Therapy Development in the Era of Team Science and Big Data: What will the future bring to the patient with epilepsy?

Program and Abstracts
Greetings Fellow Attendees,

We are honored to welcome you to the 2015 Anticonvulsant Drug Development (ADD) Program Symposium entitled “Therapy Development in the Era of Team Science and Big Data: What Will the Future Bring to the Patient with Epilepsy?” For the next 2.5 days, we have brought together basic and clinical researchers from around the world who share a special interest in team science approaches to innovation and who are passionate about translational research and therapy development. The ultimate goal of this symposium is to facilitate new knowledge, new collaborations, and new insight into state of the art therapy development that will improve the quality of life for the patient who is either at risk for developing epilepsy or who has pharmaco-resistant epilepsy.

The American anthropologist, Margaret Meade, once said “Never doubt that a small group of thoughtful, committed people can change the world; indeed, it is the only thing that ever has.” Forty years ago, a small group of thoughtful minds created the Anticonvulsant Screening Program in hopes of improving the lives of patients with epilepsy. Since then, many more clinicians, scientists, and advocates have worked together towards this common goal. We are delighted to look back on the therapeutic innovations that have come about from these collaborations and excited to look forward to those yet to come. In bringing together at this Symposium many more thoughtful, committed individuals, both junior and established, we hope that this meeting will contribute to future therapeutic advances. The members of the Organizing Committee thank each of the attendees for joining us and contributing to this exciting Symposium.

We would also like to thank the many sponsors whose generous support have made this Symposium possible.

- The Organizing Committee

**H. Steve White, Ph.D.**  
Professor and Director  
Anticonvulsant Drug Development Program  
Department of Pharmacology and Toxicology  
University of Utah

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Professor and Chair  
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**Misty D. Smith, Ph.D.**  
Research Assistant Professor  
Anticonvulsant Drug Development Program  
Department of Pharmacology and Toxicology  
University of Utah
ADD PROGRAM SYMPOSIUM SUPPORTERS

We thank the following organizations for their support of the ADD Program Symposium. Without their support this symposium would not be possible.

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UCB Pharmaceuticals
University of Utah College of Pharmacy
University of Utah Department of Pharmacology & Toxicology
University of Utah Health Sciences Center
University of Utah Vice President for Research
Upsher-Smith Laboratories

SYMPOSIUM SPEAKERS

Jeff Anderson, MD, PhD | University of Utah
Scott Baraban, PhD | University of California San Francisco
Tallie Z. Baram, MD, PhD | University of California Irvine
Melissa Barker-Haliski, PhD | University of Utah
Anne Berg, PhD | Children's Hospital of Chicago
Amy Brooks-Kayal, MD | Children's Hospital Colorado
Jeffrey Buchhalter, MD, PhD | Albert Children's Hospital
Neil Buckholtz, PhD | National Institutes of Health (NIH)
Lisa Coles, PhD* | University of Minnesota
Raimondo D’Ambrosio, PhD | University of Washington
Tracy Dixon-Salazar, PhD | Citizens United for Research in Epilepsy
Laura Ewel, PhD* | UC San Diego
Jaqueline A. French, MD | NYU Langone Medical Center
Aristea Galanopoulou, PhD | Albert Einstein College of Medicine
Alicia Goldman, MD, PhD | Baylor College of Medicine
David Henshall, PhD | Royal College of Surgeons, Dublin
Shruthi Iyer* | Creighton University
Andres Kanner, MD | University of Miami
John Kehne, PhD | NINDS Anticonvulsant Screening Program (ASP)
Henrik Klitgaard, PhD | UCB Pharmaceuticals
Hal Kohn, PhD | University of North Carolina
Harvey Kupferberg, PhD | Discussant
Jeff Loeb, PhD | University of Illinois At Chicago
Angel Lopez* | Baylor College of Medicine
Wolfgang Löscher, PhD | University of Veterinary Medicine, Hannover
James McNamara, PhD | Duke University Medical Center
Kimford Meador, MD | Stanford University
Heather Mefford, MD | University of Washington
Julie Milder, PhD | Citizens United for Research in Epilepsy
Candace Myers PhD* | University of Washington
Jeff Noebels, MD, PhD | Baylor University
Dick Normann, PhD | University of Utah
Terence O'Brien, PhD | University of Melbourne
Jack Parent, MD | University of Michigan
Manisha Patel, PhD | University of Colorado
Asla Pitkänen, MD, PhD, DSc | University of Eastern Finland
Roger Porter, MD | University of Pennsylvania, University of Maryland
Rajesh Ranganathan, PhD | National Institute for Neurological Disorders
Jong Rho, MD | University of Calgary
Mike Rogawski, MD, PhD | University of California Davis
Corinne Roucard, PhD | SynapCell SAS
Helen Scharfman, PhD | NYU Langone Medical Center
Chris Schmidt, PhD | Pfizer
Arne Schousboe, PhD | University of Copenhagen
Oleksandr Shcheglovitov, PhD | University of Utah
Graeme Sills, PhD | University of Liverpool
Robert Sloviter, PhD | Morehouse School of Medicine
Barbara Slusher, PhD | Johns Hopkins University Brain Science Institute
Misty D. Smith, PhD | University of Utah
David Swinney, PhD | Institute for Rare and Neglected Diseases
William Theodore, MD, PhD | National Institute for Neurological Disorders
Kyle Thomson, PhD | University of Utah
Andrew Tidball, PhD* | University of Michigan
Roy Twyman, PhD | Janssen Pharmaceuticals Research & Development, US
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Peter J. West, PhD | University of Utah
H. Steve White, PhD | University of Utah
Vicky H. Whittemore, PhD | National Institute for Neurological Disorders
Karen S. Wilcox, PhD | University of Utah

* Indicates Junior Investigator Awardee
Therapy Development in the Era of Team Science & Big Data:
What Will the Future Bring to the Patient with Epilepsy?

Park City Marriott
Park City, UT
May 17-20, 2015

May 17th, 2015 – Symposium Arrival Day

4:00 – 6:30 pm Symposium Check-In/Registration

6:30 – 9:30 pm Opening Reception & Dinner

Welcoming Remarks
H. Steve White, ADD Program, University of Utah
Harold H. Wolf, Professor Emeritus, University of Utah
Margo Thurman, Epilepsy Alliance of Utah

Plenary Lecture
Anne T. Berg, Ann & Robert H. Lurie Children’s Hospital of Chicago, Northwestern University Feinberg School of Medicine - Epidemiological Perspective on the State of Epilepsy Research Needs for the 21st Century

May 18th, 2015 – Symposium Day 1 – Towards Future Innovation through Public/Private Partnerships

7:45 – 8:25 am Continental Breakfast

8:25 am Tom Parks, VP Research, University of Utah - Welcoming Remarks

8:30 Session 1 – Panel: Translational Therapy Discovery: Future

Moderator: H. Steve White, University of Utah

8:30 – 9:00 John Kehne, NINDS - State Of The Anticonvulsant Screening Program Then, Now, And Beyond

9:00 – 9:30 Mike Rogawski, University of California, Davis - Antiepileptic Drugs: Needs For New Treatments And Targets

9:30 – 10:00 Henrik Klitgaard, UCB Pharmaceuticals - Translational Therapies In Epilepsy: Industry Perspective

10:00 – 10:15 Coffee Break
10:15 – 12:00 pm  
**Session 2 – Team Science Approach To Treating The Whole Patient**

Moderator: Vicky H. Whittemore, NINDS

*Each panelist will provide a 10 min overview that describes their program and how their organization approaches team science and innovation.*

- Julie Milder, CURE - Infantile Spasms Initiative
- Chris Schmidt, Pfizer Neuroscience - Pfizer Industry Perspectives On Team Science
- Barbara Slusher, Johns Hopkins University Brain Science Institute - Navigating Private And Academic Drug Discovery Efforts
- Neil Buckholtz, NIA - Accelerating Medicines Partnership Initiative

Panel Discussion - Team Science in the 21st Century – When Does It Work; When Does It Fail? (Julie Milder, Chris Schmidt, Barbara Slusher, and Neil Buckholtz).

12:00 – 1:30 pm  
**Lunch (Junior Investigators to meet with Mentors)**

1:30 – 1:50 pm  
Rajesh Ranganathan, NINDS - NINDS Therapy Development Funding Programs: Lessons Learned And The Road Ahead

2:00 pm  
**Session 3 – How Will Genetics Inform Therapy Development?**

Moderator: Jeff Noebels, Baylor University

- Heather Mefford, University of Washington - Epi4K Updates And What Has Come About From Team Science
- Jack Parent, University of Michigan - Pluripotent Stem Cell Models Of Epilepsy
- Scott Baraban, UCSF - Zebrafish Models For High-Throughput Drug Discovery

3:00 – 3:30 pm  
**Break**

3:30 – 3:50 pm  
David Swinney, Institute for Rare and Neglected Diseases - Discovering New Medicines For Rare Diseases/Mutations

3:50 – 4:30 pm  
Panel Discussion - How Do These New Approaches Inform Future Therapy Development? (Graeme Sills, David Swinney, Scott Baraban, Jeff Buchhalter)

5:00 – 7:00 pm  
**Session 4 – Young Investigators Poster Session**

Cash Bar, Cocktails and Light Hors d’Oeuvres

Note: Please have all Posters hanging by 8 am Monday, May 18th and available for viewing through symposium close on Wednesday, May 20th

(Dinner on your own)
May 19th, 2015 – Symposium Day 2 – Health Matters in Epilepsy

7:45 – 8:30 am Continental Breakfast

8:30 am

**Session 5 – Comorbid Cognitive And Neuropsychiatric Disorders Of Epilepsy And CNS Disorders With Seizures**

Moderator: Amy Brooks-Kayal, University of Colorado

8:35 – 8:55 Kimford Meador, Stanford University - General Overview: Epilepsy And Neuropsychological Comorbidities: Drug Effects On Cognition

8:55 – 9:10 Laura Ewell*, University of California, San Diego - Dentate-Dependent Memory Loss As A Biomarker For Epileptogenesis: Network Level Mechanisms

9:10 – 9:30 Helen Scharfman, NYU Langone Medical Center - Effects Of Epilepsy On Cognition And Cognitive Disorders Associated With Seizures In The Elderly

9:30 - 9:50 Coffee Break

9:50 – 10:05 Angel Lopez*, Baylor College of Medicine - ANK3 Mutations In Bipolar Disorder And Epilepsy

10:05 – 10:25 Oleksandr Shcheglovitov, University of Utah - Phelan-McDermid Syndrome And iPSCs

10:25 – 10:45 Andres Kanner, University of Miami - Overview Of The Impact Of Psychiatric Comorbidities On The Course And Treatment Of Seizure Disorders

10:45 – 11:30 Panel Discussion Wrap-Up - How Does Knowledge About Shared Pathologies And Comorbidities Drive Therapy Development? (Andres Kanner, Amy Brooks-Kayal, Helen Scharfman, Misty D. Smith, Peter West)

11:30 - 1:30 Lunch (Junior Investigators to meet with Mentors)

1:30 pm

**Session 6 – Clinical Considerations, Biomarkers, and Pathways Forward**

Moderator: Terence O’Brien, University of Melbourne

1:30 – 1:50 Manisha Patel, University of Colorado - Metabolomics of Epilepsy

1:50 – 2:10 Alicia Goldman, Baylor University - SUDEP: Pathways, Biomarkers, And Opportunities For Therapeutic Interventions

2:10 – 2:25 Lisa Coles*, University of Minnesota - Academic Drug Discovery And Development Teams: The Power Of Pharmacokinetic And Pharmacodynamic Modeling And Simulation

2:25 – 2:45 William Theodore, NINDS - Imaging Neuroinflammation And Other Biomarkers In The Brain Of Patients With Epilepsy

2:45 – 3:05 Asla Pitkänen, University of Eastern Finland - Gender Issues In Antiepileptogenic Therapy Development

3:05 – 3:30 Panel Discussion - Biomarkers For Therapy Discovery (Manisha Patel, Alicia Goldman, William Theodore)
3:30 – 3:45 Break

Session 7 – Data Blitz - Emerging Models

Moderator: Roger Porter

3:45 – 4:00 Wolfgang Löscher, University of Veterinary Medicine, Hannover - State Of Animal Models – Redux

4:00 – 4:15 Raimondo D’Ambrosio, University of Washington - Updates On The FPI-Model As Antiepileptogenic Screen

4:15 – 4:30 Arista Galanopoulou, Albert Einstein College of Medicine - Towards The Development Of Pediatric Epilepsy Models

4:30 – 4:45 Melissa Barker-Haliski, University of Utah - The TMEV Model Of Inflammation-Induced Seizures For Screening Of Investigational Drugs

4:45 – 5:00 Andrew Tidball*, University of Michigan - Modeling SCN8A Mutant Epilepsy In Patient-Derived Cortical And Autonomic Neurons

5:00 – 5:15 Corinne Roucard, SynapCell SAS - The MTLE Mouse For Drug Screening

5:15 – 5:30 Robert Sloviter, Morehouse School of Medicine - The Perforant Path Stimulation Model; Unilateral Hippocampal-Onset Epilepsy With Hippocampal Sclerosis In Rats

5:30 – 5:45 Kyle Thomson, University of Utah - A Clinically Relevant Model For Screening Anticonvulsants In Chronically Epileptic Rats

5:45 – 6:00 Roger Porter and Wolfgang Löscher - Wrap-Up – What Do Future Models Need?

