorating severity and occurrence of seizures but may not

necessarily answer the question in this case (Baraban et al. 2009). It is possible that this GABAergic reintroduction can simply compensate for hyperexcitability caused by other forces, not necessarily an interneuron population loss. More direct electrophysiological studies may be necessary to determine if this difference in interneuron loss is sufficient to account for changes in epileptiform activity.

The contribution of MFS to epilepsy is challenging to unravel, but the increase we see in pilocarpine-treated DCLK1 knockout mice could be increasing the occurrence of the epileptiform activity. Early work in the field has repeatedly shown that these sprouted axons can form functional synapses and recurrent networks (Wuarin et al. 1996, Molnar et al. 1999, Lynch et al. 2000, Buckmaster et al. 2002, Scharfman et al. 2003). However, the synapses formed by sprouted mossy fibers differ from those synapses connecting with the CA3 and can rapidly depress after repeated activation (Cavazos et al. 2003, Sutula et al. 1998, Hendricks et al. 2017). Recent work has shown optogenetically activating the MF will elicit bursts and spikes in the dentate granule cells of epileptic mice but activate little or no activity in healthy mice (Hendricks et al. 2019). Hendricks et al. also found that this could trigger recurrent network excitation in slices even without the addition of compounds to induce hyperexcitability.

While these models of recurrent aberrant activation from MFS are compelling they do not necessarily encapsulate the epileptic system as a whole. Perhaps the most contrary piece of evidence illustrating this comes from work using high doses of rapamycin to block MFS in epileptic mice (Heng et al. 2013). Blocking MFS in this pilocarpine model of epilepsy did not appear to alter the amount of seizures at all and rapamycin did not appear to reduce the hilar neuron loss caused by SE. This is significant

because early models attribute initiation of MFS to a loss of hilar neuron targets from SE-induced damage (Schmeiser et al. 2017). However, the researchers noted rapamycin dosage significantly inhibited hypertrophy of the dentate gyrus, indicating that perhaps other less noticeable off-target effects could exist increasing seizure susceptibility. One group has shown that rapamycin supplementation in the pilocarpine model suppresses somatostatin axon branching in the granule cell layer, which could offset the diminishing of excitability from a lack of MFS (Buckmaster et al. 2011). Admittedly, this is the less parsimonious of the reasons.

An important detail in understanding the differences between the pilocarpine-treated DCLK1 knockout and pilocarpine-treated wild type mice is the relative lack of seizure occurrence in the pilocarpine-treated wild type animals. The DCLK1 knockout's vulnerability to pilocarpine necessitated a smaller administration which may have been sufficient to generate epileptiform activity in these mice but to a lesser extent in wild type mice. Pilocarpine concentrations used for induction vary widely from 250mg/kg to 400 mg/kg, and using the lower range at a later time of injection probably lead to fewer cases of wild type mice developing SRS (Walter et al. 2007, da Silva 2014, Jain 2023). More severe occurrence of SE and increased damage from SE are correlated with more severe epilepsy characteristics, and many mice are not used for epilepsy evaluation unless they experience SE of sufficient severity (Walter et al. 2007, Hosford et al. 2016). This differential between experimental mice that did and did not develop SRS because of the difference in pilocarpine vulnerability could explain the downstream phenotypic differences in epileptiform activity both asleep and awake.

4.6 Pilocarpine-treated DCLK1 knockout mice exhibit abnormal sleep architecture

Sleep and epilepsy are intimately connected concepts, with each affecting the other significantly. Our setup to record sleep/wake involved surgical depth electrode EEG implantation and video observation over the course of multiple days. A previous study analyzed the percentage of sleep and wakefulness in different strains of mice that also underwent surgical EEG implantation and were recorded in a similar manner (McShane et al. 2011). They found that wild type C57/Bl6 mice sleep approximately 54% of the time and are awake 46% of the time. In our studies our wild type mice sleep approximately 53.5% of the time and are awake 46.5% of the time, in great agreement with the literature. We were initially surprised to find that the number of sleep/wake bouts in the untreated DCLK1 knockout mice were actually significantly different from the pilocarpine-treated groups but not the wild type untreated group. This difference was also perhaps opposite to what would be expected, as fewer bouts may represent less fragmented sleep (Trammell et al. 2014, Dulko et al. 2023, Kalume et al. 2015). It is important to note that researchers characterize bouts of wakefulness differently depending on experimental context. One may record every instance where the mouse appears to have sleep interrupted even fractionally to characterize acute affectation of sleep, resulting in hundreds of bouts over a 24 hr period (Kalume et al. 2015, McShane et al. 2011). We are more interested in the gross architecture of sleep, so short periods of readjustment or stretching during sleep did not trigger a bout change.

The decrease in bout number in untreated DCLK1 knockout mice may actually be more reflective of the slight change in the pilocarpine-treated mice, as we see no difference in the average sleep bout length between any of the groups. Interestingly,

these untreated DCLK1 knockout mice have much longer average waking periods than the pilocarpine-treated mice before they end up sleeping for a similar period, although there is still no deviation from untreated wild type. This may be a case where subtle and almost imperceptible differences from the baseline in opposite directions reveal a phenotypic difference. These characteristics must be considered with the observation that pilocarpine-treated DCLK1 knockout mice are the only treatment group with changes in total sleep/wake time, spending much more time asleep and less time awake than any other treatment group.

When we look at other animal models of TLE and genetic epilepsies we find a mix of often contradictory results. One study found that pilocarpine-treated rats experience less time awake and more time in slow wave sleep, while a study in electrically-stimulated SE rats showed they have more time awake, less time in paradoxical sleep, and no change in slow wave sleep (Matos et al. 2010, Bastlund et al. 2005). Examination of Kcna1-null mice, a mouse model of SUDEP, found significant alterations in circadian rhythm with increases in wakefulness and decreases in sleep (Wallace et al. 2018). What both these animal models and human patients all appear to have in common is significant sleep disruption, whether its circadian rhythmicity, changes in REM vs non-REM sleep, alterations of sleep characteristics such as sleep spindles, or differences in acute waking bouts (Zanzmera et al. 2012, Proost et al. 2022). We see these characteristics reflected in our own power spectrum analysis of these mice showing changes in delta power for epileptic mice consistently (data not shown). It is possible that the stark changes we see in total sleep and wakefulness in the pilocarpine-treated DCLK1 knockout mice are the result of a decrease in sleep quality. These mice may be increasing the duration of their

sleep to compensate for the lack of quality in the sleep. Detailed characterization of the sleep architecture could be useful for determining exactly how compromised the sleep quality of these mice is and whether it is related to the phenotypic difference.

4.7 Future directions

While we have demonstrated the significant effect that lack of DCLK1 has on hallmarks of epilepsy, there are still many questions that remain unanswered. The mechanistic cause for the worsening of these hallmarks remains unclear but experiments can be designed to clarify. To determine if the knockout phenotype is from more severe SE, mice can be recorded as they are undergoing SE and graded on the Racine scale. This would help to establish if mice are acutely responding to pilocarpine more severely and possibly incurring more damage than wild type mice, leading to the phenotypic difference. It would also provide a more specific line of inquiry as the main question then revolves around mechanisms DCLK1 may be involved in that would modulate this pilocarpine vulnerability.

How DCLK1 is expressed and whether deleting expression in specific cell types may also be of interest. Genetic manipulation of the dentate granule cells has often revealed circuit weaknesses and deletion or overexpression in just this cell type has been shown to greatly increase epilepsy occurrence and severity (Pun et al. 2012, Santos et al. 2017). Selectively deleting DCLK1 in certain interneuron subtypes or pyramidal neurons would also begin to narrow down how loss modulates specific circuit pathways. Deleting DCLK1 in a cell-specific manner and testing for changes in epileptic hallmarks could reveal which areas of the hippocampus and which cell types rely on DCLK1 for excitability maintenance.

Along with deletion, modeling DCLK1 overexpression in the hippocampus could be useful. Some experiments have shown how overexpression of DCLK1 and its isoforms can decrease excitability in the CA3/CA1 network and reduce glutamatergic synaptic transmission (Shin et al. 2013, Schenk et al. 2010). If the upregulation of DCLK1 in epileptic brains is a method to dampen excitability in the brain, options should be explored on how experimental overexpression may mediate epilepsy pathology. It is possible that experimental increase prior to SE could mitigate damage to the hippocampus, leading to a less severe phenotype. Chronic overexpression in the early phases after status epilepticus when remodeling activity is high could also potentially lead to favorable rewiring that mitigates future excitotoxic damage. Whether this overexpression reduces other hallmarks of TLE such as mossy fiber sprouting and inhibitory interneuron populations would be informative as well.

The circuit dysfunction can be further clarified by using electrophysiological techniques to determine cell-specific and field level changes in the circuit in response to loss of DCLK1. If DCLK1 is altering resting membrane potential, threshold firing rate, or other important cell-specific characteristics of excitability this should be apparent through testing with patch-clamp electrophysiology. Broader changes in ion pools can be measured using stimulation and recording of local field potentials. Attempts were previously made to record effects of these stimulations using glass pipettes, but more success may be had from using a visual method such as calcium imaging that results in a more transparent readout. Electrode recordings do allow for specificity of region for measuring certain characteristics of ion pools by measuring at just one spot, but this also introduces significant variation based on consistently recording the exact same point. The

regions of interest around the CA3 are incredibly complex, with connectivity differences based on the layer being recorded, and layers being indistinguishable based on size and morphology (Skucas et al. 2013).

When examining the effects of pilocarpine-treated DCLK1 knockout mice we see meaningful changes to sleep architecture that are likely the result of increased epileptiform activity. Other changes in DCLK1 knockout mice that alter behavior may be exacerbated in these pilocarpine-treated mice. DCLK1 and its isoforms have been implicated in a few mental health related disorder screens including ADHD and schizophrenia, and one group found that overexpression of a specific DCLK1 isoform increases parameters of anxiety in mice (Schenk 2010, Håvik et al. 2012). It would be interesting to examine whether pilocarpine-treated DCLK1 knockout mice exhibited changes to these behavioral parameters. Other research has shown DCLK1 may be involved in memory formation and learning based on association studies in humans and its relationship with BDNF (le Hellard 2009). It is possible that dysfunction in the hippocampus from epilepsy could further compromise these abilities in a DCLK1 knockout mouse.

References

Acsády L, Kamondi A, Sík A, Freund T, Buzsáki G. GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. J Neurosci. 1998 May 1;18(9):3386-403. doi: 10.1523/JNEUROSCI.18-09-03386.1998. PMID: 9547246; PMCID: PMC6792657.