(Dinner on your own)
May 20th, 2015 – Symposium Day 3: Lessons Learned and Pathways Forward

7:45 – 8:30 am Continental Breakfast

8:30 – 10:10 am Session 8: Lessons Learned for Drug Discovery Past, Present, Future

Moderator: Roy Twyman, Janssen Pharmaceuticals Research and Development, US

New Therapeutic Targets For Epilepsy – 10 min each:

- Arne Schousboe, University of Copenhagen - GABAergic Mechanisms
- Shruthi Iyer*, Creighton University - Antiseizure Effects Of Ketogenic Diet Are Mediated Via Regulation Of Brain PPAR-gamma, A Nutritionally Responsive Transcription Factor
- Jong Rho, University of Calgary - Bioenergetic Substrates: More Than Just Cellular Fuels
- Annamaria Vezzani, Mario Negri Institute for Pharmacological Research - miR146a-Based Therapy Against Neuroinflammation Has Anti-Ictogenic And Disease-Modifying Effects In Murine Models Of Seizures And Epilepsy
- Tallie Z. Baram, University of California, Irvine - Epigenetic Targets For Epilepsy / Seizure Prevention
- James McNamara, Duke University - Targeting TrkB To Prevent Epileptogenesis
- David Henshall, Royal College of Surgeons in Ireland - MicroRNAs For Antiepileptogenesis

9:40-10:10 Panel Discussion with Presenters

10:10 – 10:30 Coffee Break

10:30 – 12:00 pm Session 9 – Big Data

Moderator: Karen Wilcox, University of Utah

10:30 – 10:45 Tracy Dixon-Salazar, CURE - CURE Updates On Future Directions Based On Epilepsy Genetics: Towards Personalized Medicine

10:45 – 11:00 Richard Normann, University of Utah - Brain Mapping Initiative

11:00 – 11:15 Jeff Loeb, University of Illinois - Systems Biology Approach To Therapeutic Development

11:15 – 11:30 Candace Myers*, University of Washington - EPI4K, Gene Discovery In Epileptic Encephalopathies

11:30 – 11:45 Jeff Anderson, University of Utah - Imaging Neurophysiology Of Distributed Brain Networks

11:45 – 12:00 Panel Wrap-Up - How To Integrate Big Data To Design Big Drugs

12:00 – 12:30 pm Closing Remarks and Pathways Forward

Jacqueline French, NYU Langone Medical Center - Moving Preclinical Research To Clinical Treatments

Patient Advocate - Remembering Who We Fight For!
PARK CITY INFORMATION

Adjusting to the Higher Altitude in Utah

Adjusting from a low-altitude locale to the higher altitude of Park City (6,900+ feet/2,103+ meters) may cause some visitors to exhibit some mildly uncomfortable symptoms like these:

- headaches
- dehydration
- body aches (“flu”-like symptoms in the muscles and joints)

How can you adjust comfortably to the higher altitude and avoid or diminish these kinds of symptoms?

First and foremost: Drink plenty of water! Utah’s water—right from the faucet—is clean, pure, healthy, and delightful. You’ll enjoy drinking LOTS of Utah water!

Keeping your body hydrated is very important because high altitudes can dehydrate your system. This can be further complicated in arid regions like Utah. AND “jet-lag” can make matters worse! Water assists your body in flushing toxins, which is critical because altitude affects the body’s ability to dispose of carbon dioxide through breathing. Keep drinking water. Remember that if you feel thirsty, you have waited too long to drink.

If possible, on the first day you arrive, REST—and avoid strenuous exercise—to give your body time to adjust. Small and frequent meals of protein and complex carbohydrates can help keep symptoms to a minimum. Drink water BEFORE you feel thirsty!

At the higher altitude, limit alcohol, caffeine, and simple carbohydrates like sugar. Instead, drink plenty of water. You should also limit heavy meals and smoking. Caffeine, alcohol, tobacco, and simple carbohydrates affect your body’s ability to metabolize, and can bring more symptoms or make them worse.

Oh yes, and did we say, “DRINK LOTS OF WATER!”?

Internet access on Marriott Networks
Group Code: ADD2015
Park City Restaurants on Main Street

**Grappa** – Fine Italian food – Grappa is located in a former boarding house and features an intimate setting for classic Italian fare & panoramic upstairs views. $$$
151 Main Street
(435) 645-0636
Open Monday, Thursday 5 – 9 pm, Friday & Saturday, 5 pm – 10 pm. Closed Tue-Wed. Monday is ‘locals’ night, with $5 glasses of wine and entrees for $12-$14. 2 for 1’s online through ‘Bill White Enterprises’, billwhiterestaurantgroup.com/

**Handle** – American cuisine, mostly small plates, is served in a lively energetic space with fun cocktails. $$$
136 Heber Ave
435-602-1155
Open Wednesday – Sunday 5 – 11 pm; Closed Monday and Tuesday. Don’t miss the Rattlesnake cocktail, Roasted Shishito peppers as a starter, and the Smoked Trout Sausage for a unique dining experience!

**High West** – A must-visit! The only such facility in the entire state of Utah – rich with history $$
703 Park Ave
(435) 649-8300
Open Sunday through Thursday, 11am - 9pm; Friday & Saturday, 11am - 10pm
Featured dinner specials and live music on Wednesday nights. Distillery Tours Monday through Thursday, 2:15pm & 3:30pm; Friday through Sunday, 1, 2:15, & 3:30pm

**Rock & Reilly’s** – The centrally located establishment invokes the kindred spirits of a Prohibition-era speakeasy, infused with mining history and ski town flair. $$
427 Main St.
435-655-2926
Open Everyday 11:30 am – 1:00 am
Don’t miss New Taco Tues, Slider Night Wed, and 3 Hour Happy Hour Monday – Friday from 3 – 6 pm.

**Talisker** – Warm atmosphere. Fresh, local food, thoughtfully prepared.$$$$
515 Main Street
(435) 658-5479
Open through end of September. Hours: nightly, 5:30 to 9:00pm. (flexible closing)
“Tapas Tuesday,” 5:30 to 7:30pm : 3 options for $6-$10, changes weekly; also, half off appetizers.

**Wasatch Brew Pub** – This is a popular venue crafting unique local beers along with eclectic pub grub & cocktails in a relaxed setting. $$
250 Main St.
435-649-0900
Open Monday – Friday 11 am – 10 pm; Saturday – Sunday 10 am – 10 pm;
Award winning Wasatch ales or lagers are available by the bottle or on draft. Traditional pub grub is served with more eclectic fare such as Braised Chicken Thighs and Whiskey Salt Tater Tots.
Zoom – Sundance’s Park City restaurant. Less expensive, home-cookin’ $$$
660 Main St
(435) 649-9108
Open Tuesday - Saturday 5:30 - 9:30 pm; Closed Sunday – Monday.
Nightly dinner specials available.

OTHERS:
Butcher’s Chop House – elegant steak house at the Town Lift $$$
Bangkok Thai – Excellent, authentic & affordable Thai cuisine in an historic landmark building $$
Shabu - Freestyle Asian $$$
The Flying Sumo – Sushi $$$
Riverhorse on Main – Transcontinental Eclectic – classy, upscale, chic ambiance $$$$  
Chimayo – Southwestern rustic; lots of atmosphere $$$$  
Wahso – Tasteful curtained booths and luscious desserts $$ $
350 Main Brasserie– Excellent service and creative menu in renovated historic building $$$

Park City Bars

Cisero’s in Park City  No Name Saloon and Grill  Sky Blue
306 Main Street 447 Historic Main Street 201 Heber Ave
Downstairs O’Shucks Bar & Grill The Cabin
625 Main Street 427 Main Street 825 S. Main Street
Epic (Former Star Bar) Park City Live The Spur
268 Main Street 427 Main Street 352 Main Street
High West Distillery Sidecar Bar Wasatch Brewery
703 Park City 333 Main Street 250 Main Street

Things To Do In Park City

Park City Gallery Stroll:
On the last Friday of each month, from 6:00 to 9:00 p.m., members of the Park City Gallery Association offer a unique showcase highlighting artists, special exhibits and art events. The Gallery Stroll is a free community event that gives locals and Park City visitors alike the opportunity to enjoy light refreshments while exploring Park City's exciting art scene.

Park City Museum:
For more information, including current admission prices see:
http://www.parkcityhistory.org/
Olympic Park:
Free admission includes access to Olympic Museum and access to hiking trails. Day pass tickets are required to ride the zipline, alpine slide, chairlifts, bobsled, etc. For current pass and ticket pricing see: http://www.utah.com/parkcity/olympic_park.htm

Park City Outdoors

Fly Fishing

Trout Bum 2
4343 N. Hwy 224 Suite 101
Park City, Ut. 84098
http://www.troutbum2.com/
1-877-878-2862; 435-658-1166

Park City Anglers
P.O. Box 683155
Park City, Ut 84068
Phone: 435-658-3474
E-Mail: flyfishing@parkcityanglers.com

Golf

Park City Golf Course
1541 Thaynes Canyon Drive
Park City, Utah
435-615-5800

Crater Springs Golf Course at Homestead
700 Homestead Dr.
Midway, Utah 84049
866-931-3097
www.CraterSpringsGolf.com

Soldier Hollow Golf Course
1370 West Soldier Hollow Lane
Midway, Utah 84049
435-654-7442
soldierhollowgolf.com

Wasatch Golf Course
Wasatch Mountain State Park
Midway, Utah 84049
435-654-0532
http://www.wasatchgolfcourse.com

Hiking / Biking

With over 400 miles of trails for hiking and biking, Park City couldn't be better for outdoor enthusiasts. From scenic strolls to adrenaline-raising terrain, Park City is a great place for a mountain biking vacation. If you prefer a lift up the mountain; some of the Resorts do provide lift-served mountain biking and hiking.

Mountain Bike/Road Bike Rentals in Park City

Cole Sport
435-649-4806
(800) 345-2938
1615 Park Ave
Park City, UT 84060

Jans Mountain Outfitters
435-649-4949
(800) 745-1020
1600 Park Avenue
Park City, UT 84060

White Pine Touring
435-649-8710
1790 Bonanza Drive
Park City, UT 84060
ABSTRACTS

Genetic and pharmacological targeting of α2δ-1 signaling to prevent epileptogenic circuit reorganization, pathological synaptogenesis, and cell death following neonatal cortical insult

Lauren Andresen, Danielle Croker, David Hampton and Chris Dulla

Epigenetic Targets for Epilepsy / Seizure Prevention

Tallie Z. Baram

Therapeutic treatment of acute behavioral seizures in the Theiler’s murine encephalomyelitis virus model of acquired epilepsy improves long-term behavioral outcome


Short-term synaptic reorganization in the rat neocortex after perinatal hypoxia-ischemia

J.D. Bastar, J. Spampanato, F.E. Dudek

An animal model of comorbid epilepsy and autism based on the combination of maternal stress and a perinatal teratogen: A new platform for anti-epileptogenic/anti-autism drug development.

Florencia Bercum, Krista M. Rodgers, Alex M. Benison, Zachary Smith, Elise Kornreich, Elizabeth Woodruff and Daniel S. Barth

Scavenging seizure-induced reactive oxygen species with a catalytic antioxidant attenuates neuroinflammation in experimental temporal lobe epilepsy

Pallavi Bhuyan, Li-Ping Liang, Brian J. Day and Manisha Patel

Predictive biomarkers during early epileptogenesis in post-SE rats

Sonja Bröer, Claudia Brandt, Kathrin Töllner, Rebecca Klee, and Wolfgang Löschner

Deletion of PDE11A4, an enzyme with expression restricted to the hippocampus, alters glutamatergic signaling and produces transient amnesia

Will Capell, Shweta Hegde, Joseph Meyers, Michy P. Kelly, Ph.D.

Academic Drug Discovery and Development Teams: The Power of Pharmacokinetic and Pharmacodynamic Modeling and Simulation

Lisa D Coles, Edward E Patterson, Illo E Leppik, Irene Vuu, Usha Mishra, Andrea Eckert, Gregory Worrell, Daniel Crepeau, Aristeia S Galanopoulou, James C Cloyd

Preliminary evidence of adaptive immune system cell infiltration into the brain of a genetic model of chronic epilepsy

Malavika Deodhar, Ankita Aggarwal, Timothy A. Someone, Kristina A. Simeone
Comparison of spike-wave discharges and other oscillatory activity in normal animals with spontaneous recurrent seizures across several animal models of acquired epilepsy
F.E. Dudek, K.M. Rodgers, W.A. Pouliot, S. Kadam, E.H. Bertram, D.S. Barth

Dentate-Dependent Memory Loss as a Biomarker for Epileptogenesis: Network Level Mechanisms

Anticonvulsant Effects Of A Galanin Receptor 3 (GalR3) Antagonist Snap 37889 in Mouse Models Of Seizures
Saurabh A Gagangras, E. Jill Dahle, Cameron S. Metcalf, Kyle E. Thompson, H. Steve White

In utero electroporation of the novel plasmid, NASTIE, for distinguishing neurons and astrocytes during calcium imaging experiments in rat hippocampus
M.B. Gibbons, S.W.A. Titen, P. Tvrdik, J.A. White and K.S. Wilcox

Precision editing of zebrafish syntaxin-binding protein 1 (STXBP1) to model human neurodevelopmental disease
Brian P. Grone, Maria Marchese, Kyla R. Hamling, Federico Sicca, Filippo M. Santorelli, and Scott C. Baraban

Plasma Metabolomic Analysis of (1-3)IGF-1 Treatment in Rats
Svenja Heischmann, Chong Lee, John Le, Nichole Reisdorph, John Swann, Manisha Patel

Vorinostat reduces hyperexcitability and epileptiform events in a novel drug-screening platform
Ibhazehiebo K, Gavrilevici C, Scott L, Rho JM²,², Kurrasch DM¹,³

Molecular mechanism of astroglial swelling and neuronal overactivation in the retina
Anthony Iuso, Daniel Ryskamp, Andrew Jo, Maxim Kozhemyakin, Oleg Yarishkin and David Križaj

An international, curated KCNQ2 database and website: a tool set for elucidation of genotype-phenotype relationships and development of precision medicines
Nishtha Joshi, Edward C. Cooper, Maurizio Tagliatela, Sarah Weckhuysen

Improvement in Seizure Control During Conversion to Eslicarbazepine Acetate Monotherapy: A Pooled Analysis of Two Trials in Adults with Refractory Partial-Onset Seizures
Gregory Krauss, Victor Biton, Hailong Cheng, David Blum
Known genes explain a significant proportion of cases of early onset epileptic encephalopathy with burst-suppression
Christopher M. LaCoursiere, Heather E. Olson, MD, Dimira Tambunan, Rebecca Pinsky, Beth Sheidly, Annapurna Poduri, MD MPH

Neurodegeneration, Gliosis, and Glial Proliferation in Two Mouse Models of Temporal Lobe Epilepsy
Jaycie L. Loewen, Melissa L. Barker-Haliski, E. Jill Dahle, H. Steve White, Karen S. Wilcox

Ankyrin-G: A novel mechanistic link between epilepsy and bipolar disorder
Angel Y. Lopez, Mingxuan Xu, Atul Maheshwari, Jeffrey L. Noebels, Edward C. Cooper

NAX 810-2, A Novel Galanin-Based Therapy for Epilepsy
Cameron S. Metcalf, Brian D. Klein, Daniel R. McDougle, Liuyin Zhang, Grzegorz Bulaj, H. Steve White

Clinical spectrum of epilepsy secondary to KCNQ2 variants and initial response to ezogabine
John J. Millichap, MD and Edward C. Cooper, MD, PhD on behalf of the RIKEE Network.

Gene discovery and high-throughput resequencing of candidate genes in epileptic encephalopathies
Candace T. Myers, Jacinta M. McMahon, Amy Schneider, Rikke S. Moller, Gemma L. Carvill, Ingrid E. Scheffer, Heather C. Mefford, Epi4K Investigators

The Norzler method: a reliable, SE-free animal model of acquired human temporal lobe epilepsy
Braxton A. Norwood, Friederike Kienzler-Norwood, Felix Rosenow

Synaptic scaling in the hippocampus in a mouse model of viral-induced temporal lobe epilepsy
Dipan C. Patel, Pallavi Bhuyan, E. Jill Dahle, Robert S. Fujinami, H. Steve White, Manisha Patel, Karen S. Wilcox

Eslocarbazepine acetate monotherapy in adults with partial-onset seizures: A pooled analysis of two randomized double-blind studies with use of a historical control.
Ladislav Pazdera, Jacqueline French, Michael Sperling, Mercedes Jacobson, Hailong Cheng, David Blum

Mechanisms of action of anti-seizure drugs and the Anticonvulsant Screening Program of the National Institute of Neurological Disorders and Stroke
Roger J. Porter, Harvey J. Kupferberg, Bettie Jean Hessie

TRPV4 mediates changes in microglial morphology and process dynamics during treatment with hypotonic stimuli
Sarah N. Redmon, Oleg Yarishkin, Taylor Drake, David Križaj
5-HT6 receptor ligands modulate seizure thresholds and inhibitory synaptic transmission in the dentate gyrus
Gregory J. Remigio, Gerald W. Saunders, Peter J. West

Systemic delivery of antagomir-134 produces long-lasting seizure-suppressive effects
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Genetic and pharmacological targeting of α2δ-1 signaling to prevent epileptogenic circuit reorganization, pathological synaptogenesis, and cell death following neonatal cortical insult

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Developmental cortical malformations, such as polymicrogyria, have a high incidence of drug-resistant epilepsy, but the underlying mechanisms by which these lesions contribute to the onset of seizure activity remain poorly understood. Using the neonatal freeze-lesion (FL) model in mice, we have shown that FL induces a focal cortical malformation consisting of a microgyrial zone and a hyperexcitable paramicrogyrial zone after a 2-week latent period. FL-cortex also shows an upregulation of reactive astrocytes and the astrocyte-secreted protein thrombospondin (TSP) for one week, prior to the onset of epileptiform activity. TSP is known to induce excitatory synapse formation, which we hypothesize contributes to the pathological reorganization of the FL cortex. The neuronal receptor for TSP is the calcium channel subunit α2δ-1 and we have found that α2δ-1 is also transiently upregulated during the same time window as increased TSP expression. We hypothesized that TSP and α2δ-1 upregulation leads to aberrant excitatory synaptogenesis, pathological network formation, and cell death and that targeting TSP/α2δ-1 signaling will be protective against epileptogenic processes following neonatal cortical insult. To address this hypothesis, we disrupted the interaction of TSP with α2δ-1 using a treatment paradigm of once daily, I.P. injections of gabapentin (GBP), an antagonist of TSP/α2δ1 signaling, to coincide with the time period of TSP/α2δ-1 upregulation. In vitro hyperexcitability was assessed by recording evoked cortical fields potentials (fEPSP) and by performing extracellular glutamate imaging with a FRET-based biosensor. In vivo seizure susceptibility was further investigated by recording EEG following acute kainate injection. These experiments demonstrated that pharmacologically disrupting TSP/α2δ-1 signaling for one week post-injury, with GBP, prevented the later onset of both in vitro and in vivo hyperexcitability. Importantly, in line with the known mechanism of TSP/α2δ-1-driven synaptogenesis, GBP treatment also blocked the rise in excitatory synapses following FL. Lastly, we showed that GBP treatment attenuated anatomical cortical reorganization as assessed by changes in GFAP+ reactive astrocytes, cortical layer specific neuronal markers and markers of cell death. Next, we utilized a genetic approach to address the same question but avoid the potential non-specific drug effects of GBP. Using a germ-line, global knockout of α2δ-1, we again performed FL, but now in the absence of α2δ-1. Following injury, α2δ-1 KO mice have less epileptiform activity as measured by fEPSP recordings. Interestingly, the FL α2δ-1 KO mice have an intermediate phenotype compared to GBP treated wild-type animals, which suggests that KO of α2δ-1 during development leads to genetic compensation, perhaps of other pro-synaptogenic pathways, that contribute to pathological network formation following FL. Finally, α2δ-1 KO mice appear insensitive to GBP treatment, providing compelling evidence that the therapeutic effects of GBP are specific to its interactions at α2δ-1. These results shed new light on how hyperexcitable networks are formed after injury and suggest the use of GBP, or other modulators of TSP/α2δ-1 signaling, as potential therapeutic agents to minimize epileptogenesis associated with developmental cortical malformations and other post-traumatic epileptic conditions.
Epigenetic Targets for Epilepsy / Seizure Prevention

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The group of neurological disorders characterized by spontaneous seizures (Epilepsy) result from numerous causes and mechanisms. Temporal lobe (limbic) epilepsy often follows identified brain insults such as trauma, long febrile seizures, or brain infections. The process by which spontaneous seizures arise after these insults involves the transformation of normal neurons and circuits into an epileptic network. However, the underlying mechanisms remain unknown.

Four decades of research have shown changes in the expression of crucial neuronal genes, (e.g., ion channels), that take place during the epileptogenic process. These contribute to altered neuronal function and to changes in neuronal connectivity that promote network hyperexcitability. More recently, evidence is accumulating for large-scale changes in the expression of numerous genes within neurons, and for associated epigenetic chromatin alterations during the epileptogenic process. These insult-provoked epigenetic and transcriptional events seem to involve a limited number of transcriptional ‘master switches’ and signaling cascades, which, in turn, influence the expression of large numbers of neuronal genes. Therefore, these regulatory molecules and pathways, alone and in combination, offer enticing targets for intervention, with the goal of preventing the transformation of ‘normal’ into ‘epileptic’ neurons.
Therapeutic treatment of acute behavioral seizures in the Theiler’s murine encephalomyelitis virus model of acquired epilepsy improves long-term behavioral outcome


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Inflammation represents a significant risk factor for seizure induction and maintenance, with pro-inflammatory cytokines being highly expressed in various animal seizure models and human patients with epilepsy. Infections of the CNS can contribute to the development of chronic epilepsy due to an increased risk of seizures and status epilepticus. Theiler’s murine encephalomyelitis virus (TMEV), when injected into brains of C57/Bl6 mice, provides a novel model of infection-induced epilepsy. Approximately 50-65% of infected mice develop acute, handling-induced seizures during the infection and subsequently develop spontaneous, recurrent seizures and behavioral impairment weeks later. It is unknown whether treatment during the acute infection can attenuate handling-induced seizures, as well as ameliorate long-term behavioral comorbidities associated with epilepsy. This study investigated the efficacy of the antiseizure drugs (ASDs) valproic acid (VPA) and carbamazepine (CBZ), and the anti-inflammatory antibiotic, minocycline (MIN) on TMEV-induced seizures and behavioral comorbidity. On Day 0, male C57/Bl6 mice were infected with TMEV, then treated daily (Day 0-8) with CBZ (n = 28; 20 mg/kg b.i.d., i.p.), VPA (n = 28; 100 mg/kg q.d., i.p.), MIN (n = 28; 50 mg/kg q.d., i.p.), or vehicle control (n = 28). Mice were assessed twice daily for 7 days for handling-induced seizures by an experimenter blinded to treatment. Relative to controls, significantly more CBZ-treated mice presented with seizures; VPA reduced the proportion with seizures, and MIN conferred no change. In animals with seizures, VPA, but not CBZ or MIN, increased the latency to the first observed seizure; only MIN-treated mice demonstrated improved open-field exploratory behavior, a measure of anxiety-like behavior. Furthermore, only MIN significantly reduced the proportion of mice displaying tonic-extension seizures or mortality with PTZ. These data suggest that while treatment with prototype ASDs may alter acute seizure expression in the TMEV model, treatment with anti-inflammatory agents during the acute infection period may mitigate long-term behavioral comorbidities and seizure threshold changes. Such information supports a growing body of evidence suggesting a role for inflammation in seizure disorders and associated comorbidities.