Agulto RL, Rogers MM, Tan TC, Ramkumar A, Downing AM, Bodin H, Castro J, Nowakowski DW, Ori-McKenney KM. Autoregulatory control of microtubule binding in doublecortin-like kinase 1. Elife. 2021 Jul 26;10:e60126. doi: 10.7554/eLife.60126. PMID: 34310279; PMCID: PMC8352597.

Ahmed Juvale II, Che Has AT. The evolution of the pilocarpine animal model of status epilepticus. Heliyon. 2020 Jul 28;6(7):e04557. doi: 10.1016/j.heliyon.2020.e04557. PMID: 32775726; PMCID: PMC7393986.

Amaral DG, Witter MP. The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience. 1989;31(3):571-91. doi: 10.1016/0306-4522(89)90424-7. PMID: 2687721.

André V, Marescaux C, Nehlig A, Fritschy JM. Alterations of hippocampal GAbaergic system contribute to development of spontaneous recurrent seizures in the rat lithium-pilocarpine model of temporal lobe epilepsy. Hippocampus. 2001;11(4):452-68. doi: 10.1002/hipo.1060. PMID: 11530850.

Annegers JF, Hauser WA, Coan SP, Rocca WA. A population-based study of seizures after traumatic brain injuries. N Engl J Med. 1998 Jan 1;338(1):20-4. doi: 10.1056/NEJM199801013380104. PMID: 9414327.

Arabadzisz D, Antal K, Parpan F, Emri Z, Fritschy JM. Epileptogenesis and chronic seizures in a mouse model of temporal lobe epilepsy are associated with distinct EEG patterns and selective neurochemical alterations in the contralateral hippocampus. Exp Neurol. 2005 Jul;194(1):76-90. doi: 10.1016/j.expneurol.2005.01.029. PMID: 15899245.

Araki T, Simon RP, Taki W, Lan JQ, Henshall DC. Characterization of neuronal death induced by focally evoked limbic seizures in the C57BL/6 mouse. J Neurosci Res. 2002 Sep 1;69(5):614-21. doi: 10.1002/jnr.10356. PMID: 12210827.

Asadi-Pooya AA, Stewart GR, Abrams DJ, Sharan A. Prevalence and Incidence of Drug-Resistant Mesial Temporal Lobe Epilepsy in the United States. World Neurosurg. 2017 Mar;99:662-666. doi: 10.1016/j.wneu.2016.12.074. Epub 2016 Dec 27. PMID: 28034810.

Austin JE, Buckmaster PS. Recurrent excitation of granule cells with basal dendrites and low interneuron density and inhibitory postsynaptic current frequency in the dentate gyrus of macaque monkeys. J Comp Neurol. 2004 Aug 23;476(3):205-18. doi: 10.1002/cne.20182. PMID: 15269966.

Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF. Synaptic reorganization by mossy fibers in human epileptic fascia dentata. Neuroscience. 1991;42(2):351-63. doi: 10.1016/0306-4522(91)90380-7. PMID: 1716744.
Bahi-Buisson N, Souville I, Fourniol FJ, Toussaint A, Moores CA, Houdusse A, Lemaitre JY, Poirier K, Khalaf-Nazzal R, Hully M, Leger PL, Elie C, Boddaert N, Beldjord C, Chelly J, Francis F; SBH-LIS European Consortium. New insights into genotype-phenotype correlations for the doublecortin-related lissencephaly spectrum. Brain. 2013 Jan;136(Pt 1):223-44. doi: 10.1093/brain/aws323. PMID: 23365099; PMCID: PMC3562079.

Baraban SC, Southwell DG, Estrada RC, Jones DL, Sebe JY, Alfaro-Cervello C, García-Verdugo JM, Rubenstein JL, Alvarez-Buylla A. Reduction of seizures by transplantation of cortical GABAergic interneuron precursors into Kv1.1 mutant mice. Proc Natl Acad Sci U S A. 2009 Sep 8;106(36):15472-7. doi: 10.1073/pnas.0900141106. Epub 2009 Aug 24. PMID: 19706400; PMCID: PMC2741275.

Barfuss JD, Nascimento FA, Duhaime E, Kapur S, Karakis I, Ng M, Herlopian A, Lam A, Maus D, Halford JJ, Cash S, Brandon Westover M, Jing J. On-demand EEG education through competition - A novel, app-based approach to learning to identify interictal epileptiform discharges. Clin Neurophysiol Pract. 2023 Aug 19;8:177-186. doi: 10.1016/j.cnp.2023.08.003. PMID: 37681118; PMCID: PMC10480673.

Bartolomei F, Khalil M, Wendling F, Sontheimer A, Régis J, Ranjeva JP, Guye M, Chauvel P. Entorhinal cortex involvement in human mesial temporal lobe epilepsy: an electrophysiologic and volumetric study. Epilepsia. 2005 May;46(5):677-87. doi: 10.1111/j.1528-1167.2005.43804.x. PMID: 15857433.

Beghi E, Carpio A, Forsgren L, Hesdorffer DC, Malmgren K, Sander JW, Tomson T, Hauser WA. Recommendation for a definition of acute symptomatic seizure. Epilepsia. 2010 Apr;51(4):671-5. doi: 10.1111/j.1528-1167.2009.02285.x. Epub 2009 Sep 3. PMID: 19732133.

Ben-Ari Y, Crepel V, Represa A. Seizures beget seizures in temporal lobe epilepsies: the boomerang effects of newly formed aberrant kainatergic synapses. Epilepsy Curr. 2008 May-Jun;8(3):68-72. doi: 10.1111/j.1535-7511.2008.00241.x. PMID: 18488058; PMCID: PMC2384153.

Bender RA, Dubé C, Gonzalez-Vega R, Mina EW, Baram TZ. Mossy fiber plasticity and enhanced hippocampal excitability, without hippocampal cell loss or altered neurogenesis, in an animal model of prolonged febrile seizures. Hippocampus. 2003;13(3):399-412. doi: 10.1002/hipo.10089. PMID: 12722980; PMCID: PMC2927853.

Bennett V, Healy J. Membrane domains based on ankyrin and spectrin associated with cell-cell interactions. Cold Spring Harb Perspect Biol. 2009 Dec;1(6):a003012. doi:

10.1101/cshperspect.a003012. Epub 2009 Aug 19. PMID: 20457566; PMCID: PMC2882121.

Bergoglio E, Suzuki IK, Togashi K, Tsuji M, Takeuchi S, Koizumi H, Emoto K. Spatial and temporal diversity of DCLK1 isoforms in developing mouse brain. Neurosci Res. 2021 Sep;170:154-165. doi: 10.1016/j.neures.2020.12.004. Epub 2021 Jan 22. PMID: 33485913.

Bergoglio E, Suzuki IK, Togashi K, Tsuji M, Takeuchi S, Koizumi H, Emoto K. Spatial and temporal diversity of DCLK1 isoforms in developing mouse brain. Neurosci Res. 2021 Sep;170:154-165. doi: 10.1016/j.neures.2020.12.004. Epub 2021 Jan 22. PMID: 33485913.

Berkovic SF, McIntosh A, Howell RA, Mitchell A, Sheffield LJ, Hopper JL. Familial temporal lobe epilepsy: a common disorder identified in twins. Ann Neurol. 1996 Aug;40(2):227-35. doi: 10.1002/ana.410400214. PMID: 8773604.

Bernard C. Alterations in synaptic function in epilepsy. In: Noebels JL, Avoli M, Rogawski MA, et al., editors. Jasper's Basic Mechanisms of the Epilepsies [Internet]. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US); 2012. Available from: https://www.ncbi.nlm.nih.gov/books/NBK98161/

Binder DK, Croll SD, Gall CM, Scharfman HE. BDNF and epilepsy: too much of a good thing? Trends Neurosci. 2001 Jan;24(1):47-53. doi: 10.1016/s0166-2236(00)01682-9. PMID: 11163887.

Blair RD. Temporal lobe epilepsy semiology. Epilepsy Res Treat. 2012;2012:751510. doi: 10.1155/2012/751510. Epub 2012 Mar 7. PMID: 22957241; PMCID: PMC3420439.

Blumcke I, Spreafico R, Haaker G, Coras R, Kobow K, Bien CG, Pfäfflin M, Elger C, Widman G, Schramm J, Becker A, Braun KP, Leijten F, Baayen JC, Aronica E, Chassoux F, Hamer H, Stefan H, Rössler K, Thom M, Walker MC, Sisodiya SM, Duncan JS, McEvoy AW, Pieper T, Holthausen H, Kudernatsch M, Meencke HJ, Kahane P, Schulze-Bonhage A, Zentner J, Heiland DH, Urbach H, Steinhoff BJ, Bast T, Tassi L, Lo Russo G, Özkara C, Oz B, Krsek P, Vogelgesang S, Runge U, Lerche H, Weber Y, Honavar M, Pimentel J, Arzimanoglou A, Ulate-Campos A, Noachtar S, Hartl E, Schijns O, Guerrini R, Barba C, Jacques TS, Cross JH, Feucht M, Mühlebner A, Grunwald T, Trinka E, Winkler PA, Gil-Nagel A, Toledano Delgado R, Mayer T, Lutz M, Zountsas B, Garganis K, Rosenow F, Hermsen A, von Oertzen TJ, Diepgen TL, Avanzini G; EEBB Consortium. Histopathological Findings in Brain Tissue Obtained during Epilepsy Surgery. N Engl J Med. 2017 Oct 26;377(17):1648-1656. doi: 10.1056/NEJMoa1703784. PMID: 29069555.

Blume WT. The progression of epilepsy. Epilepsia. 2006;47 Suppl 1:71-8. doi: 10.1111/j.1528-1167.2006.00665.x. PMID: 17044831.

Blumenfeld H, Klein JP, Schridde U, Vestal M, Rice T, Khera DS, Bashyal C, Giblin K, Paul-Laughinghouse C, Wang F, Phadke A, Mission J, Agarwal RK, Englot DJ, Motelow J, Nersesyan H, Waxman SG, Levin AR. Early treatment suppresses the development of spike-wave epilepsy in a rat model. Epilepsia. 2008 Mar;49(3):400-9. doi: 10.1111/j.1528-1167.2007.01458.x. Epub 2007 Dec 6. PMID: 18070091; PMCID: PMC3143182.

Botterill JJ, Lu YL, LaFrancois JJ, Bernstein HL, Alcantara-Gonzalez D, Jain S, Leary P, Scharfman HE. An Excitatory and Epileptogenic Effect of Dentate Gyrus Mossy Cells in a Mouse Model of Epilepsy. Cell Rep. 2019 Nov 26;29(9):2875-2889.e6. doi: 10.1016/j.celrep.2019.10.100. PMID: 31775052; PMCID: PMC6905501.