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Short-term synaptic reorganization in the rat neocortex after perinatal hypoxia-ischemia

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Perinatal hypoxia-ischemia (PHI) is common and predisposes the infant to subsequent neurological impairments, such as cognitive deficits, cerebral palsy, and epilepsy. The PHI-induced, anatomical and physiological changes in the neocortex that subsequently contribute to the development of spontaneous recurrent seizures (i.e., chronic epilepsy) remain unknown. Previous data from our group (Kadam et al., 2010 J. Neurosci. 30: 404-15) demonstrate that a commonly used model of hypoxic-ischemic encephalopathy can produce a robust infarct, and rats with PHI-induced brain damage develop epilepsy. We hypothesized that the neocortical pyramidal cells in the peri-infarct region have reduced synaptic innervation, due to local neuronal death, when compared to sham controls. PHI was induced in rat pups at post-natal day 7 (P7) using the Rice-Vannucci model. Whole-cell recordings were performed on brain slices from PHI-treated animals and sham controls at 24-48 hr (i.e., P8-9) after PHI and at a time hypothesized to be during the latent period (P21-23). Specifically, we recorded miniature inhibitory post-synaptic currents (mIPSCs), miniature excitatory post-synaptic currents (mEPSCs), and tonic inhibition converging onto principal cells within the peri-infarct region that surrounds the damage. Immunohistochemistry for GAD67 and NeuN labeling was used to confirm the loss of interneurons and principal cells in the damaged area and the concentration of cells remaining in the peri-infarct region. The frequency of both mIPSCs and mEPSCs onto superficial cortical pyramidal cells in the PHI-treated animals was significantly decreased within 24-48 hr after PHI. At 2 weeks after PHI, however, the frequency of the mIPSCs and mEPSCs had recovered to control levels. The kinetics, amplitude and decay constant of the mIPSCs and mEPSCs were unchanged at both time points. Likewise, tonic inhibition did not differ between PHI and sham-control animals for either time. At the later time period, isolated groups of cells could clearly be seen in and around the cortical infarct. The GAD67 and NeuN labeling confirmed that these islands of cortex contained both pyramidal cells and interneurons; however, their contribution to normal and abnormal cortical function remains unclear. Our data suggest that one of the first functional changes in the damaged cortex following PHI is a loss of inhibitory and excitatory synaptic innervation, which appears to recover within 2 weeks after PHI. Considering the extensive damage that results from PHI, it is remarkable that so much functional recovery in synaptic innervation occurs within 2 weeks. The initial spontaneous recurrent seizures that begin to occur a few weeks after PHI appear to be generated primarily within the peri-infarct region (Kadam et al., 2010); the present studies provide evidence that substantial reorganization of both excitatory and inhibitory synaptic connections has already occurred by this point, and it is plausible that continued synaptic reorganization contributes to an hyperexcitable network and progressive epileptogenesis.

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An animal model of comorbid epilepsy and autism based on the combination of maternal stress and a perinatal teratogen: A new platform for anti-epileptogenic/anti-autism drug development.

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It has long been known that autism spectrum disorder (ASD) is strikingly comorbid with epilepsy, with over 30% of ASD patients also experiencing chronic recurrent seizures and a similar fraction of epilepsy patients with behavioral symptoms of ASD. These numbers rise as high as 60% when sub-convulsive epileptiform spikes are included in assessment of epilepsy. While the comorbidity of ASD and epilepsy could hold clues to possible common mechanisms for both of these disorders and point the way to intervention strategies, there is a need for animal models to facilitate this work.

Here we examined the effects of combining maternal stress with a perinatal teratogen terbutaline (used to arrest pre-term labor), both recognized risk factors in humans for ASD in offspring, on both ASD-like behavior (deficits in social exploration and vocalization and enhanced repetitive behavior) and spontaneous recurrent seizures in rats. We found that either treatment alone resulted in a subset of ASD-like behavior (vocalization deficits) and no signs of epilepsy. The combination of maternal stress and terbutaline produced the full triad of ASD-like behaviors and 50% of the rats also developed recurrent convulsive temporal lobe seizures. Furthermore, the remaining half of these animals displayed sub-convulsive spikes in the absence of seizures.

We conclude that: 1) Combinations of teratogens are far more powerful than single treatments, a conclusion that should be extended to human studies of risk factors, 2) Both convulsive seizures and non-convulsive epileptiform spikes are closely associated with severe behavioral disturbances associated with ASD, and finally, 3) The present results represent not only a new and highly realistic animal model of epilepsy and ASD, but provide a platform for exploring mechanisms and developing anti-epileptiform/anti-ASD compounds.

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Scavenging seizure-induced reactive oxygen species with a catalytic antioxidant attenuates neuroinflammation in experimental temporal lobe epilepsy

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Inflammation has been identified as an epileptogenic factor in temporal lobe epilepsy (TLE). Understanding the mechanisms underlying seizure-induced neuroinflammation could lead to the development of novel anti-epileptogenic therapies. Reactive oxygen species (ROS) are recognized as key mediators of seizure-induced neuronal damage and known to increase in models of TLE. Recent literature suggests that ROS are important mediators of inflammation. Therefore, we tested the role of ROS in seizure-induced neuroinflammation (pro-inflammatory cytokines and microgliosis) using a potent antioxidant which catalytically scavenges ROS but is devoid of direct anti-inflammatory actions. Adult male Sprague-Dawley rats were subjected to pilocarpine-induced status epilepticus (SE) following pretreatment with scopolamine and post-treatment with diazepam. Proinflammatory cytokine production was assessed in the hippocampus and piriform cortex 6, 24, 48 hours (h) and 1 week post-pilocarpine using a multiplex cytokine array (Mesoscale Discovery). The time course study revealed increases in the levels of TNF-α, IL-1β, IL-6 and KC/GRO at 6, 24 and 48 hours after pilocarpine in the hippocampus and piriform cortex with a return to control values at the 1 week time-point. To determine the role of ROS in SE-induced cytokine production, a separate cohort of rats were treated with AEOL10150 [Mn(III) tetrakis (N,N'-diethylimidazolium-2-y1) porphyrin], a catalytic antioxidant with high superoxide dismutase (SOD) and catalase activities beginning 60 min after pilocapine and every 4h until sacrifice (24h). Hippocampus and piriform cortex was collected 24 hours after pilocarpine injection for HPLC analysis of the glutathione redox status (GSH/GSSG), 3-nitrotyrosine/tyrosine (3NT/Tyr) ratios and multiplex cytokine measurement. Whole brains were collected for immunohistochemical staining for microgliosis (Iba-1). Treatment with pilocarpine significantly increased 3NT/Tyr ratio and decreased GSH/GSSG ratio in both brain regions which were significantly reversed by AEOL10150. AEOL10150 treatment attenuated pro-inflammatory cytokine production in the hippocampus and piriform cortices as well as microgliosis without altering pilocarpine-induced SE. In order to rule out that these effects were due to a direct anti-inflammatory action, murine microglial BV2 cells were stimulated with the inflammasogen lipopolysaccharide (LPS) and the effect of AEOL10150 on cytokine release was measured. There was no change in LPS-induced TNF-α release upon treatment with the compound. Additionally, this effect was sustained even when cells were treated with the NADPH oxidase (Nox) inhibitor apocynin to inhibit Nox-induced ROS production, suggesting that AEOL10150 does not have direct anti-inflammatory properties in-vitro. These results demonstrate that scavenging ROS can decrease indices of seizure-induced neuroinflammation and highlight the importance of redox mechanisms in controlling inflammation in TLE.

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Predictive biomarkers during early epileptogenesis in post-SE rats

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A widely used model to study the mechanisms of epileptogenesis and develop strategies for epilepsy prevention is the lithium–pilocarpine rat model, in which status epilepticus (SE) leads to epilepsy with spontaneous recurrent seizures (SRS). We have recently developed a drug cocktail, consisting of diazepam, phenobarbital, and the cholinergic antagonist scopolamine that allows complete and persistent SE termination in the pilocarpine model (Brandt et al., Neurobiol. Dis. 2015). Unexpectedly, in contrast to previous methods of SE termination, an SE terminated after 60 min by this cocktail did not induce development of SRS, but an SE duration of 90 or 120 min was needed for epilepsy to develop. In the search for biomarkers that predict development of epilepsy after SE, we evaluated seizure threshold (determined by i.v. infusion of pentylenetetrazole [PTZ]) and occurrence of behavioral hyperexcitability (determined by approach–response, touch–response, finger-snap, and pick-up test as described by Rice et al. 1998) in rats with 60 vs. 90 min SE. In the 3 weeks following SE, significant seizure threshold decrease and behavioral hyperexcitability was only seen in rats with 90 min SE, whereas no significant difference to pre-SE control was observed in the 60 min SE group (Brandt et al. 2015). The pick-up test was most sensitive for hyperexcitable behavior in epileptic vs. non-epileptic rats.

Based on the hypothesis that a lowered PTZ threshold and an elevated behavioral excitability are predictive biomarkers for epilepsy development, we performed a prospective study with larger groups of control, 60 min and 90 min SE animals. PTZ tests were performed prior to SE, and again 1, 2, and 3 weeks post SE following the behavioral test battery. SE rats were repeatedly monitored for the development of SRS up to 6 months post SE. Animals developing SRS were compared with animals without observed SRS.

In this study, PTZ thresholds were lowered in SE rats with and without SRS in comparison to their individual thresholds prior to SE, but only epileptic rats had a significantly lower seizure threshold than control rats without SE at all investigated time-points. Additionally, animals with SRS were significantly more hyperexcitable in the pick-up test 1 and 3 weeks post SE.

Our data validate the PTZ threshold as well as the hyperexcitability test as predictive biomarkers for SRS development in groups of animals; however they are not predictive for the individual animal. In this respect, it is interesting to note that recent studies in rodent models of traumatic brain injury and stroke have indicated PTZ as a highly sensitive biomarker for increased seizure susceptibility, but not all animals with decreased seizure threshold seem to develop epilepsy (Kharatishvili and Pitkänen, 2010; Bolkvadze and Pitkänen, 2012; Brima et al., 2013; Mishra et al., 2014). There is need for a combination of different biomarkers in epileptogenesis; seizure threshold alone is not a reliable predictor of epilepsy for the individual animal, but has to be supplemented by additional biomarkers such as behavior, biochemical parameters, and EEG alterations.

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Deletion of PDE11A4, an enzyme with expression restricted to the hippocampus, alters glutamatergic signaling and produces transient amnesia

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Common complaints expressed by individuals with transient epileptic amnesia and other forms of temporal lobe epilepsy include a transient inability to form new memories, an increased rate of forgetting recent long-term memories (LTMs), and/or an inability to retrieve remote LTMs from long ago (c.f., Butler and Zeman, 2008). No medications currently treat the memory deficits associated with TLE; therefore, a more complete understanding of the molecular mechanisms underlying recent versus remote long-term memory are required to develop more effective therapeutics.

LTM for certain types of information (e.g., social experiences) appear to require the hippocampal formation initially but then become independent of the hippocampal formation and, instead, require the cortex (c.f., Frankland and Bontempi, 2005). This transition in the neuroanatomical circuitry of recent vs. remote LTM is termed systems level consolidation. It is thought that elements of a memory are initially encoded simultaneously within both the hippocampal formation and cortex, but hippocampal-mediated replay of the memory (either online during rehearsal or offline during sleep) is required to permanently consolidate the reorganization of cortico-cortico connections and, at the same time, erase the memory from the hippocampal formation. Very little is known of the molecular mechanisms that are required for systems levels consolidation, but what has been described implicates NMDA receptor and CaMKII signaling as well as de novo protein synthesis. It is highly likely that systems level consolidation is altered in temporal lobe epilepsy, given the circuitry and biochemistry underlying this memory storage process.

Our lab focuses on Phosphodiesterase 11A (PDE11A), 1 of 11 PDE families that break down cAMP and cGMP. We were the first to identify PDE11A expression in brain and have shown it is the ONLY PDE with expression restricted to the hippocampal formation (CA1 and subiculum). This expression pattern positions PDE11A to play a key role in systems level consolidation. In addition, PDE11A appears to regulate glutamatergic and CaMKII signaling, which also positions PDE11A to play a key role in systems level consolidation. New and exciting data from our lab show that deletion of PDE11A paradoxically impairs recent LTM for social odor recognition (SOR) and social transmission of food preference (STFP) but enhances remote LTM for SOR and STFP. This transient amnesia may be related to decreases in phosphorylation of the AMPA receptor subunit GluR1 and/or increases in phosphorylation of the NMDA receptor subunit NR2A within the ventral hippocampus. Together, these data suggest that PDE11A4 is a key regulator of both recent and remote long-term memory, suggesting that a PDE11A4-targeted compound may hold therapeutic potential for memory deficits associated with TLE.
Academic Drug Discovery and Development Teams: The Power of Pharmacokinetic and Pharmacodynamic Modeling and Simulation

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The development of new therapies for epilepsy is hindered by the limitations of efficiently and successfully translating results from animals to humans. Use of pharmacokinetic (PK) and pharmacodynamics (PD) methods and modeling tools can accelerate drug discovery and early development by helping to identify lead compounds, selecting appropriate animal models, accelerating dose finding, more accurately scaling dose from one species to the next, decreasing costs of expensive later-stage animal studies, and providing guidance in the design of human studies. While pharmacokinetics is integrated early in industry approaches to drug discovery and development, this is often lacking in academic research programs. At the University of Minnesota, we have formed trans-disciplinary teams with the goal of developing new therapies for seizure emergencies with programs ranging from early discovery to clinical development. The team members include some combination of a neurologist, electrophysiologist, veterinarian, clinical pharmacologist, analytical scientist, chemist, engineer, pharmaceutical scientist, biostatistician, and pharmacokineticist. As one example, we are evaluating the utility of canine status epilepticus (CSE) as a translational platform to evaluate the safety and efficacy of novel compounds and inform the design of human status epilepticus trials. Our approach is to conduct PK studies in a small number of dogs, the results of which are used to construct models and perform simulations to determine dosing for randomized, blinded, placebo-controlled trials in CSE. We have recently completed two PK/PD studies, one with fosphenytoin (FOS) and one with topiramate, to determine the intravenous loading dose needed for dogs to attain target drug concentrations. For each study, four dogs were used to characterize the pharmacokinetics. Blood samples were collected from which plasma drug concentrations were measured using HPLC-MS. Pharmacokinetic modeling and simulations were used to select the dose for the clinical trial based on a target concentration. Based on the simulations, a dose of 15mg/kg of FOS was selected and used in the CSE clinical trial. With this approach, we attained targeted plasma phenytoin concentrations in 15/16 dogs randomized to the treatment arm. Treatment with FOS was statistically superior to placebo and the response rate was approximately the same as report in controlled trials in humans. As another example, we participating in a research team headed by Dr. Galanopoulou at Albert Einstein School of Medicine to evaluate novel therapies for infantile spasms using rodent models of infantile spasms. For this work, PK/PD models are being developed using single dose data to support simulation of multiple dose regimens used to determine optimal dosing for long-term efficacy and safety studies. The goal of this work is to use PK modeling and simulation to determine safe and effective dosage regimens for subsequent animal clinical studies, which in turn can, inform design of human clinical studies.
Preliminary evidence of adaptive immune system cell infiltration into the brain of a genetic model of chronic epilepsy

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Neuroinflammation is well established as a major factor promoting neurodegeneration after seizures and may contribute to the generation and worsening of seizures. Inflammatory responses in the CNS are regulated by innate (e.g. astrocytes and microglia) and adaptive (e.g. infiltrating leukocytes) immune systems. The contribution of the innate immune system has been well described in multiple models of seizures, epileptogenesis and chronic epilepsy as well as in human epilepsy. To date, a role for the adaptive immune system has only been explored in acute seizure models. Here, we sought to determine whether peripheral immune cells infiltrate the brain of a genetic model of chronic epilepsy with severe seizures, Kv1.1alpha knockout (KO) mice. Using flow cytometry, we examined markers of leukocytes - CD4 (T helper cell), CD8 (T cytotoxic cell), B220 (B cells), Gr1 (neutrophils), Mac1/CD11b (monocytes, neutrophils, natural killer cells, granulocytes and macrophages) and MHC-II-Ia (immune antigen presenting cells) in brain and spleen from wildtype (WT) and KO mice. Our preliminary data suggests an approximately 2-fold elevation in CD8+ and B220+ cell populations and a 5-fold increase in CD8+ cell populations in the brain of KO mice as compared to WT mice. In the spleen, the CD8+ population increased by approximately 50%. The difference in populations of the other markers was not significant in the brain or spleen of KO or WT mice. Thus, our preliminary results suggest that the adaptive immune system is chronically activated in epileptic Kv1.1 KO mice. Future experiments will examine specific brain regions (e.g. cortex and hippocampus), developmental time-points during epileptogenesis and further delineate the character of the infiltrating leukocytes.

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Comparison of spike-wave discharges and other oscillatory activity in normal animals with spontaneous recurrent seizures across several animal models of acquired epilepsy

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\textbf{Rationale:} Several publications have reported spontaneous recurrent seizures in animal models of acquired epilepsy based on brain insults proposed to be more realistic than previous models. These studies have reported brief periods of oscillatory, spike-and-wave discharges (SWDs) with blank-stare behaviors, which have been considered to represent the non-convulsive seizures of acquired epilepsy. A critical problem, however, is that similar events are also seen in \textit{normal animals} (i.e., non-injured controls). The present studies aim to compare these normal electrographic events with non-convulsive and convulsive seizures recorded in several diverse animal models of acquired epilepsy.

\textbf{Methods:} In addition to pilocarpine- and kainate-induced status epilepticus, animal models (rats) included unilateral carotid occlusion with hypoxia at postnatal day 7, controlled cortical impact in adults, and a model of penetrating brain injury in adults. Several chronic-recording techniques were used across the different laboratories and animal models.

\textbf{Results:} SWD-like events in normal rats were much shorter in duration than the non-convulsive and convulsive seizures in the models of acquired epilepsy (see Methods, above). Normal SWD events were typically <5-10 sec, but could be longer; in contrast, the convulsive/non-convulsive seizures in diverse models of acquired epilepsy were routinely 20-40 sec, but could also be longer (e.g., 2-3 min). The SWD-like events typically started abruptly with little or no “build-up”, whereas the non-convulsive/convulsive seizures in the brain-injured rats typically began with a progressive increase in amplitude and/or frequency. The inter-spike intervals were relatively homogeneous during normal SWD, while the frequency of EEG spikes in the non-convulsive and convulsive seizures was typically more variable with distinct shifts in pattern. The non-convulsive/convulsive seizures often had post-ictal depression, which was not seen with the SWDs or other rhythmic events in normal rats.

\textbf{Conclusions:} Readily observable and quantifiable electrographic properties of SWD in normal rats (and after postnatal hypoxia or fluid percussion injury, see abstract by Barth, Dudek and Rodgers) could be readily distinguished from the seizures characteristic of acquired epilepsy. The non-convulsive and convulsive seizures in these diverse models of brain injury (see Methods, above) were much more similar to each other than they were to the normal SWDs.

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Dentate-Dependent Memory Loss as a Biomarker for Epileptogenesis: Network Level Mechanisms

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One of the most common comorbidities of Temporal Lobe Epilepsy (TLE) is memory impairment, yet little is understood about the underlying network level mechanisms, or how early in the disease the impairments manifest. Understanding at this level would be essential for utilizing cognitive tests as biomarkers for TLE and for developing treatments for ameliorating memory impairment. It is well known that the memory system of the brain, the hippocampus, undergoes severe structural reorganization during epileptogenesis, which is especially profound in the dentate gyrus (DG). Therefore it seems probable that memory processes that depend on the DG would be impaired during epileptogenesis, before the emergence of recurrent seizures.

In health, the DG performs pattern separation, the process of encoding similar experiences with distinct and separate neural codes during learning. We hypothesized that reorganization of the DG during epileptogenesis would undermine DG mediated pattern separation, which could be used as an early indicator that epileptogenesis is occurring, and may therefore provide an essential window for preventative care. To test this we employed parallel techniques of in vivo behavioral testing, in vivo single unit recording, and post-hoc histology. We employed the low-dose kainate model for chronic epilepsy, in which dentate reorganization by way of cell loss and mossy fiber sprouting has been characterized to begin after insult and increase during epileptogenesis leading to the chronic phase marked by spontaneous seizures. Four months after induction, we tested rats on a DG dependent behavioral pattern separation task (Morris et al., 2012) and then assayed structural changes post-hoc. During the span of behavioral testing no seizures were witnessed in any of the induced rats. Induced rats (n=8) took significantly more trials to distinguish between two adjacent arms on an 8 radial-arm maze than control rats (n=5) (p≤0.01), and took the same number of trials as rats with colchicine-DG lesions. In line with the time-point tested, we found significant, but subtle aberrant axonal sprouting in induced rats (n=8) compared to control rats (n=5) (p≤0.05). The learning impairment did not reflect gross hippocampal dysfunction because there was no difference between induced (n=5) and control rats (n=3) in the number of trials required for distinguishing non-adjacent arms, a paradigm that is also spared in rats with dentate lesions. To uncover network mechanisms of the observed pattern separation impairment, we examined DG network encoding mechanisms in awake-behaving rats. We found that DG neurons in induced rats displayed less precise spatial coding. Neurons had larger place fields in induced rats compared to neurons in control rats (n=31 fields, n= 73 fields, p≤ 0.001); place fields were less stable in induced rats (p ≤ 0.002); and place fields displayed improper rate coding between different environments. These findings suggest that DG function is impaired early in epileptogenesis, and can be measured behaviorally and physiologically. The use of behavioral pattern separation tests in patients as a marker for ongoing epileptogenic processes may therefore be valid. We predict that therapies that target the deteriorating spatial coding in the DG could rescue function.

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Anticonvulsant Effects Of A Galanin Receptor 3 (GalR3) Antagonist Snap 37889 In Mouse Models Of Seizures

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The neuropeptide galanin is widely considered an endogenous anticonvulsant by virtue of its inhibitory effects on excitatory neurotransmission. Galanin and its synthetic analogs have been previously shown to control seizures, and play a crucial role in ictogenesis (seizure onset) and epileptogenesis (the development of epilepsy). Galanin exerts its physiological effects through three G-protein receptor subtypes, which differ in their localization and signaling pathways. Although the anticonvulsant activity of galanin is ascribed to the activation of central GalR1 and GalR2 receptors, the role GalR3 in the control of neuronal excitation remains unknown. The distinct localization of GalR3 mRNA in the regions critical for seizure activity suggests the potential modulatory role for GalR3 in neurotransmission. In the absence of selective agonists for GalR3, specific antagonists offer a promising tool to understand the contribution of GalR3 in various physiological and pathological events. To assess the role of GalR3 in the manifestation of seizure activity, we studied the effect of systemic administration of centrally active GalR3 antagonist SNAP 37889 on various seizure phenotypes in CF-1 mice (n=8 or more/group). SNAP 37889 (in doses ranging from 15 mg/kg to 45 mg/kg) was administered i.p. 60 minutes prior to seizure induction. Pre-treatment with SNAP 37889 significantly increased the i.v. Metrazol-seizure threshold compared to vehicle; an effect that suggests increased inhibitory tone. SNAP 37889 also displayed a non-dose related anticonvulsant effect in the minimal clonic test, but not in 6 Hz psychomotor seizure test. These results suggest that the GalR3 receptor plays a role in modulating seizure activity and threshold. Importantly, anticonvulsant effect was observed at doses that were devoid of sedative or motor impairment. These results have implications on identification of new drug targets to treat epilepsy in future.

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Conflict of interest: Dr. H. Steve White is a scientific co-founder of NeuroAdjuvants, Inc., Salt Lake City, Utah.
In utero electroporation of the novel plasmid, NASTIE, for distinguishing neurons and astrocytes during calcium imaging experiments in rat hippocampus

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Traditionally considered only as passive agents in the brain, astrocytes are now recognized to be active participants in neurotransmission, thus making the study of their function vital to better understand epilepsy. The recent availability of genetically encoded calcium indicators (GECIs), such as the GCaMP family of proteins have made it possible to monitor the activity of neurons and astrocytes in large brain networks. However, there currently exist no tool that can selectively distinguish astrocyte processes from neuronal processes, seriously limiting our ability to assess local interactions between these cells. In order to address this gap in research tools, we recently constructed a new plasmid called NASTIE (Neuron Astrocyte Specific Targeting with In utero Electroporation) that contains the gene for the GECI GCaMP6 and utilizes Cre/lox DNA recombination technology to enable the genetic identification of astrocytes with the fluorescent protein tdTomato and neurons with the fluorescent protein Cerulean. To achieve expression of NASTIE in the adult rat brain, we used in utero electroporation (IUE) along with the piggyBac (PB) transposon system for functional and stable expression. IUE surgery was performed on embryonic day 14 rats. DNA was injected into the lateral ventricles of embryonic brains, and an electric field was applied across the uterine walls to facilitate transfection of DNA into hippocampal progenitor cells. Embryos were returned to the mother and dams were allowed to give birth. To characterize NASTIE transgene expression in the rat brain, we dissected and post-fixed brains from postnatal day 9 and 35 rats that had previously undergone IUE. Immunohistochemistry was performed on the tissue to label NeuN (neuronal marker), GFAP (astrocyte marker), and GFP (GCaMP marker) and was assessed for colocalization with Cerulean, tdTomato and GCaMP6 expression. To increase overall expression levels of the NASTIE transgene, we implemented a hyperactive mutant of the piggyBac transposase (hyPBase). Future directions for this project include investigating calcium activity in reactive astrocytes in acute brain slices prepared from adult rats treated with kainic acid in order to evaluate neuronal-glial signaling in the epileptogenic brain.