Brito MR, Prado TS, Alvim MKM, Santos LSR, Morita-Sherman M, Yasuda CL, Cendes F. Longitudinal analysis of interictal electroencephalograms in patients with temporal lobe epilepsy with hippocampal sclerosis. Seizure. 2021 Aug;90:141-144. doi: 10.1016/j.seizure.2021.02.008. Epub 2021 Feb 12. PMID: 33608133.

Brodie MJ, Zuberi SM, Scheffer IE, Fisher RS. The 2017 ILAE classification of seizure types and the epilepsies: what do people with epilepsy and their caregivers need to know? Epileptic Disord. 2018 Apr 1;20(2):77-87. doi: 10.1684/epd.2018.0957. PMID: 29620013.

Buckmaster PS, Wen X. Rapamycin suppresses axon sprouting by somatostatin interneurons in a mouse model of temporal lobe epilepsy. Epilepsia. 2011 Nov;52(11):2057-64. doi: 10.1111/j.1528-1167.2011.03253.x. Epub 2011 Aug 29. PMID: 21883182; PMCID: PMC3204172.

Buckmaster PS, Zhang GF, Yamawaki R. Axon sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. J Neurosci. 2002 Aug 1;22(15):6650-8. doi: 10.1523/JNEUROSCI.22-15-06650.2002. PMID: 12151544; PMCID: PMC6758164.

Buckmaster PS. Does mossy fiber sprouting give rise to the epileptic state? Adv Exp Med Biol. 2014;813:161-8. doi: 10.1007/978-94-017-8914-1_13. PMID: 25012375.

Bui AD, Nguyen TM, Limouse C, Kim HK, Szabo GG, Felong S, Maroso M, Soltesz I. Dentate gyrus mossy cells control spontaneous convulsive seizures and spatial memory. Science. 2018 Feb 16;359(6377):787-790. doi: 10.1126/science.aan4074. PMID: 29449490; PMCID: PMC6040648.

Burgess HA, Reiner O. Alternative splice variants of doublecortin-like kinase are differentially expressed and have different kinase activities. J Biol Chem. 2002 May 17;277(20):17696-705. doi: 10.1074/jbc.M111981200. Epub 2002 Mar 7. PMID: 11884394.

Burgess HA, Reiner O. Cleavage of doublecortin-like kinase by calpain releases an active kinase fragment from a microtubule anchorage domain. J Biol Chem. 2001 Sep 28;276(39):36397-403. doi: 10.1074/jbc.M105153200. Epub 2001 Jul 25. PMID: 11473121.

Carli ALE, Hardy JM, Hoblos H, Ernst M, Lucet IS, Buchert M. Structure-Guided Prediction of the Functional Impact of DCLK1 Mutations on Tumorigenesis. Biomedicines. 2023 Mar 22;11(3):990. doi: 10.3390/biomedicines11030990. PMID: 36979969; PMCID: PMC10046695.

Cavalheiro EA, Riche DA, Le Gal La Salle G. Long-term effects of intrahippocampal kainic acid injection in rats: a method for inducing spontaneous recurrent seizures. Electroencephalogr Clin Neurophysiol. 1982 Jun;53(6):581-9. doi: 10.1016/0013-4694(82)90134-1. PMID: 6177503.

Cavalheiro EA, Leite JP, Bortolotto ZA, Turski WA, Ikonomidou C, Turski L. Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures. Epilepsia. 1991 Nov-Dec;32(6):778-82. doi: 10.1111/j.1528-1157.1991.tb05533.x. PMID: 1743148.

Cavazos JE, Golarai G, Sutula TP. Mossy fiber synaptic reorganization induced by kindling: time course of development, progression, and permanence. J Neurosci. 1991 Sep;11(9):2795-803. doi: 10.1523/JNEUROSCI.11-09-02795.1991. PMID: 1880549; PMCID: PMC6575263.

Cavazos JE, Zhang P, Qazi R, Sutula TP. Ultrastructural features of sprouted mossy fiber synapses in kindled and kainic acid-treated rats. J Comp Neurol. 2003 Apr 7;458(3):272-92. doi: 10.1002/cne.10581. PMID: 12619081.

Chakravarthy N, Tsakalis K, Sabesan S, Iasemidis L. Homeostasis of brain dynamics in epilepsy: a feedback control systems perspective of seizures. Ann Biomed Eng. 2009 Mar;37(3):565-85. doi: 10.1007/s10439-008-9625-6. Epub 2009 Jan 6. PMID: 19125333; PMCID: PMC3120843.

Cherian A, Thomas SV. Status epilepticus. Ann Indian Acad Neurol. 2009 Jul;12(3):140-53. doi: 10.4103/0972-2327.56312. PMID: 20174493; PMCID: PMC2824929.

Chi ZH, Wang X, Cai JQ, Stoltenberg M, Danscher G, Wang ZY. Zinc transporter 3 immunohistochemical tracing of sprouting mossy fibres. Neurochem Int. 2008 Jun;52(7):1305-9. doi: 10.1016/j.neuint.2008.02.008. Epub 2008 Mar 6. PMID: 18406010.

Cobos I, Calcagnotto ME, Vilaythong AJ, Thwin MT, Noebels JL, Baraban SC, Rubenstein JL. Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. Nat Neurosci. 2005 Aug;8(8):1059-68. doi: 10.1038/nn1499. Epub 2005 Jul 10. PMID: 16007083.

Cole AR, Causeret F, Yadirgi G, Hastie CJ, McLauchlan H, McManus EJ, Hernández F, Eickholt BJ, Nikolic M, Sutherland C. Distinct priming kinases contribute to differential regulation of collapsin response mediator proteins by glycogen synthase kinase-3 in vivo. J Biol Chem. 2006 Jun 16;281(24):16591-8. doi: 10.1074/jbc.M513344200. Epub 2006 Apr 12. PMID: 16611631; PMCID: PMC1805471.

Colin E, Huynh Cong E, Mollet G, Guichet A, Gribouval O, Arrondel C, Boyer O, Daniel L, Gubler MC, Ekinci Z, Tsimaratos M, Chabrol B, Boddaert N, Verloes A, Chevrollier A, Gueguen N, Desquiret-Dumas V, Ferré M, Procaccio V, Richard L, Funalot B, Moncla A, Bonneau D, Antignac C. Loss-of-function mutations in WDR73 are responsible for microcephaly and steroid-resistant nephrotic syndrome: Galloway-Mowat syndrome. Am J Hum Genet. 2014 Dec 4;95(6):637-48. doi: 10.1016/j.ajhg.2014.10.011. Epub 2014 Nov 13. PMID: 25466283; PMCID: PMC4259970.

Corbo JC, Deuel TA, Long JM, LaPorte P, Tsai E, Wynshaw-Boris A, Walsh CA. Doublecortin is required in mice for lamination of the hippocampus but not the neocortex. J Neurosci. 2002 Sep 1;22(17):7548-57. doi: 10.1523/JNEUROSCI.22-17-07548.2002. PMID: 12196578; PMCID: PMC6757990.

Corey LA, Pellock JM, Kjeldsen MJ, Nakken KO. Importance of genetic factors in the occurrence of epilepsy syndrome type: a twin study. Epilepsy Res. 2011 Nov;97(1-2):103-11. doi: 10.1016/j.eplepsyres.2011.07.018. Epub 2011 Aug 31. PMID: 21885256; PMCID: PMC3215843.

Coulter DA, Carlson GC. Functional regulation of the dentate gyrus by GABA-mediated inhibition. Prog Brain Res. 2007;163:235-43. doi: 10.1016/S0079-6123(07)63014-3. PMID: 17765722.

Crompton DE, Scheffer IE, Taylor I, Cook MJ, McKelvie PA, Vears DF, Lawrence KM, McMahon JM, Grinton BE, McIntosh AM, Berkovic SF. Familial mesial temporal lobe epilepsy: a benign epilepsy syndrome showing complex inheritance. Brain. 2010 Nov;133(11):3221-31. doi: 10.1093/brain/awq251. Epub 2010 Sep 23. PMID: 20864493.

Cronin J, Dudek FE. Chronic seizures and collateral sprouting of dentate mossy fibers after kainic acid treatment in rats. Brain Res. 1988 Nov 22;474(1):181-4. doi: 10.1016/0006-8993(88)90681-6. PMID: 3214710.

Curia G, Longo D, Biagini G, Jones RS, Avoli M. The pilocarpine model of temporal lobe epilepsy. J Neurosci Methods. 2008 Jul 30;172(2):143-57. doi: 10.1016/j.jneumeth.2008.04.019. Epub 2008 Apr 26. PMID: 18550176; PMCID: PMC2518220.

da Silva AP, Lopes JS, Vieira Pde S, Pinheiro EE, da Silva ML, Silva Filho JC, da Costa JS Jr, David JM, de Freitas RM. Behavioral and neurochemical studies in mice pretreated with garcinielliptone FC in pilocarpine-induced seizures. Pharmacol Biochem

Behav. 2014 Sep;124:305-10. doi: 10.1016/j.pbb.2014.05.021. Epub 2014 Jun 6. PMID: 24911645.

Danzer S. Mossy Fiber Sprouting in the Epileptic Brain: Taking on the Lernaean Hydra. Epilepsy Curr. 2017 Jan-Feb;17(1):50-51. doi: 10.5698/1535-7511-17.1.50. PMID: 28331473; PMCID: PMC5340559.

de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res. 1989 Aug 28;495(2):387-95. doi: 10.1016/0006-8993(89)90234-5. PMID: 2569920.

de Vries WN, Binns LT, Fancher KS, Dean J, Moore R, Kemler R, Knowles BB. Expression of Cre recombinase in mouse oocytes: a means to study maternal effect genes. Genesis. 2000 Feb;26(2):110-2. PMID: 10686600.

Denkena J, Zaisser A, Merz B, Klinger B, Kuhl D, Blüthgen N, Hermey G. Neuronal activity regulates alternative exon usage. Mol Brain. 2020 Nov 10;13(1):148. doi: 10.1186/s13041-020-00685-3. PMID: 33172478; PMCID: PMC7656758.

des Portes V, Pinard JM, Billuart P, Vinet MC, Koulakoff A, Carrié A, Gelot A, Dupuis E, Motte J, Berwald-Netter Y, Catala M, Kahn A, Beldjord C, Chelly J. A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. Cell. 1998 Jan 9;92(1):51-61. doi: 10.1016/s0092-8674(00)80898-3. PMID: 9489699.

Deuel TA, Liu JS, Corbo JC, Yoo SY, Rorke-Adams LB, Walsh CA. Genetic interactions between doublecortin and doublecortin-like kinase in neuronal migration and axon outgrowth. Neuron. 2006 Jan 5;49(1):41-53. doi: 10.1016/j.neuron.2005.10.038. PMID: 16387638.

Dhir A. Pentylenetetrazol (PTZ) kindling model of epilepsy. Curr Protoc Neurosci. 2012;Chapter 9:Unit9.37. doi: 10.1002/0471142301.ns0937s58. PMID: 23042503.

Di Donato N, Chiari S, Mirzaa GM, Aldinger K, Parrini E, Olds C, Barkovich AJ, Guerrini R, Dobyns WB. Lissencephaly: Expanded imaging and clinical classification. Am J Med Genet A. 2017 Jun;173(6):1473-1488. doi: 10.1002/ajmg.a.38245. Epub 2017 Apr 25. PMID: 28440899; PMCID: PMC5526446.

Ding J, Li X, Tian H, Wang L, Guo B, Wang Y, Li W, Wang F, Sun T. SCN1A Mutation-Beyond Dravet Syndrome: A Systematic Review and Narrative Synthesis. Front Neurol. 2021 Dec 24;12:743726. doi: 10.3389/fneur.2021.743726. PMID: 35002916; PMCID: PMC8739186.

do Nascimento AL, Dos Santos NF, Campos Pelágio F, Aparecida Teixeira S, de Moraes Ferrari EA, Langone F. Neuronal degeneration and gliosis time-course in the mouse hippocampal formation after pilocarpine-induced status epilepticus. Brain Res.

2012 Aug 27;1470:98-110. doi: 10.1016/j.brainres.2012.06.008. Epub 2012 Jul 8. PMID: 22781142.

Drexel M, Preidt AP, Sperk G. Sequel of spontaneous seizures after kainic acid-induced status epilepticus and associated neuropathological changes in the subiculum and entorhinal cortex. Neuropharmacology. 2012 Oct;63(5):806-17. doi: 10.1016/j.neuropharm.2012.06.009. Epub 2012 Jun 18. PMID: 22722023; PMCID: PMC3409872.

Drexel M, Romanov RA, Wood J, Weger S, Heilbronn R, Wulff P, Tasan RO, Harkany T, Sperk G. Selective Silencing of Hippocampal Parvalbumin Interneurons Induces Development of Recurrent Spontaneous Limbic Seizures in Mice. J Neurosci. 2017 Aug 23;37(34):8166-8179. doi: 10.1523/JNEUROSCI.3456-16.2017. Epub 2017 Jul 21. PMID: 28733354; PMCID: PMC6596787.

Dulko E, Jedrusiak M, Osuru HP, Atluri N, Illendula M, Davis EM, Beenhakker MP, Lunardi N. Sleep Fragmentation, Electroencephalographic Slowing, and Circadian Disarray in a Mouse Model for Intensive Care Unit Delirium. Anesth Analg. 2023 Jul 1;137(1):209-220. doi: 10.1213/ANE.0000000000006524. Epub 2023 May 16. PMID: 37192134.

Engel J Jr; International League Against Epilepsy (ILAE). A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. Epilepsia. 2001 Jun;42(6):796-803. doi: 10.1046/j.1528-1157.2001.10401.x. PMID: 11422340.

Fernández G, Effenberger O, Vinz B, Steinlein O, Elger CE, Döhring W, Heinze HJ. Hippocampal malformation as a cause of familial febrile convulsions and subsequent hippocampal sclerosis. Neurology. 1998 Apr;50(4):909-17. doi: 10.1212/wnl.50.4.909. PMID: 9566371.

Fisher RS, Bonner AM. The Revised Definition and Classification of Epilepsy for Neurodiagnostic Technologists. Neurodiagn J. 2018;58(1):1-10. doi: 10.1080/21646821.2018.1428455. PMID: 29562876.

Fisher RS, Cross JH, D'Souza C, French JA, Haut SR, Higurashi N, Hirsch E, Jansen FE, Lagae L, Moshé SL, Peltola J, Roulet Perez E, Scheffer IE, Schulze-Bonhage A, Somerville E, Sperling M, Yacubian EM, Zuberi SM. Instruction manual for the ILAE 2017 operational classification of seizure types. Epilepsia. 2017 Apr;58(4):531-542. doi: 10.1111/epi.13671. Epub 2017 Mar 8. PMID: 28276064.

Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, Lagae L, Moshé SL, Peltola J, Roulet Perez E, Scheffer IE, Zuberi SM. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia. 2017 Apr;58(4):522-530. doi: 10.1111/epi.13670. Epub 2017 Mar 8. PMID: 28276060.

French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, Spencer DD. Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. Ann Neurol. 1993 Dec;34(6):774-80. doi: 10.1002/ana.410340604. PMID: 8250525.

Fu X, Brown KJ, Yap CC, Winckler B, Jaiswal JK, Liu JS. Doublecortin (Dcx) family proteins regulate filamentous actin structure in developing neurons. J Neurosci. 2013 Jan 9;33(2):709-21. doi: 10.1523/JNEUROSCI.4603-12.2013. PMID: 23303949; PMCID: PMC3711551.

Gleeson JG, Allen KM, Fox JW, Lamperti ED, Berkovic S, Scheffer I, Cooper EC, Dobyns WB, Minnerath SR, Ross ME, Walsh CA. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell. 1998 Jan 9;92(1):63-72. doi: 10.1016/s0092-8674(00)80899-5. PMID: 9489700.

Gleeson JG, Lin PT, Flanagan LA, Walsh CA. Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. Neuron. 1999 Jun;23(2):257-71. doi: 10.1016/s0896-6273(00)80778-3. PMID: 10399933.

Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. Exp Neurol. 1969 Nov;25(3):295-330. doi: 10.1016/0014-4886(69)90128-9. PMID: 4981856.

Graham ME, Ruma-Haynes P, Capes-Davis AG, Dunn JM, Tan TC, Valova VA, Robinson PJ, Jeffrey PL. Multisite phosphorylation of doublecortin by cyclin-dependent kinase 5. Biochem J. 2004 Jul 15;381(Pt 2):471-81. doi: 10.1042/BJ20040324. PMID: 15099191; PMCID: PMC1133854.

Gray WP, Sundstrom LE. Kainic acid increases the proliferation of granule cell progenitors in the dentate gyrus of the adult rat. Brain Res. 1998 Apr 20;790(1-2):52-9. doi: 10.1016/s0006-8993(98)00030-4. PMID: 9593820.

Gröticke I, Hoffmann K, Löscher W. Behavioral alterations in a mouse model of temporal lobe epilepsy induced by intrahippocampal injection of kainate. Exp Neurol. 2008 Sep;213(1):71-83. doi: 10.1016/j.expneurol.2008.04.036. Epub 2008 May 13. PMID: 18585709.

Håvik B, Degenhardt FA, Johansson S, Fernandes CP, Hinney A, Scherag A, Lybæk H, Djurovic S, Christoforou A, Ersland KM, Giddaluru S, O'Donovan MC, Owen MJ, Craddock N, Mühleisen TW, Mattheisen M, Schimmelmann BG, Renner T, Warnke A, Herpertz-Dahlmann B, Sinzig J, Albayrak Ö, Rietschel M, Nöthen MM, Bramham CR, Werge T, Hebebrand J, Haavik J, Andreassen OA, Cichon S, Steen VM, Le Hellard S. DCLK1 variants are associated across schizophrenia and attention deficit/hyperactivity

disorder. PLoS One. 2012;7(4):e35424. doi: 10.1371/journal.pone.0035424. Epub 2012 Apr 23. PMID: 22539971; PMCID: PMC3335166.

Håvik B, Degenhardt FA, Johansson S, Fernandes CP, Hinney A, Scherag A, Lybæk H, Djurovic S, Christoforou A, Ersland KM, Giddaluru S, O'Donovan MC, Owen MJ, Craddock N, Mühleisen TW, Mattheisen M, Schimmelmann BG, Renner T, Warnke A, Herpertz-Dahlmann B, Sinzig J, Albayrak Ö, Rietschel M, Nöthen MM, Bramham CR, Werge T, Hebebrand J, Haavik J, Andreassen OA, Cichon S, Steen VM, Le Hellard S. DCLK1 variants are associated across schizophrenia and attention deficit/hyperactivity disorder. PLoS One. 2012;7(4):e35424. doi: 10.1371/journal.pone.0035424. Epub 2012 Apr 23. PMID: 22539971; PMCID: PMC3335166.

Hendricks WD, Chen Y, Bensen AL, Westbrook GL, Schnell E. Short-Term Depression of Sprouted Mossy Fiber Synapses from Adult-Born Granule Cells. J Neurosci. 2017 Jun 7;37(23):5722-5735. doi: 10.1523/JNEUROSCI.0761-17.2017. Epub 2017 May 11. PMID: 28495975; PMCID: PMC5469308.

Hendricks WD, Westbrook GL, Schnell E. Early detonation by sprouted mossy fibers enables aberrant dentate network activity. Proc Natl Acad Sci U S A. 2019 May 28;116(22):10994-10999. doi: 10.1073/pnas.1821227116. Epub 2019 May 13. PMID: 31085654; PMCID: PMC6561181.

Heng K, Haney MM, Buckmaster PS. High-dose rapamycin blocks mossy fiber sprouting but not seizures in a mouse model of temporal lobe epilepsy. Epilepsia. 2013 Sep;54(9):1535-41. doi: 10.1111/epi.12246. Epub 2013 Jul 12. PMID: 23848506; PMCID: PMC3769425.

Henze DA, Wittner L, Buzsáki G. Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo. Nat Neurosci. 2002 Aug;5(8):790-5. doi: 10.1038/nn887. PMID: 12118256.

Hosford BE, Liska JP, Danzer SC. Ablation of Newly Generated Hippocampal Granule Cells Has Disease-Modifying Effects in Epilepsy. J Neurosci. 2016 Oct 26;36(43):11013-11023. doi: 10.1523/JNEUROSCI.1371-16.2016. PMID: 27798182; PMCID: PMC5098838.

Houser CR, Harris AB, Vaughn JE. Time course of the reduction of GABA terminals in a model of focal epilepsy: a glutamic acid decarboxylase immunocytochemical study. Brain Res. 1986 Sep 24;383(1-2):129-45. doi: 10.1016/0006-8993(86)90014-4. PMID: 3094829.

Houser CR, Miyashiro JE, Swartz BE, Walsh GO, Rich JR, Delgado-Escueta AV. Altered patterns of dynorphin immunoreactivity suggest mossy fiber reorganization in human hippocampal epilepsy. J Neurosci. 1990 Jan;10(1):267-82. doi: 10.1523/JNEUROSCI.10-01-00267.1990. PMID: 1688934; PMCID: PMC6570337.