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Precision editing of zebrafish syntaxin-binding protein 1 (STXBP1) to model human neurodevelopmental disease

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Mutations in the synaptic machinery gene syntaxin-binding protein 1, STXBP1, also known as MUNC18-1, have been linked to childhood epilepsy and other neurodevelopmental problems. We identified a family with a mutation in STXBP1 that was associated with a range of clinical outcomes. To study loss-of-function mutations in syntaxin-binding protein, we used CRISPR/Cas9 gene editing to disrupt zebrafish stxbp1a. The mutant zebrafish exhibited profound developmental problems including reduced movement, developmental delay, and decreased responses to light/dark transitions.

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Plasma Metabolomic Analysis of (1-3)IGF-1 Treatment in Rats

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Infantile Spasms (IS) is a devastating seizure disorder with onset in early infancy. Therapies to achieve seizure control, improve developmental potential, and quality of life in children with IS are an urgent need. Insulin-like growth factor-1 (IGF-1) and its tripeptides (1-3)IGF-1 have been shown to provide beneficial effects in animal models of various neurological disorders. Effects are attributed to anti-apoptotic effects, decreases in astrogliosis and neuroinflammation, and neurotrophic actions of the peptide. (1-3)IGF-1 is being considered as potential therapy for IS (Lee et al., AES Meeting Abstract 2014). To better understand the signaling pathways targeted by (1-3)IGF-1 in IS models, it is important to first evaluate its effect on metabolic pathways in naïve rats. Here we conducted a plasma metabolomics analysis to determine the metabolic changes induced by chronic (1-3)IGF-1 in rats.

Plasma was collected from Sprague Dawley rats that were injected daily with (1-3)IGF-1 (10mg/kg/day) or saline for three weeks starting on postnatal day 35. Animal grouping was as follows: 2 male controls, 2 female controls, 2 male (1-3)IGF-1-treated, 2 (1-3)IGF-1-treated. Samples were extracted and analyzed by high performance liquid chromatography-mass spectrometry. Metabolites were tentatively identified using exact mass and isotope ratios. Fold-change and pathway enrichment analysis was performed on compounds exhibiting a > 2-fold change.

Metabolites that were > 2-fold changed in rats subjected to (1-3)IGF-1 therapy vs. controls (compounds changing > 2-fold in males (N=2) and/or females (N=2) were pooled for pathway enrichment analysis) were phospholipids of various classes, vitamins, steroid hormones, and compounds linked to neurotransmission and inflammation. Pathway analysis revealed several pathways to be significantly changed (padjusted < 0.05), “Retrograde Endocannabinoid signaling” (p = 6.69E-05), “Defective adenocorticotropic hormone (ACTH) causes Obesity and Pro-opiomelanocortinin deficiency” (p = 6.40E-05), “Opioid Signaling” (p = 1.33E-04), and “Serotonergic Synapse” (p = 0.016) among other pathways with potential involvement in IS pathophysiology were found to be targets of (1-3)IGF-1 therapy. Changes in levels of anandamides (anandamide (20:l, n-9) and anandamide (20:2, n-6)), arachidonic acid, 2-arachidonoylglycerol, and prostaglandins are responsible for changes in endocannabinoid signaling. Effects on a pathway describing defective ACTH, which is a hormone established as a treatment for IS, are driven by changes in prostaglandins (prostaglandin E2 and D2), eicosatrienoic acid, tetracdecanoic acid, palmitamide, anandamide (20:l, n-9), and 2-arachidonoylglycerol.

According to the results of this study (1-3)IGF-1 exerts major effects on endocannabinoid signaling, sphingolipid and arachidonic acid metabolism, and the biosynthesis of unsaturated fatty acids among others. Many of the identified compound groups and pathways show links to seizure disorders and their established treatment options (ketogenic diet, cannabinoids, ACTH). Our data shows that metabolomics analysis is a powerful approach to determine target metabolites and pathways of new therapies for IS. In the case of (1-3)IGF-1 therapy plasma has proven to be a valuable biofluid for the determination of (1-3)IGF-1-triggered changes in several key metabolites and pathways.

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VORINOSTAT REDUCES HYPEREXCITABILITY AND EPILEPTIFORM EVENTS IN A NOVEL DRUG-SCREENING PLATFORM

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Epilepsy is a common neurological disorder that affects approximately 1% of the global population. Approximately a third of epileptic patients are refractory to medical therapy and continue to have unremitting recurrent seizures and attendant life-long cognitive, behavioral and mental health problems. Here, we employed gene-editing tools to create an epileptic zebrafish model that we then used to uncover novel anti-seizure drugs. Using TALEN technology, we introduced mutations into \textit{kcna1}, the zebrafish ortholog of \textit{KV1.1} that is mutated in a human form of focal-onset epilepsy. We validated this \textit{kcna1} mutant using a combination of commonly accepted behavioral and cellular electrophysiological assays. A shelf screen was conducted using a small library of 143 FDA-approved compounds in two zebrafish models: our \textit{kcna1} mutant and the commonly-employed pentylentetrazole (PTZ)-induction paradigm. We employed a high-throughput behavioral assay that monitors locomotor activity and identifies compounds that decreased hyperactivity in these models. Specifically, swim activity at baseline and after the addition of drug was analyzed in both models using the 96-well Zebrabox System (Viewpoint, France). High-velocity movements (>20 mm/s) have been previously shown to correspond to paroxysmal seizure-like convulsions and we assayed for drugs that blocked high-velocity swim movements in each model by >40%. Interestingly and as expected, both models – i.e., PTZ-induced and \textit{kcna1} mutants – displayed increased locomotor activity, but only 9 of the 143 compounds were efficacious in blocking this hyperexcitability behavior in \textit{kcna1} mutants, whereas 33 of the 143 compounds were efficacious only in the PTZ-induced model. Two compounds were efficacious in both. Vorinostat, an HDAC-inhibitor that is currently used in human cancer patients, was our top hit in both models. We then examined the effect of Vorinostat on epileptiform events in \textit{kcna1} zebrafish brains using electroencephalogram (EEG) recording, and observed that vorinostat effectively reduced the frequency and duration of seizure-like activities to WT levels. Currently, we are examining the effects of vorinostat on brain electrical activities in \textit{Kv1.1} mutant rodent models using video EEG recordings. Preliminary data suggest Vorinostat is effective in reducing the frequency of seizures in epileptic rodents, which remains the gold standard for anti-seizure drug discovery validation. Our screening platform illustrates the ability to make genetic mutants in zebrafish that can be used for identifying investigational agents for monogenic forms of epilepsy.

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Molecular mechanism of astroglial swelling and neuronal overactivation in the retina

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Dysregulation in astroglial osmo-regulation may promote neuronal hyperexcitability, seizures and epileptogenesis. While hyperexcitability is exacerbated by pathological ion buffering and water transport, the osmosensing mechanisms and their relationship to neuronal activation in the brain remains largely undefined. This is in part due to the complexity of the networks within the CNS. We therefore took advantage of the retina, an approachable and well-defined CNS tissue – in which volume regulation is dominated by a specific type of astroglia, the Müller cell – to determine the molecular mechanism of hypotonic swelling. We also determined the molecular mechanism that mediates the (patho)physiological response to swelling in retinal neurons and characterized its function in 2nd messenger signaling, excitability and neuronal survival.

Acutely dissociated mouse retinas, loaded with calcium indicator dyes, were stimulated with anisosmotic solutions and pharmacological modulators of TRP channels. Alternatively, transmembrane currents were measured in whole-cell clamped cells. We found that the polymodal, nonselective cation channel, TRPV4 (transient receptor potential isoform 4) represents the main osmosensor for Müller cells and retinal ganglion cells (RGCs). In astroglia, swelling induced large \([Ca^{2+}]\) elevations that were associated with transcellular Ca\textsuperscript{2+} waves and antagonized by TRPV4 blockers. The extent of swelling was much lower in Trpv4\textsuperscript{-/-} cells and in the absence of extracellular Ca\textsuperscript{2+}. Thus indicating that Ca\textsuperscript{2+} influx – elicited by channel activation – suffices to facilitate glial swelling. TRPV4 activation was also required for regulatory volume decrease (RVD).

It is noteworthy that swelling-induced, TRPV4-mediated signals were blocked by genetic elimination of aquaporin 4 (AQP4), suggesting that swelling drives TRPV4 activation via AQP4-driven water influx. We confirmed this hypothesis through heterologous expression of TRPV4 and AQP4 in \textit{Xenopus} oocytes. The importance of TRPV4 signaling for glial physiology was further underscored by the observation that both TRPV4 overactivation and inhibition can elicit reactive gliosis, suggesting that glial TRPV4 channels represent a hub that links swelling, Ca\textsuperscript{2+} homeostasis and inflammatory responsiveness. Consistent with this, we found that TRPV4 activation in Müller cells requires concomitant activation of proinflammatory phospholipase A2 signaling mediates the eicosanoid metabolites of cytochrome P450.

Our data also suggest that TRPV4 modulates neuronal excitability itself. TRPV4 activation through selective agonists, temperature, swelling or pressure dramatically potentiated RGC excitability and, if sustained, triggered massive cell death under \textit{in vitro} and \textit{in vivo} conditions. Taken together, we have identified a novel mechanism that regulates both positive and negative feedback loops associated with glial swelling and neuronal excitability in the vertebrate retina. We propose that targeting TRPV4 might simultaneously alleviate cytotoxic edema and neuronal overexcitation in brain injury models such as TBI and epilepsy.

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An international, curated KCNQ2 database and website: a tool set for elucidation of genotype-phenotype relationships and development of precision medicines

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Rich data sets describing individual differences among patients are becoming available, potentially enabling more specific targeting of therapies to the underlying pathophysiology, or “precision medicine”. Mendelian disorders represent appealing models for developing such approaches, since at least one primary mechanistic factor is shared among the patients. However, such disorders are uncommon, requiring team efforts to identify patient populations sufficiently large to power close observational and interventional studies. KCNQ2 related epilepsy has some advantages for this approach: much is known about the molecular and cellular neurobiology of the encoded voltage-gated potassium channels, and KCNQ channels are targets of an already-approved anti-seizure drug, ezogabine, and many additional agents in development. Our initial objective was to see if a sufficiently large number of patients with KCNQ2 variants could be identified to facilitate clinical research. Subsequent objectives included improving understanding of genotype-phenotype relationships, developing better outcome measures, and identifying likely responder/non-responder subgroups useful for subsequent therapeutic trials.

Individuals with KCNQ2 variants enter the database via 4 pathways: (A) Families may self-refer, and be “registered” after informed consent to us. (B) Treating physicians can “enroll” patients anonymously, after informed consent to the physician. (C) We enter variants and associated clinical and functional data published by others. (D) We enter variants reported to us by individual testing labs or disclosed to aggregation sites (e.g., ClinVar). When a new case is identified, we check for potential redundant reporting. Data collected includes clinical history, family history, test results (e.g., relevant negative studies, genetics, EEG, MRI scans), therapeutic trials and responses, as available. Each entry is reviewed by a multi-institutional, multidisciplinary expert panel. A gene-specific scoring matrix for pathogenicity and prognosis has been developed, based on the latest American College for Medical Genetics guidelines. An online scoring calculator is being developed to optimize the reliability of variant classification (non-pathogenic, likely non-pathogenic, uncertain significance, likely pathogenic/self-limiting, likely pathogenic/epileptic encephalopathy, pathogenic/self-limiting, pathogenic/epileptic encephalopathy). Scores are reported to a locus specific website (www.rikee.org) and to ClinVar (www.ncbi.nlm.nih.gov/clinvar/).

We have so far identified 106 individuals initially classified as epileptic encephalopathy and 100 unrelated cases (or pedigrees) classified as self-limiting neonatal or infantile epilepsy (benign familial neonatal/infantile epilepsy). An additional 28 cases are recently entered via path A or B and not yet classified. Significant correlations have been identified between phenotype and variant characteristics, namely; variant type (e.g., missense vs. larger deletions/truncations), variant genetic location, and molecular functional change in the variant.