Ikegaya Y. Abnormal targeting of developing hippocampal mossy fibers after epileptiform activities via L-type Ca2+ channel activation in vitro. J Neurosci. 1999 Jan 15;19(2):802-12. doi: 10.1523/JNEUROSCI.19-02-00802.1999. PMID: 9880600; PMCID: PMC6782205.

Iyengar SS, LaFrancois JJ, Friedman D, Drew LJ, Denny CA, Burghardt NS, Wu MV, Hsieh J, Hen R, Scharfman HE. Suppression of adult neurogenesis increases the acute effects of kainic acid. Exp Neurol. 2015 Feb;264:135-49. doi: 10.1016/j.expneurol.2014.11.009. Epub 2014 Dec 2. PMID: 25476494; PMCID: PMC4800819.

Jain S, LaFrancois JJ, Botterill JJ, Alcantara-Gonzalez D, Scharfman HE. Adult neurogenesis in the mouse dentate gyrus protects the hippocampus from neuronal injury following severe seizures. Hippocampus. 2019 Aug;29(8):683-709. doi: 10.1002/hipo.23062. Epub 2019 Jan 23. PMID: 30672046; PMCID: PMC6640126.

Jallon P. Epilepsy in developing countries. Epilepsia. 1997 Oct;38(10):1143-51. doi: 10.1111/j.1528-1157.1997.tb01205.x. PMID: 9579962.

Jean DC, Baas PW, Black MM. A novel role for doublecortin and doublecortin-like kinase in regulating growth cone microtubules. Hum Mol Genet. 2012 Dec 15;21(26):5511-27. doi: 10.1093/hmg/dds395. Epub 2012 Sep 21. PMID: 23001563; PMCID: PMC3516135.

Jessberger S, Römer B, Babu H, Kempermann G. Seizures induce proliferation and dispersion of doublecortin-positive hippocampal progenitor cells. Exp Neurol. 2005 Dec;196(2):342-51. doi: 10.1016/j.expneurol.2005.08.010. Epub 2005 Oct 5. PMID: 16168988.

Jinde S, Zsiros V, Jiang Z, Nakao K, Pickel J, Kohno K, Belforte JE, Nakazawa K. Hilar mossy cell degeneration causes transient dentate granule cell hyperexcitability and impaired pattern separation. Neuron. 2012 Dec 20;76(6):1189-200. doi: 10.1016/j.neuron.2012.10.036. PMID: 23259953; PMCID: PMC3530172.

Jordan BA, Fernholz BD, Boussac M, Xu C, Grigorean G, Ziff EB, Neubert TA. Identification and verification of novel rodent postsynaptic density proteins. Mol Cell Proteomics. 2004 Sep;3(9):857-71. doi: 10.1074/mcp.M400045-MCP200. Epub 2004 May 28. PMID: 15169875.

Kalume F, Oakley JC, Westenbroek RE, Gile J, de la Iglesia HO, Scheuer T, Catterall WA. Sleep impairment and reduced interneuron excitability in a mouse model of Dravet Syndrome. Neurobiol Dis. 2015 May;77:141-54. doi: 10.1016/j.nbd.2015.02.016. Epub 2015 Mar 10. PMID: 25766678; PMCID: PMC4402280.

Kaur S, Ghuman MS, Devarajan LJ. A pediatric epilepsy classic: "Double cortex" syndrome. J Pediatr Neurosci. 2015 Apr-Jun;10(2):125-6. doi: 10.4103/1817-1745.159201. PMID: 26167214; PMCID: PMC4489054.

Keays DA, Tian G, Poirier K, Huang GJ, Siebold C, Cleak J, Oliver PL, Fray M, Harvey RJ, Molnár Z, Piñon MC, Dear N, Valdar W, Brown SD, Davies KE, Rawlins JN, Cowan NJ, Nolan P, Chelly J, Flint J. Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. Cell. 2007 Jan 12;128(1):45-57. doi: 10.1016/j.cell.2006.12.017. PMID: 17218254; PMCID: PMC1885944.

Kimura T, Watanabe H, Iwamatsu A, Kaibuchi K. Tubulin and CRMP-2 complex is transported via Kinesin-1. J Neurochem. 2005 Jun;93(6):1371-82. doi: 10.1111/j.1471-4159.2005.03063.x. PMID: 15935053.

Kjeldsen MJ, Corey LA, Christensen K, Friis ML. Epileptic seizures and syndromes in twins: the importance of genetic factors. Epilepsy Res. 2003 Jun-Jul;55(1-2):137-46. doi: 10.1016/s0920-1211(03)00117-7. PMID: 12948623.

Koizumi H, Fujioka H, Togashi K, Thompson J, Yates JR 3rd, Gleeson JG, Emoto K. DCLK1 phosphorylates the microtubule-associated protein MAP7D1 to promote axon elongation in cortical neurons. Dev Neurobiol. 2017 Apr;77(4):493-510. doi: 10.1002/dneu.22428. Epub 2016 Nov 24. PMID: 27503845.

Koizumi H, Tanaka T, Gleeson JG. Doublecortin-like kinase functions with doublecortin to mediate fiber tract decussation and neuronal migration. Neuron. 2006 Jan 5;49(1):55-66. doi: 10.1016/j.neuron.2005.10.040. PMID: 16387639.

Koyama R, Yamada MK, Fujisawa S, Katoh-Semba R, Matsuki N, Ikegaya Y. Brain-derived neurotrophic factor induces hyperexcitable reentrant circuits in the dentate gyrus. J Neurosci. 2004 Aug 18;24(33):7215-24. doi: 10.1523/JNEUROSCI.2045-04.2004. PMID: 15317847; PMCID: PMC6729760.

Krook-Magnuson E, Armstrong C, Bui A, Lew S, Oijala M, Soltesz I. In vivo evaluation of the dentate gate theory in epilepsy. J Physiol. 2015 May 15;593(10):2379-88. doi: 10.1113/JP270056. Epub 2015 Mar 31. PMID: 25752305; PMCID: PMC4457198.

Lapray D, Popova IY, Kindler J, Jorquera I, Becq H, Manent JB, Luhmann HJ, Represa A. Spontaneous epileptic manifestations in a DCX knockdown model of human double cortex. Cereb Cortex. 2010 Nov;20(11):2694-701. doi: 10.1093/cercor/bhq014. Epub 2010 Feb 17. PMID: 20164125.

Le Hellard S, Håvik B, Espeseth T, Breilid H, Løvlie R, Luciano M, Gow AJ, Harris SE, Starr JM, Wibrand K, Lundervold AJ, Porteous DJ, Bramham CR, Deary IJ, Reinvang I, Steen VM. Variants in doublecortin- and calmodulin kinase like 1, a gene up-regulated by BDNF, are associated with memory and general cognitive abilities. PLoS One. 2009

Oct 21;4(10):e7534. doi: 10.1371/journal.pone.0007534. PMID: 19844571; PMCID: PMC2760101.

Lévesque M, Avoli M. The kainic acid model of temporal lobe epilepsy. Neurosci Biobehav Rev. 2013 Dec;37(10 Pt 2):2887-99. doi: 10.1016/j.neubiorev.2013.10.011. Epub 2013 Oct 30. PMID: 24184743; PMCID: PMC4878897.

Lewis DV, Shinnar S, Hesdorffer DC, Bagiella E, Bello JA, Chan S, Xu Y, MacFall J, Gomes WA, Moshé SL, Mathern GW, Pellock JM, Nordli DR Jr, Frank LM, Provenzale J, Shinnar RC, Epstein LG, Masur D, Litherland C, Sun S; FEBSTAT Study Team. Hippocampal sclerosis after febrile status epilepticus: the FEBSTAT study. Ann Neurol. 2014 Feb;75(2):178-85. doi: 10.1002/ana.24081. Epub 2014 Mar 1. PMID: 24318290; PMCID: PMC3980500.

Li P, Fu X, Smith NA, Ziobro J, Curiel J, Tenga MJ, Martin B, Freedman S, Cea-Del Rio CA, Oboti L, Tsuchida TN, Oluigbo C, Yaun A, Magge SN, O'Neill B, Kao A, Zelleke TG, Depositario-Cabacar DT, Ghimbovschi S, Knoblach S, Ho CY, Corbin JG, Goodkin HP, Vicini S, Huntsman MM, Gaillard WD, Valdez G, Liu JS. Loss of CLOCK Results in Dysfunction of Brain Circuits Underlying Focal Epilepsy. Neuron. 2017 Oct 11;96(2):387-401.e6. doi: 10.1016/j.neuron.2017.09.044. PMID: 29024662; PMCID: PMC6233318.

Li Y, Tong F, Zhang Y, Cai Y, Ding J, Wang Q, Wang X. Neuropilin-2 Signaling Modulates Mossy Fiber Sprouting by Regulating Axon Collateral Formation Through CRMP2 in a Rat Model of Epilepsy. Mol Neurobiol. 2022 Nov;59(11):6817-6833. doi: 10.1007/s12035-022-02995-0. Epub 2022 Aug 31. PMID: 36044155; PMCID: PMC9525442.

Lin PT, Gleeson JG, Corbo JC, Flanagan L, Walsh CA. DCAMKL1 encodes a protein kinase with homology to doublecortin that regulates microtubule polymerization. J Neurosci. 2000 Dec 15;20(24):9152-61. doi: 10.1523/JNEUROSCI.20-24-09152.2000. PMID: 11124993; PMCID: PMC6773030.

Lindquist BE, Timbie C, Voskobiynyk Y, Paz JT. Thalamocortical circuits in generalized epilepsy: Pathophysiologic mechanisms and therapeutic targets. Neurobiol Dis. 2023 Jun 1;181:106094. doi: 10.1016/j.nbd.2023.106094. Epub 2023 Mar 27. PMID: 36990364; PMCID: PMC10192143.

Lipka J, Kapitein LC, Jaworski J, Hoogenraad CC. Microtubule-binding protein doublecortin-like kinase 1 (DCLK1) guides kinesin-3-mediated cargo transport to dendrites. EMBO J. 2016 Feb 1;35(3):302-18. doi: 10.15252/embj.201592929. Epub 2016 Jan 12. PMID: 26758546; PMCID: PMC4741305.

Liu JS, Schubert CR, Fu X, Fourniol FJ, Jaiswal JK, Houdusse A, Stultz CM, Moores CA, Walsh CA. Molecular basis for specific regulation of neuronal kinesin-3 motors by doublecortin family proteins. Mol Cell. 2012 Sep 14;47(5):707-21. doi:

10.1016/j.molcel.2012.06.025. Epub 2012 Aug 1. PMID: 22857951; PMCID: PMC3549492.

Löscher W, Potschka H, Sisodiya SM, Vezzani A. Drug Resistance in Epilepsy: Clinical Impact, Potential Mechanisms, and New Innovative Treatment Options. Pharmacol Rev. 2020 Jul;72(3):606-638. doi: 10.1124/pr.120.019539. PMID: 32540959; PMCID: PMC7300324.

Lothman EW, Stringer JL, Bertram EH. The dentate gyrus as a control point for seizures in the hippocampus and beyond. Epilepsy Res Suppl. 1992;7:301-13. PMID: 1334669.

Lowenstein DH. Epilepsy after head injury: an overview. Epilepsia. 2009 Feb;50 Suppl 2:4-9. doi: 10.1111/j.1528-1167.2008.02004.x. PMID: 19187288.

Lynch M, Sutula T. Recurrent excitatory connectivity in the dentate gyrus of kindled and kainic acid-treated rats. J Neurophysiol. 2000 Feb;83(2):693-704. doi: 10.1152/jn.2000.83.2.693. PMID: 10669485.

Majewski L, Wojtas B, Maciąg F, Kuznicki J. Changes in Calcium Homeostasis and Gene Expression Implicated in Epilepsy in Hippocampi of Mice Overexpressing ORAI1. Int J Mol Sci. 2019 Nov 6;20(22):5539. doi: 10.3390/ijms20225539. PMID: 31698854; PMCID: PMC6888010.

Makinson CD, Tanaka BS, Sorokin JM, Wong JC, Christian CA, Goldin AL, Escayg A, Huguenard JR. Regulation of Thalamic and Cortical Network Synchrony by Scn8a. Neuron. 2017 Mar 8;93(5):1165-1179.e6. doi: 10.1016/j.neuron.2017.01.031. Epub 2017 Feb 23. PMID: 28238546; PMCID: PMC5393918.

Marco P, Sola RG, Pulido P, Alijarde MT, Sánchez A, Ramón y Cajal S, DeFelipe J. Inhibitory neurons in the human epileptogenic temporal neocortex. An immunocytochemical study. Brain. 1996 Aug;119 (Pt 4):1327-47. doi: 10.1093/brain/119.4.1327. PMID: 8813295.

Masukawa LM, Uruno K, Sperling M, O'Connor MJ, Burdette LJ. The functional relationship between antidromically evoked field responses of the dentate gyrus and mossy fiber reorganization in temporal lobe epileptic patients. Brain Res. 1992 May 1;579(1):119-27. doi: 10.1016/0006-8993(92)90750-4. PMID: 1623399.

Matos G, Tsai R, Baldo MV, de Castro I, Sameshima K, Valle AC. The sleep-wake cycle in adult rats following pilocarpine-induced temporal lobe epilepsy. Epilepsy Behav. 2010 Mar;17(3):324-31. doi: 10.1016/j.yebeh.2009.11.015. Epub 2010 Feb 4. PMID: 20133208.

Mátyás A, Borbély E, Mihály A. Hippocampal Sclerosis in Pilocarpine Epilepsy: Survival of Peptide-Containing Neurons and Learning and Memory Disturbances in the Adult

NMRI Strain Mouse. Int J Mol Sci. 2021 Dec 24;23(1):204. doi: 10.3390/ijms23010204. PMID: 35008630; PMCID: PMC8745054.
McShane BB, Galante RJ, Jensen ST, Naidoo N, Pack AI, Wyner A. Characterization of the bout durations of sleep and wakefulness. J Neurosci Methods. 2010 Nov 30;193(2):321-33. doi: 10.1016/j.jneumeth.2010.08.024. Epub 2010 Sep 15. PMID: 20817037; PMCID: PMC2970733.

Mello LE, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, Finch DM. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. Epilepsia. 1993 Nov-Dec;34(6):985-95. doi: 10.1111/j.1528-1157.1993.tb02123.x. PMID: 7694849.

Menezes LFS, Sabiá Júnior EF, Tibery DV, Carneiro LDA, Schwartz EF. Epilepsy-Related Voltage-Gated Sodium Channelopathies: A Review. Front Pharmacol. 2020 Aug 18;11:1276. doi: 10.3389/fphar.2020.01276. PMID: 33013363; PMCID: PMC7461817.

Molnár P, Nadler JV. Mossy fiber-granule cell synapses in the normal and epileptic rat dentate gyrus studied with minimal laser photostimulation. J Neurophysiol. 1999 Oct;82(4):1883-94. doi: 10.1152/jn.1999.82.4.1883. PMID: 10515977.

Momen AA, Momen M. Double Cortex Syndrome (Subcortical Band Heterotopia): A Case Report. Iran J Child Neurol. 2015 Spring;9(2):64-8. PMID: 26221167; PMCID: PMC4515345.

Moslehi M, Ng DCH, Bogoyevitch MA. Doublecortin X (DCX) serine 28 phosphorylation is a regulatory switch, modulating association of DCX with microtubules and actin filaments. Biochim Biophys Acta Mol Cell Res. 2019 Apr;1866(4):638-649. doi: 10.1016/j.bbamcr.2019.01.003. Epub 2019 Jan 6. PMID: 30625347.

Muramatsu R, Nakahara S, Ichikawa J, Watanabe K, Matsuki N, Koyama R. The ratio of 'deleted in colorectal cancer' to 'uncoordinated-5A' netrin-1 receptors on the growth cone regulates mossy fibre directionality. Brain. 2010 Jan;133(Pt 1):60-75. doi: 10.1093/brain/awp266. Epub 2009 Oct 25. PMID: 19858080.

Murphy KE, Zhang EY, Wyatt EV, Sperringer JE, Duncan BW, Maness PF. Doublecortin-Like Kinase 1 Facilitates Dendritic Spine Growth of Pyramidal Neurons in Mouse Prefrontal Cortex. Neuroscience. 2023 Jan 1;508:98-109. doi: 10.1016/j.neuroscience.2022.08.020. Epub 2022 Sep 3. PMID: 36064052; PMCID: PMC10317307.

Nagamine T, Shimomura S, Sueyoshi N, Kameshita I. Influence of Ser/Pro-rich domain and kinase domain of double cortin-like protein kinase on microtubule-binding activity. J Biochem. 2011 May;149(5):619-27. doi: 10.1093/jb/mvr013. Epub 2011 Jan 28. PMID: 21278387.

National Center for Biotechnology Information (NCBI), corp-author NCBI; Bethesda, MD: 2020. [June 8; 2020]. DCLK1 doublecortin like kinase 1 [Homo sapiens (human)]

Nawabi H, Belin S, Cartoni R, Williams PR, Wang C, Latremolière A, Wang X, Zhu J, Taub DG, Fu X, Yu B, Gu X, Woolf CJ, Liu JS, Gabel CV, Steen JA, He Z. Doublecortin-Like Kinases Promote Neuronal Survival and Induce Growth Cone Reformation via Distinct Mechanisms. Neuron. 2015 Nov 18;88(4):704-19. doi: 10.1016/j.neuron.2015.10.005. Epub 2015 Oct 29. PMID: 26526391; PMCID: PMC10069300.

Niwa S, Nakamura F, Tomabechi Y, Aoki M, Shigematsu H, Matsumoto T, Yamagata A, Fukai S, Hirokawa N, Goshima Y, Shirouzu M, Nitta R. Structural basis for CRMP2-induced axonal microtubule formation. Sci Rep. 2017 Sep 6;7(1):10681. doi: 10.1038/s41598-017-11031-4. PMID: 28878401; PMCID: PMC5587665.

Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Jasper's Basic Mechanisms of the Epilepsies [Internet]. 4th ed. Bethesda (MD): National Center for Biotechnology Information (US); 2012. PMID: 22787592.

Nosten-Bertrand M, Kappeler C, Dinocourt C, Denis C, Germain J, Phan Dinh Tuy F, Verstraeten S, Alvarez C, Métin C, Chelly J, Giros B, Miles R, Depaulis A, Francis F. Epilepsy in Dcx knockout mice associated with discrete lamination defects and enhanced excitability in the hippocampus. PLoS One. 2008 Jun 25;3(6):e2473. doi: 10.1371/journal.pone.0002473. PMID: 18575605; PMCID: PMC2429962.

Ouyang Q, Lizarraga SB, Schmidt M, Yang U, Gong J, Ellisor D, Kauer JA, Morrow EM. Christianson syndrome protein NHE6 modulates TrkB endosomal signaling required for neuronal circuit development. Neuron. 2013 Oct 2;80(1):97-112. doi: 10.1016/j.neuron.2013.07.043. Epub 2013 Sep 12. PMID: 24035762; PMCID: PMC3830955.

Panayiotopoulos CP. The Epilepsies: Seizures, Syndromes and Management. Oxfordshire (UK): Bladon Medical Publishing; 2005. Chapter 10, Idiopathic Generalised Epilepsies. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2608/

Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci. 1997 May 15;17(10):3727-38. doi: 10.1523/JNEUROSCI.17-10-03727.1997. PMID: 9133393; PMCID: PMC6573703.

Patel O, Dai W, Mentzel M, Griffin MD, Serindoux J, Gay Y, Fischer S, Sterle S, Kropp A, Burns CJ, Ernst M, Buchert M, Lucet IS. Biochemical and Structural Insights into Doublecortin-like Kinase Domain 1. Structure. 2016 Sep 6;24(9):1550-61. doi: 10.1016/j.str.2016.07.008. Epub 2016 Aug 18. PMID: 27545623.

Perucca P. Genetics of Focal Epilepsies: What Do We Know and Where Are We Heading? Epilepsy Curr. 2018 Nov-Dec;18(6):356-362. doi: 10.5698/1535-7597.18.6.356. PMID: 30568546; PMCID: PMC6278742.

Phillips GR, Anderson TR, Florens L, Gudas C, Magda G, Yates JR 3rd, Colman DR. Actin-binding proteins in a postsynaptic preparation: Lasp-1 is a component of central nervous system synapses and dendritic spines. J Neurosci Res. 2004 Oct 1;78(1):38-48. doi: 10.1002/jnr.20224. PMID: 15372503.

Proost R, Lagae L, Van Paesschen W, Jansen K. Sleep in children with refractory epilepsy and epileptic encephalopathies: A systematic review of literature. Eur J Paediatr Neurol. 2022 May;38:53-61. doi: 10.1016/j.ejpn.2022.03.010. Epub 2022 Mar 29. PMID: 35395626.