In summary, we have used a team approach to develop a comprehensive KCNQ2 database that is already proving useful for understanding genotype-phenotype relationships and pathophysiology. Such understanding is needed for developing targeted treatments for this catastrophic epileptic encephalopathy. Our approach can be used for other Mendelian epilepsy syndromes that are uncommon at any one clinical center. As “big data” is used to characterize patients with more prevalent, genetically complex epilepsies, subgroups will likely be identified with shared causes. Our work provides a model of the challenges in and approaches for working with such subgroups.

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Improvement in Seizure Control During Conversion to Eslicarbazepine Acetate Monotherapy: A Pooled Analysis of Two Trials in Adults with Refractory Partial-Onset Seizures

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Eslicarbazepine acetate (ESL) is not approved for monotherapy use. Two identical phase III studies (093-045 and 093-046) suggest that conversion to ESL monotherapy is effective and well tolerated in patients with partial-onset seizures (POS) (Pazdera et al, Epilepsy Curr 2014;14[Suppl.1]:108; Sperling et al, Epilepsy Curr 2014;14[Suppl.1]:431–2). This analysis uses pooled data from these studies to determine the proportion of patients with improvements in seizure control during ESL monotherapy (1200mg and 1600mg once-daily [QD]). METHODS: Studies 093-045 and -046 were 18-wk, randomized, double-blind, conversion-to-monotherapy studies which evaluated ESL 1200mg and 1600mg QD, compared with a historical control (as described by French et al, Epilepsia 2010;51:1936–43). Patients aged 16–70 years with POS not well controlled (≥4 POS in the 8 wks before screening, with no 4-wk seizure-free period) by 1–2 antiepileptic drugs (AEDs) were randomized (2:1) to ESL 1600mg or 1200mg QD (2-wk titration; 6-wk conversion [other AEDs withdrawn]; 10-wk monotherapy). The primary endpoint was the proportion of patients meeting ≥1 of 5 exit criteria (related to worsening of seizure control) by wk 16 (French et al, AES 2014). In both trials, seizure improvement endpoints (reduction in standardized seizure frequency [SSF] and seizure freedom) were prospectively specified as secondary endpoints. RESULTS: Overall, 365 patients began ESL therapy (1600mg, n=242; 1200mg, n=123). Median age was 38 years; 52.1% were female. The pooled efficacy population (patients who began monotherapy conversion) comprised 332 patients (1600mg, n=218; 1200mg, n=114). For the 18-wk double-blind period there were improvements in SSF with both doses of ESL; median reductions vs baseline were 43.2% for ESL 1600mg and 35.7% for ESL 1200mg. The 50% responder (≥50% reduction from baseline in seizure frequency) rates were 43.1% and 36.0%, respectively. 8.7% and 7.9% of patients experienced seizure freedom (100% reduction in seizures) during the 10-wk ESL monotherapy period, and 15.6% and 14.9%, respectively, during the last 4 wks of the monotherapy period. There were improvements in seizure control with ESL monotherapy in patients with different categories of POS, in US patients and non-US patients, and in patients who switched from various different baseline AEDs. Compared with patients who switched from carbamazepine or oxcarbazepine, more marked improvements in seizures occurred in patients who converted from other AEDs (Figure 1). Similar proportions of US and non-US patients had seizure freedom during the monotherapy period (US: 8.7% [ESL 1600 mg] and 6.8% [ESL 1200 mg]; non-US: 8.8% and 9.8%). CONCLUSIONS: Conversion to ESL monotherapy (1200mg and 1600mg QD) led to reductions in median seizure frequency in patients with POS not well controlled by 1–2 other AEDs. A substantial proportion of patients had ≥50% reduction in seizure frequency.

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**Known genes explain a significant proportion of cases of early onset epileptic encephalopathy with burst-suppression**

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Ohtahara syndrome (OS) is a severe early onset epilepsy with a heterogeneous etiology. OS is considered an epileptic encephalopathy- characterized by intractable tonic seizures, a burst suppression electroencephalogram (EEG) pattern, and severe intellectual disability. Onset of OS typically occurs during the first month of life and often presents within the first ten days. Many cases of OS progress into infantile spasms (West syndrome) or Lennox-Gastaut syndrome. Based on current literature, genetic mutations explain approximately 10-20% of epileptic encephalopathies. Known genetic causes of OS include brain malformations (e.g. polymicrogyria) and inborn errors of metabolism, but little is known about the distribution of genetic etiologies in patients without brain malformations.

We ascertained 21 patients with OS without defects on structural neuroimaging and performed whole exome sequencing (WES) on the probands and parents when available. Analysis of WES variants and Sanger sequencing validation were conducted using standard methods. We identified a pathogenic or likely pathogenic variant in 15/26 cases: 35% with a *de novo* STXBP1 variant, 4% with a SCN2A variant, and one case with a pathogenic homozygous PNPO variant. All variants in known genes were predicted to be pathogenic *in silico* and there was no phenotypic distinction between the genetic subgroups. In addition, WES data revealed possible *de novo* heterozygous mutations in novel genes GRIN3B, DISC1, and SLC6A8. These findings together suggest that early and efficient genetic testing for a targeted small panel of genes would likely be high yield and clinically beneficial for patients with OS. Coming to a diagnosis early would prevent extensive diagnostic evaluations, allow for appropriate genetic counseling, resolve parental fears regarding alternative causes, and in many cases directly guide treatment. In addition our study and others highlight genes that are important to model to understand epileptogenesis and to serve as the basis for pre-clinical drug discovery.

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Neurodegeneration, gliosis, and glial proliferation in two models of temporal lobe epilepsy

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Despite the introduction of many new anti-seizure medications over the last two decades, it is estimated that 30–40% of patients with epilepsy do not have their seizures adequately controlled. In the Anticonvulsant Drug Development (ADD) Program, considerable research is ongoing to identify animal models appropriate for the identification of novel compounds that are more efficacious in treatment-resistant patients. The corneal kindled mouse is a model of temporal lobe epilepsy (TLE) that allows for the rapid screening of investigational compounds. However, lack of information as to the specific inflammatory pathology resulting from corneal kindling in mice leaves research towards development of antiseizure drugs (ASDs) in this model uninformed. Likewise, the Thelier’s murine encephalomyelitis virus (TMEV) model of infection-induced TLE may prove to be a useful model for screening, but quantitative assessment of neurodegeneration, glial reactivity, and cell-type-specific proliferation is lacking. Therefore, the current study set out to test the hypothesis that these two very different models would display contrasting and distinctive neuropathological characteristics.

C57BL/6 mice were injected intracranially with PBS or TMEV (3 X 10\textsuperscript{5} PFU), monitored for seizures, and sacrificed 4 and 14d post-injection (n=8 per group). CF1 mice were corneaually stimulated until fully kindled, and sacrificed with untreated controls 24h and 7d after kindling (n=8 per group). Mice were transcardially perfused, and 25\textmu m sections through the hippocampus were collected on a freezing microtome. Serial brain sections from each group were stained with antibodies directed against NeuN, GFAP, and Iba1 to evaluate neurodegeneration and the extent of gliosis in both astrocytes and microglia, respectively. In addition, antibodies directed against Ki-67 were used to assess proliferation in glial cells. Z-stacks were collected in CA1, cortex, and dentate gyrus (DG) with a confocal microscope and analyzed using ImageJ.

In the hippocampus of TMEV-injected mice (4 and 14dpi,) both microglia and astrocytes were found to have significantly increased expression of Iba-1 and GFAP, respectively; indicative of reactive gliosis. As previously described, there was also a significant degree of cell death in the CA1 stratum pyramidale region. In contrast, there was no significant neurodegeneration or activation of microglia in the hippocampus of corneal kindled mice. Increases in GFAP expression were seen, however, suggesting that astrocytes are reactive in this model. Significant increases in the number of Ki-67+ nuclei combined with colocalization analyses demonstrated that there was proliferation of microglia and astrocytes in the dentate gyrus of TMEV-infected mice at 4dpi. These results, coupled with previous findings, suggest that histochemical measures of inflammation are significantly greater in the TMEV model of infection-induced TLE as compared to the corneal kindled model. Thus, different classes of ASDs could have varied effects on seizures observed in these two models and the use of multiple mouse models of TLE in drug screening efforts is likely to be beneficial.

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Ankyrin-G: A novel mechanistic link between epilepsy and bipolar disorder

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The co-morbidity of mood disorders and epilepsy is well-established epidemiologically but poorly understood. Genetics offers a strategy for revealing underlying mechanisms and pathways responsible for this co-morbidity. ANK3 is a gene that has been widely implicated in psychiatric disease. Single nucleotide polymorphisms (SNPs) in ANK3 have shown association with bipolar disorder in multiple, independent genome wide association studies (GWAS). The majority of these SNPs are located in the intronic regions near two alternative first exons of ANK3, exons 1e and 1b. Post mortem human brain tissue studies have shown that individuals containing these SNPs have significantly reduced levels of ANK3 exon 1b transcripts in many brain regions. However, the mechanism(s) by which ANK3 contributes to this disorder is still unknown. ANK3 is a highly conserved, large gene encoding multiple isoforms of a very large (180-480 kDa) protein, Ankyrin-G (AnkG). AnkG isoforms are expressed in almost all tissues and function generally as a molecular linker between integral membrane proteins and the cytoskeleton. In neurons, AnkG is concentrated at the axon initial segments (AISs) and nodes of Ranvier (NRs) where it binds voltage-gated sodium (NaV) and potassium (Kv) channels necessary for action potential generation and conduction. Consequently, AnkG is essential for the assembly, maintenance, and function of neuronal AISs and NRs. We generated antibodies specifically detecting the two alternative N-terminal peptides encoded by exons 1e and 1b. We found that parvalbumin-positive (PV+) interneurons exclusively express isoforms of AnkG encoded by transcripts that include exon 1b. This pattern was observed in many different brain regions, including limbic system circuits that have roles in mood and emotion. Using quantitative analysis of images obtained by confocal microscopy, we found that heterozygous mice lacking the exon 1b isoforms of ANK3 have significantly reduced AnkG and NaV channel densities at the AIS of PV+ interneurons. Using video EEG monitoring, we observed spontaneous generalized seizures in both heterozygous and homozygous exon 1b-KO mice. The mice also showed marked susceptibility to audiogenic seizures absent in littermate controls. Thus loss of AnkG from the AISs of PV+ interneurons leads to hyperexcitability in the cortex and epilepsy. We hypothesize that imbalanced inhibition and excitation may underlie the genetic association of ANK3 and mood disorder. Further studies of ANK3 provide an opportunity to examine the nexus between epilepsy and disorders of mood, which is clinically very important and very poorly understood. A role for axonal AnkG-ion channel protein complexes in mood is appealing, given the reciprocal epidemiological association of epilepsy and psychiatric disorders, and the frequent and sometimes efficacious clinical use of NaV channel blockers in both types of disorders.

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NAX 810-2, A Novel Galanin-Based Therapy for Epilepsy.