Pun RY, Rolle IJ, Lasarge CL, Hosford BE, Rosen JM, Uhl JD, Schmeltzer SN, Faulkner C, Bronson SL, Murphy BL, Richards DA, Holland KD, Danzer SC. Excessive activation of mTOR in postnatally generated granule cells is sufficient to cause epilepsy. Neuron. 2012 Sep 20;75(6):1022-34. doi: 10.1016/j.neuron.2012.08.002. PMID: 22998871; PMCID: PMC3474536.

Qin L, Liu X, Liu S, Liu Y, Yang Y, Yang H, Chen Y, Chen L. Differentially expressed proteins underlying childhood cortical dysplasia with epilepsy identified by iTRAQ proteomic profiling. PLoS One. 2017 Feb 21;12(2):e0172214. doi: 10.1371/journal.pone.0172214. PMID: 28222113; PMCID: PMC5319751.

Quesnel-Vallières M, Dargaei Z, Irimia M, Gonatopoulos-Pournatzis T, Ip JY, Wu M, Sterne-Weiler T, Nakagawa S, Woodin MA, Blencowe BJ, Cordes SP. Misregulation of an Activity-Dependent Splicing Network as a Common Mechanism Underlying Autism Spectrum Disorders. Mol Cell. 2016 Dec 15;64(6):1023-1034. doi: 10.1016/j.molcel.2016.11.033. PMID: 27984743.

R. V. Sharan and S. Berkovsky, "Epileptic Seizure Detection Using Multi-Channel EEG Wavelet Power Spectra and 1-D Convolutional Neural Networks," 2020 42nd Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC), Montreal, QC, Canada, 2020, pp. 545-548, doi: 10.1109/EMBC44109.2020.9176243.

Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol. 1972 Mar;32(3):281-94. doi: 10.1016/0013-4694(72)90177-0. PMID: 4110397.

Ratté S, Lacaille JC. Selective degeneration and synaptic reorganization of hippocampal interneurons in a chronic model of temporal lobe epilepsy. Adv Neurol. 2006;97:69-76. PMID: 16383116.

Reddy C, Saini AG. Metabolic Epilepsy. Indian J Pediatr. 2021 Oct;88(10):1025-1032. doi: 10.1007/s12098-020-03510-w. Epub 2020 Oct 16. PMID: 33063276.

Reiner O, Carrozzo R, Shen Y, Wehnert M, Faustinella F, Dobyns WB, Caskey CT, Ledbetter DH. Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats. Nature. 1993 Aug 19;364(6439):717-21. doi: 10.1038/364717a0. PMID: 8355785.

Roliz, A.H., Kothare, S. The Interaction Between Sleep and Epilepsy. Curr Neurol Neurosci Rep 22, 551–563 (2022). https://doi.org/10.1007/s11910-022-01219-1

Rosen GD, Jacobs KM, Prince DA. Effects of neonatal freeze lesions on expression of parvalbumin in rat neocortex. Cereb Cortex. 1998 Dec;8(8):753-61. doi: 10.1093/cercor/8.8.753. PMID: 9863702.

Rüttimann E, Vacher CM, Gassmann M, Kaupmann K, Van der Putten H, Bettler B. Altered hippocampal expression of calbindin-D-28k and calretinin in GABA(B(1))-deficient mice. Biochem Pharmacol. 2004 Oct 15;68(8):1613-20. doi: 10.1016/j.bcp.2004.07.019. PMID: 15451404.

Santos VR, Pun RYK, Arafa SR, LaSarge CL, Rowley S, Khademi S, Bouley T, Holland KD, Garcia-Cairasco N, Danzer SC. PTEN deletion increases hippocampal granule cell excitability in male and female mice. Neurobiol Dis. 2017 Dec;108:339-351. doi: 10.1016/j.nbd.2017.08.014. Epub 2017 Sep 21. PMID: 28855130; PMCID: PMC5675774.

Sarmast ST, Abdullahi AM, Jahan N. Current Classification of Seizures and Epilepsies: Scope, Limitations and Recommendations for Future Action. Cureus. 2020 Sep 20;12(9):e10549. doi: 10.7759/cureus.10549. PMID: 33101797; PMCID: PMC7575300.

Schaar BT, Kinoshita K, McConnell SK. Doublecortin microtubule affinity is regulated by a balance of kinase and phosphatase activity at the leading edge of migrating neurons. Neuron. 2004 Jan 22;41(2):203-13. doi: 10.1016/s0896-6273(03)00843-2. PMID: 14741102.

Scharfman HE, Sollas AL, Berger RE, Goodman JH. Electrophysiological evidence of monosynaptic excitatory transmission between granule cells after seizure-induced mossy fiber sprouting. J Neurophysiol. 2003 Oct;90(4):2536-47. doi: 10.1152/jn.00251.2003. PMID: 14534276.

Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, Hirsch E, Jain S, Mathern GW, Moshé SL, Nordli DR, Perucca E, Tomson T, Wiebe S, Zhang YH, Zuberi SM. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia. 2017 Apr;58(4):512-521. doi: 10.1111/epi.13709. Epub 2017 Mar 8. PMID: 28276062; PMCID: PMC5386840.

Schenk GJ, Veldhuisen B, Wedemeier O, McGown CC, Schouten TG, Oitzl M, de Kloet ER, Vreugdenhil E. Over-expression of δC-DCLK-short in mouse brain results in a more anxious behavioral phenotype. Physiol Behav. 2010 Nov 2;101(4):541-8. doi: 10.1016/j.physbeh.2010.08.002. Epub 2010 Aug 10. PMID: 20705078.

Schenk GJ, Werkman T, Wadman W, Veldhuisen B, Dijkmans TF, Blaas E, Kegel L, de Kloet ER, Vreugdenhil E. Over-expression of the DCLK gene transcript CARP decreases CA3/CA1 network excitability. Brain Res. 2010 Sep 17;1352:21-34. doi: 10.1016/j.brainres.2010.07.068. Epub 2010 Jul 24. PMID: 20659433.

Schmeiser B, Li J, Brandt A, Zentner J, Doostkam S, Freiman TM. Different mossy fiber sprouting patterns in ILAE hippocampal sclerosis types. Epilepsy Res. 2017 Oct;136:115-122. doi: 10.1016/j.eplepsyres.2017.08.002. Epub 2017 Aug 8. PMID: 28822872.

Scholl EA, Dudek FE, Ekstrand JJ. Neuronal degeneration is observed in multiple regions outside the hippocampus after lithium pilocarpine-induced status epilepticus in the immature rat. Neuroscience. 2013 Nov 12;252:45-59. doi: 10.1016/j.neuroscience.2013.07.045. Epub 2013 Jul 27. PMID: 23896573; PMCID: PMC3916148.

Shang L, Kwon YG, Nandy S, Lawrence DS, Edelman AM. Catalytic and regulatory domains of doublecortin kinase-1. Biochemistry. 2003 Feb 25;42(7):2185-94. doi: 10.1021/bi026913i. PMID: 12590608.

Shibata K, Nakahara S, Shimizu E, Yamashita T, Matsuki N, Koyama R. Repulsive guidance molecule a regulates hippocampal mossy fiber branching in vitro. Neuroreport. 2013 Aug 7;24(11):609-15. doi: 10.1097/WNR.0b013e3283632c08. PMID: 23744345.

Shimada T, Yamagata K. Pentylenetetrazole-Induced Kindling Mouse Model. J Vis Exp. 2018 Jun 12;(136):56573. doi: 10.3791/56573. PMID: 29985308; PMCID: PMC6101698.

Shin E, Kashiwagi Y, Kuriu T, Iwasaki H, Tanaka T, Koizumi H, Gleeson JG, Okabe S. Doublecortin-like kinase enhances dendritic remodelling and negatively regulates synapse maturation. Nat Commun. 2013;4:1440. doi: 10.1038/ncomms2443. PMID: 23385585; PMCID: PMC4017031.

Shorvon S, Guerrini R. Acute symptomatic seizures--should we retain the term? Epilepsia. 2010 Apr;51(4):722-3. doi: 10.1111/j.1528-1167.2010.02501.x. PMID: 20394648.

Singhi P. Infectious causes of seizures and epilepsy in the developing world. Dev Med Child Neurol. 2011 Jul;53(7):600-9. doi: 10.1111/j.1469-8749.2011.03928.x. Epub 2011 Apr 19. PMID: 21518343.

Sirven JI. Epilepsy: A Spectrum Disorder. Cold Spring Harb Perspect Med. 2015 Sep 1;5(9):a022848. doi: 10.1101/cshperspect.a022848. PMID: 26328931; PMCID: PMC4561391.

Skucas VA, Duffy AM, Harte-Hargrove LC, Magagna-Poveda A, Radman T, Chakraborty G, Schroeder CE, MacLusky NJ, Scharfman HE. Testosterone depletion in adult male rats increases mossy fiber transmission, LTP, and sprouting in area CA3 of hippocampus. J Neurosci. 2013 Feb 6;33(6):2338-55. doi: 10.1523/JNEUROSCI.3857-12.2013. PMID: 23392664; PMCID: PMC3711621.

Spampanato J, Sullivan RK, Turpin FR, Bartlett PF, Sah P. Properties of doublecortin expressing neurons in the adult mouse dentate gyrus. PLoS One. 2012;7(9):e41029. doi: 10.1371/journal.pone.0041029. Epub 2012 Sep 5. PMID: 22957010; PMCID: PMC3434174.

Spreafico R, Battaglia G, Arcelli P, Andermann F, Dubeau F, Palmini A, Olivier A, Villemure JG, Tampieri D, Avanzini G, Avoli M. Cortical dysplasia: an immunocytochemical study of three patients. Neurology. 1998 Jan;50(1):27-36. doi: 10.1212/wnl.50.1.27. PMID: 9443453.

Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. Ann Neurol. 1989 Sep;26(3):321-30. doi: 10.1002/ana.410260303. PMID: 2508534.

Sutula T, Zhang P, Lynch M, Sayin U, Golarai G, Rod R. Synaptic and axonal remodeling of mossy fibers in the hilus and supragranular region of the dentate gyrus in kainate-treated rats. J Comp Neurol. 1998 Jan 26;390(4):578-94. doi: 10.1002/(sici)1096-9861(19980126)390:4<578::aid-cne9>3.0.co;2-y. PMID: 9450537.

Thom M, Martinian L, Parnavelas JG, Sisodiya SM. Distribution of cortical interneurons in grey matter heterotopia in patients with epilepsy. Epilepsia. 2004 Aug;45(8):916-23. doi: 10.1111/j.0013-9580.2004.46603.x. PMID: 15270756.

Thurman DJ, Beghi E, Begley CE, Berg AT, Buchhalter JR, Ding D, Hesdorffer DC, Hauser WA, Kazis L, Kobau R, Kroner B, Labiner D, Liow K, Logroscino G, Medina MT, Newton CR, Parko K, Paschal A, Preux PM, Sander JW, Selassie A, Theodore W, Tomson T, Wiebe S; ILAE Commission on Epidemiology. Standards for epidemiologic studies and surveillance of epilepsy. Epilepsia. 2011 Sep;52 Suppl 7:2-26. doi: 10.1111/j.1528-1167.2011.03121.x. PMID: 21899536.

Trammell RA, Verhulst S, Toth LA. Effects of sleep fragmentation on sleep and markers of inflammation in mice. Comp Med. 2014 Feb;64(1):13-24. PMID: 24512957; PMCID: PMC3929215.

Turski WA, Cavalheiro EA, Schwarz M, Czuczwar SJ, Kleinrok Z, Turski L. Limbic seizures produced by pilocarpine in rats: behavioural, electroencephalographic and

neuropathological study. Behav Brain Res. 1983 Sep;9(3):315-35. doi: 10.1016/0166-4328(83)90136-5. PMID: 6639740.

Uchida Y, Ohshima T, Sasaki Y, Suzuki H, Yanai S, Yamashita N, Nakamura F, Takei K, Ihara Y, Mikoshiba K, Kolattukudy P, Honnorat J, Goshima Y. Semaphorin3A signalling is mediated via sequential Cdk5 and GSK3beta phosphorylation of CRMP2: implication of common phosphorylating mechanism underlying axon guidance and Alzheimer's disease. Genes Cells. 2005 Feb;10(2):165-79. doi: 10.1111/j.1365-2443.2005.00827.x. PMID: 15676027.

Vadlamudi L, Milne RL, Lawrence K, Heron SE, Eckhaus J, Keay D, Connellan M, Torn-Broers Y, Howell RA, Mulley JC, Scheffer IE, Dibbens LM, Hopper JL, Berkovic SF. Genetics of epilepsy: The testimony of twins in the molecular era. Neurology. 2014 Sep 16;83(12):1042-8. doi: 10.1212/WNL.0000000000000790. Epub 2014 Aug 8. PMID: 25107880; PMCID: PMC4166361.

Valassina N, Brusco S, Salamone A, Serra L, Luoni M, Giannelli S, Bido S, Massimino L, Ungaro F, Mazzara PG, D'Adamo P, Lignani G, Broccoli V, Colasante G. Scn1a gene reactivation after symptom onset rescues pathological phenotypes in a mouse model of Dravet syndrome. Nat Commun. 2022 Jan 10;13(1):161. doi: 10.1038/s41467-021-27837-w. PMID: 35013317; PMCID: PMC8748984.

Vezzani A. Pilocarpine-induced seizures revisited: what does the model mimic? Epilepsy Curr. 2009 Sep-Oct;9(5):146-8. doi: 10.1111/j.1535-7511.2009.01323.x. PMID: 19826509; PMCID: PMC2759049.

Vreugdenhil E, Datson N, Engels B, de Jong J, van Koningsbruggen S, Schaaf M, de Kloet ER. Kainate-elicited seizures induce mRNA encoding a CaMK-related peptide: a putative modulator of kinase activity in rat hippocampus. J Neurobiol. 1999 Apr;39(1):41-50. PMID: 10213452.

Wallace E, Wright S, Schoenike B, Roopra A, Rho JM, Maganti RK. Altered circadian rhythms and oscillation of clock genes and sirtuin 1 in a model of sudden unexpected death in epilepsy. Epilepsia. 2018 Aug;59(8):1527-1539. doi: 10.1111/epi.14513. Epub 2018 Jul 15. PMID: 30009381.

Walter C, Murphy BL, Pun RY, Spieles-Engemann AL, Danzer SC. Pilocarpine-induced seizures cause selective time-dependent changes to adult-generated hippocampal dentate granule cells. J Neurosci. 2007 Jul 11;27(28):7541-52. doi: 10.1523/JNEUROSCI.0431-07.2007. PMID: 17626215; PMCID: PMC6672603.

Wang J, Lin ZJ, Liu L, Xu HQ, Shi YW, Yi YH, He N, Liao WP. Epilepsy-associated genes. Seizure. 2017 Jan;44:11-20. doi: 10.1016/j.seizure.2016.11.030. Epub 2016 Dec 6. PMID: 28007376.

Wang X, Hu Z, Zhong K. The Role of Brain-Derived Neurotrophic Factor in Epileptogenesis: an Update. Front Pharmacol. 2021 Nov 26;12:758232. doi: 10.3389/fphar.2021.758232. PMID: 34899313; PMCID: PMC8661413.

Watanabe M, Miyai A, Danjo S, Nakamura Y, Itoh K. The threshold of pentylenetetrazole-induced convulsive seizures, but not that of nonconvulsive seizures, is controlled by the nitric oxide levels in murine brains. Exp Neurol. 2013 Sep;247:645-52. doi: 10.1016/j.expneurol.2013.02.019. Epub 2013 Mar 7. PMID: 23499834.

Williamson A, Patrylo PR, Spencer DD. Decrease in inhibition in dentate granule cells from patients with medial temporal lobe epilepsy. Ann Neurol. 1999 Jan;45(1):92-9. doi: 10.1002/1531-8249(199901)45:1<92::aid-art15>3.0.co;2-n. PMID: 9894882.

Williamson R, Hanif S, Mathews GC, Lagrange AH, Abou-Khalil B. Generalized-onset seizures with secondary focal evolution. Epilepsia. 2009 Jul;50(7):1827-32. doi: 10.1111/j.1528-1167.2009.02045.x. Epub 2009 Feb 26. PMID: 19260942.

Wittner L, Eross L, Czirják S, Halász P, Freund TF, Maglóczky Z. Surviving CA1 pyramidal cells receive intact perisomatic inhibitory input in the human epileptic hippocampus. Brain. 2005 Jan;128(Pt 1):138-52. doi: 10.1093/brain/awh339. Epub 2004 Nov 17. PMID: 15548550.

Wu JQ, Wang X, Beveridge NJ, Tooney PA, Scott RJ, Carr VJ, Cairns MJ. Transcriptome sequencing revealed significant alteration of cortical promoter usage and splicing in schizophrenia. PLoS One. 2012;7(4):e36351. doi: 10.1371/journal.pone.0036351. Epub 2012 Apr 27. PMID: 22558445; PMCID: PMC3338678.

Wuarin JP, Dudek FE. Electrographic seizures and new recurrent excitatory circuits in the dentate gyrus of hippocampal slices from kainate-treated epileptic rats. J Neurosci. 1996 Jul 15;16(14):4438-48. doi: 10.1523/JNEUROSCI.16-14-04438.1996. PMID: 8699254; PMCID: PMC6578858.

Xu X, Miller EC, Pozzo-Miller L. Dendritic spine dysgenesis in Rett syndrome. Front Neuroanat. 2014 Sep 10;8:97. doi: 10.3389/fnana.2014.00097. PMID: 25309341; PMCID: PMC4159975.

Yang L, Zhang R, Zhu H, Chen F, Yu N, Di Q. Factors influencing the long-term prognosis of patients with temporal lobe epilepsy: a single center study. Ann Palliat Med. 2020 Sep;9(5):3194-3203. doi: 10.21037/apm-20-1415. Epub 2020 Sep 12. PMID: 32954759.

Yogatheesan Varatharajah, Brent Berry, Boney Joseph, Irena Balzekas, Tal Pal Attia, Vaclav Kremen, Benjamin Brinkmann, Ravishankar Iyer, Gregory Worrell, Characterizing the electrophysiological abnormalities in visually reviewed normal EEGs

of drug-resistant focal epilepsy patients, Brain Communications, Volume 3, Issue 2, 2021, fcab102, https://doi.org/10.1093/braincomms/fcab102

Yoshimura Y, Yamauchi Y, Shinkawa T, Taoka M, Donai H, Takahashi N, Isobe T, Yamauchi T. Molecular constituents of the postsynaptic density fraction revealed by proteomic analysis using multidimensional liquid chromatography-tandem mass spectrometry. J Neurochem. 2004 Feb;88(3):759-68. doi: 10.1046/j.1471-4159.2003.02136.x. PMID: 14720225.

Yu EP, Dengler CG, Frausto SF, Putt ME, Yue C, Takano H, Coulter DA. Protracted postnatal development of sparse, specific dentate granule cell activation in the mouse hippocampus. J Neurosci. 2013 Feb 13;33(7):2947-60. doi: 10.1523/JNEUROSCI.1868-12.2013. PMID: 23407953; PMCID: PMC3711669.

Yuan Y, Duan Y, Li W, Ren J, Li Z, Yang C. Differences in the Default Mode Network of Temporal Lobe Epilepsy Patients Detected by Hilbert-Huang Transform Based Dynamic Functional Connectivity. Brain Topogr. 2023 Jul;36(4):581-594. doi: 10.1007/s10548-023-00966-9. Epub 2023 Apr 28. PMID: 37115390.

Zanzmera P, Shukla G, Gupta A, Singh H, Goyal V, Srivastava A, Behari M. Markedly disturbed sleep in medically refractory compared to controlled epilepsy - a clinical and polysomnography study. Seizure. 2012 Sep;21(7):487-90. doi: 10.1016/j.seizure.2012.04.005. Epub 2012 May 24. PMID: 22633696.

Zhang L, Wang M, Liu M, Zhang D. A Survey on Deep Learning for Neuroimaging-Based Brain Disorder Analysis. Front Neurosci. 2020 Oct 8;14:779. doi: 10.3389/fnins.2020.00779. PMID: 33117114; PMCID: PMC7578242.

Zhang S, Kou Y, Hu C, Han Y. MicroRNA profiling in the dentate gyrus in epileptic rats: The role of miR-187-3p. Medicine (Baltimore). 2017 Jun;96(22):e6744. doi: 10.1097/MD.0000000000006744. PMID: 28562528; PMCID: PMC5459693.

Zhang T, Yu F, Xu H, Chen M, Chen X, Guo L, Zhou C, Xu Y, Wang F, Yu J, Wu B. Dysregulation of REV-ERBα impairs GABAergic function and promotes epileptic seizures in preclinical models. Nat Commun. 2021 Feb 22;12(1):1216. doi: 10.1038/s41467-021-21477-w. PMID: 33619249; PMCID: PMC7900242.

Zhang W, Huguenard JR, Buckmaster PS. Increased excitatory synaptic input to granule cells from hilar and CA3 regions in a rat model of temporal lobe epilepsy. J Neurosci. 2012 Jan 25;32(4):1183-96. doi: 10.1523/JNEUROSCI.5342-11.2012. PMID: 22279204; PMCID: PMC3778651.