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Most of the available antiepileptic drugs exert their activity by modulating voltage- and/or receptor-gated ion channels. Despite treatment with currently available AEDs, approximately 25-40% of patients with refractory epilepsy continue to experience uncontrolled seizures. The endogenous neuropeptide galanin and its associated receptors play an important role in the control of seizures and are therefore an attractive therapeutic target. However, developing neuropeptides as therapeutics has been challenging due to poor metabolic stability and lack of blood-brain barrier penetration. Furthermore, activation of peripheral GalR1 receptors results in hyperglycemia due to inhibition of insulin release. We have applied a novel technology platform to develop galanin analogs that are metabolically stable, maintain low nanomolar affinity for galanin receptors, and following systemic administration are potently active in preventing induced seizures. Subsequent studies have led to the discovery of NAX 810-2, a GalR2-preferring agonist that displays potent anticonvulsant activity in several seizure and epilepsy models. NAX 810-2 is a GalR2-preferring agonist (GalR2 Ki 32 nM, GalR1 Ki 494 nM) that shows a high level of plasma protein binding (99.5%) and displays potent anti-seizure activity in the mouse 6Hz model (ED₅₀, 32mA: 2.5 mg/kg i.p.), the Frings Audiogenic model (ED₅₀: 9.2 mg/kg i.p.), and the mouse corneal kindling model (ED₅₀: 7.4 mg/kg i.p.). Pharmacokinetic studies show that the plasma half-life of the analog is approximately 1.25h following systemic (i.v.) administration. Repeated administration of NAX 810-2 (8 mg/kg b.i.d., 3 days) shows that the analog retains full efficacy. In summary, NAX 810-2 is a potential first-in-class neuropeptide therapeutic. Pre-clinical development efforts of NAX 810-2 are part of a milestone-driven translational research program with the goal of advancing this lead candidate to IND-filing. This work was supported in part by the NIH U01 grant NS066911.
Clinical spectrum of epilepsy secondary to KCNQ2 variants and initial response to ezogabine

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Variations in KCNQ2 underlie a spectrum of epilepsy from benign to severe. Current understanding remains limited and this disease best studied in the context of epilepsy with similar age of onset. The initial clinical presentation of the severe form of KCNQ2-related epilepsy is recognizable as one of the neonatal epileptic encephalopathies, Early Myoclonic Encephalopathy (EME) or Ohtahara syndrome. These syndromes are characterized by tonic and myoclonic seizures with EEG burst suppression with onset within the first week of life. Patients with EME and Ohtahara syndrome will typically have exhaustive evaluations for a metabolic or structural etiology. De novo KCNQ2 pathogenic variants have been found in neonatal-onset epileptic encephalopathy. In vitro studies of some of these variants show strongly suppressed channel activity, suggesting that phenotype severity reflects the degree of “KCNQ2-deficiency”. Since ezogabine can increase KCNQ2 channel function, its use in severe KCNQ2 encephalopathy could be beneficial. However, previous use of ezogabine in infants and children is very limited. The aim of this study was to better understand genotype-phenotype relationships in KCNQ2-related epilepsy, and begin to assess the utility of selective KCNQ channel openers as potential targeted, disease-modifying treatments. Twenty-three previously unreported patients with neonatal-onset epilepsy and KCNQ2 variants were studied. There were 11 patients treated with ezogabine. Data (demographics; birth, seizure, family, and developmental history; EEG, neuro-imaging, and genetic test results; treatments, drug levels, and treatment responses) were collected. The genotype-phenotype relationships in these and 70 previously described cases were analyzed. In this series, the mean seizure onset age was 1.8 +/- 1.6 (s.d.) days. An initial EEG was obtained within the first week of life in 20/23 patients; 11 showed suppression-burst. Brain MRI was normal in 16/23 patients. Other seizure types appeared in infancy in 15 patients, and 8 developed epileptic spasms. At last follow up, seizures persisted in 9/23 patients. Development at last follow up was abnormal in all patients and severely abnormal in 15/23. KCNQ2 variants identified included single amino acid changes (substitutions, or in one instance, a single residue deletion), and were clustered in four specific areas predicted to detrimentally affect tetrameric channel functions. Treatment with ezogabine was associated with improvement in seizures and/or development in 3 of 4 treated before 6 months of age, and 2 of 7 treated later in life. There were no serious side effects were observed. In conclusion, our findings show that KCNQ2 variants cause neonatal-onset epilepsy of varying severity, including the syndrome of Benign Familial Neonatal Convulsions and neonatal-onset encephalopathy. Pathogenic KCNQ2 variants in the epileptic encephalopathy phenotype are usually clustered in “hot spots” known from in vitro studies to be critical for channel activity. Ezogabine appeared well tolerated and potentially beneficial for the treatment of refractory seizures. Developing an effective treatment strategy remains the most challenging clinical question, but continued genotype-phenotype correlations are needed for weighing the risks and benefits of available interventions. Development of a collaborative multicenter approach can help address these challenges for studying a rare cause of disease that may applied more broadly.

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Gene discovery and high-throughput resequencing of candidate genes in epileptic encephalopathies

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Whole exome studies in patients with epileptic encephalopathies (EE) have demonstrated the breadth of genetic heterogeneity in these severe childhood epilepsy syndromes. Our previous study identified 329 \textit{de novo} mutations in 305 genes when 264 trios (affected child and unaffected parents) were sequenced. We aimed to identify additional patients with \textit{de novo} mutations in 27 of these candidate genes to confirm the role of each gene and the phenotypic spectrum in the genetic etiology of EE. We performed targeted capture and high-throughput resequencing of 27 genes in which a \textit{de novo} mutation was identified in one or more proband with Infantile Spasms (IS) or Lennox-Gastaut syndrome (LGS) in our prior study. More than 600 patients with diverse EE phenotypes were screened. We have identified at least 16 patients with \textit{de novo} mutations in 7 genes. Among these are recurrent mutations in \textit{CACNA1A}, \textit{GABRB3}, \textit{GNAO1}, and \textit{ALG13} highlighting the importance of these genes in EE. Segregation studies are pending for additional probands but it is likely that genes involved in glutamate transport, endocytic trafficking, and transcriptional regulation are important for healthy neural circuitry, and mutations in these pathways cause EE. We will report the frequency of \textit{de novo} mutations for each gene screened in our cohort as well as investigate genotype-phenotype correlations for genes in which multiple patients harbor mutations. In summary, we have confirmed the role of at least 7 additional genes in the genetic etiology of EE and expanded the phenotypic spectrum associated with these genes beyond IS and LGS in which they were first discovered.

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The Norzler method: a reliable, SE-free animal model of acquired human temporal lobe epilepsy

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Despite the value of the (systemic) KA model, which has unequivocally contributed greatly to our understanding of epilepsy, drawbacks persist: mortality, capricious neuropathology, erratic latency to spontaneous epilepsy, and significant non-responders (some surviving animals never exhibit spontaneous seizures). These problems are probably the result of the uncontrollable nature of status epilepticus (SE), an all-or-nothing response that manifests differently in each animal. Rather than attempt to incrementally increase either the reliability or survivability of SE, we approached the problem from a different angle. Our aim was to develop a simple, robust animal model of acquired TLE that closely and reliably mimics the human condition, while avoiding SE and its aforementioned complications. Here we introduce the Norzler method, which is comprised of a single dose of KA administered concurrently with a single dose of lorazepam, a benzodiazepine that is a first-line treatment for convulsive SE, to freely moving male Sprague-Dawley rats. This treatment induced acute hippocampal seizures, but not SE, as well as the defining characteristics of acquired human TLE: pronounced hippocampal neurodegeneration, spontaneous hippocampal-onset seizures after a prolonged seizure-free period, all without either morbidity or mortality. This approach may prove useful in studies on mechanisms of epileptogenesis (the development of epilepsy) and ictogenesis (manifestation of individual seizures), as well as drug discovery for the population of patients whose seizures cannot be controlled by contemporary therapies.
Synaptic scaling in the hippocampus in a mouse model of viral-induced temporal lobe epilepsy

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Introduction: CNS infection is a common cause of epilepsy that is often refractory to established antiseizure drugs. We have developed a novel mouse model of infection-induced epilepsy that offers a unique opportunity to study the molecular mechanism(s) underlying epileptogenesis and to identify novel therapeutic strategies. Theiler's murine encephalomyelitis virus (TMEV) infected C57BL/6J mice show acute behavioral seizures between 3 and 7 days post-infection (dpi), exhibit pathological and physiological changes (gliosis, neurodegeneration in CA1, and increased excitation in CA3 pyramidal neurons) in the hippocampus, survive the infection, and develop epilepsy after a latent period. TMEV infection increases mRNA expression of proinflammatory cytokines, especially tumor necrosis factor-alpha (TNF-α), in the brain during the acute seizure period. In several systems, TNF-α has been shown to increase excitatory neurotransmission by upregulating the insertion of AMPAR subunits on the post-synaptic membrane via TNFR1. Therefore, we hypothesized that an increase in TNF-α following TMEV infection would increase the levels of AMPAR subunits on the neuronal membrane and contribute to hyperexcitability in TMEV-infected mice.

Methods: Mice were injected with either TMEV or PBS and monitored for acute seizure behavior. In one group of mice, we microdissected hippocampi (n=5-6) at 5 dpi and measured the protein expression of various inflammatory modulators including TNF-α by multiplex electroluminescence immunoassay. In a second group of mice, we measured the membrane expression of GluA1 and GluA2 subunits of AMPAR by cell surface biotinylation assays in acute hippocampal slices (n=6) at 5 dpi followed by SDS-PAGE and immunoblotting.

Results & Conclusion: We found >200 fold increase in TNF-α protein levels in the hippocampus obtained from TMEV-infected mice compared to control. The levels of other inflammatory modulators such as IFNγ, IL6, IL1β, and IL10 were also increased significantly in the TMEV-infected group. In addition, biotinylation assays revealed that the ratios of surface/total protein expressions of GluA1 and GluA2 were elevated in the TMEV group, while the total protein levels of both subunits were decreased. These data suggest that increased levels of TNF-α may contribute to synaptic scaling of AMPARs in the hippocampus, which in turn may contribute to increased seizure susceptibility following TMEV infection. Future work will evaluate the role of TNF-α.

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Eslicarbazepine Acetate Monotherapy in Adults with Partial-Onset Seizures: A Pooled Analysis of Two Randomized Double-Blind Studies with Use of a Historical Control

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RATIONALE: Eslicarbazepine acetate (ESL) is a once-daily (QD) oral antiepileptic drug (AED), approved in the US and EU as adjunctive treatment of partial-onset seizures (POS). ESL is not approved as monotherapy. We present the pooled analysis of two identical studies using a historical control ‘withdrawal to monotherapy’ design (French et al. Epilepsia 2010;51:1936–43). Conducting two independent studies made it possible to reduce the total sample size, increase the statistical power of the analysis, and conduct one trial exclusively in North America (NA).

METHODS: The two randomized double-blind studies were performed in NA (study 045) and NA/Europe (study 046). Patients aged ≥16 yrs, using 1–2 AEDs, with ≥4 POS in the 8 wks before screening, and no 4-wk seizure-free period, were randomized (2:1) to ESL 1600/1200mg QD (2-wk titration; 6-wk conversion [other AEDs withdrawn]; 10-wk monotherapy). The primary endpoint was exit rate (the proportion of patients meeting ≥1 of 5 exit criteria [worsening seizure control] by wk 16). ESL was considered effective if the upper 95% confidence limit (UCL) of the Kaplan–Meier-estimated exit rate was < 65.3% (single study) or < 72.2% (two studies).

RESULTS: Overall, 365 patients began titration (1600mg, n=242; 1200mg, n=123). Median age was 38 yrs; 52.1% were female. Compared with non-US patients, US patients weighed more, had a greater duration of epilepsy, and had higher maximum 2- and 28-day seizure rates at baseline. The pooled efficacy population (patients who began conversion) comprised 332 patients (1600mg, n=218; 1200mg, n=114). Estimated exit rates (95% CI) with ESL 1600 and 1200mg respectively were: study 045, 28.7% (21.2–36.1%) and 44.4% (32.5–58.3%); study 046, 12.8% (7.5–21.5%) and 15.6% (8.1–28.7%); pooled 045/046, 20.6% (15.6–26.8%) and 30.8% (23.0–40.5%; Figure 1). The UCLs were less than the 65.3% threshold for all treatment groups; both doses of ESL monotherapy were superior to historical controls. Exit rates in the pooled population were higher in US vs non-US patients: ESL 1600mg, US 25.0%, non-US 13.1%; ESL 1200mg, US 42.9%, non-US 10.2%. UCLs for all groups were < 65.3%. Higher exit rates occurred in patients with: epilepsy duration ≥ 20 yrs; baseline use of 2 AEDs; rescue medication use. Except for phenytoin, no baseline AED significantly affected exit rate. Headache was the only treatment-emergent adverse event (TEAE) observed in ≥5% of patients during monotherapy (8.5%; Table 1). Minor differences in TEAE profile were observed by baseline AED. From baseline to study end, QOLIE-31 scores improved by 5.7 (ESL 1600mg) and 3.3 points (1200mg; clinically meaningful change: ≥ 5 points; Borghs et al. Epilepsy Behav 2012;23:230–4). There were no notable changes in MADRS scores.

CONCLUSIONS: Exit rates with ESL (1600mg and 1200mg) were superior to historical controls; differences between US and non-US patients may be due to differences in placebo response, or to more severe epilepsy in US patients. ESL was well tolerated as monotherapy.

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Mechanisms of action of anti-seizure drugs and the Anticonvulsant Screening Program of the National Institute of Neurological Disorders and Stroke

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Purpose: To determine the efficacy of the Anticonvulsant Screening Program (ASP) of the National Institute of Neurological Disorders and Stroke (NINDS) in identifying the mechanisms of action (MOA) of the currently available antiseizure drugs and those in development.

Methods: Data were tabulated from multiple sources, including the ASP and the literature.

Results: Since it was established in 1975, the ASP has contributed to the identification of at least 9 new antiseizure drugs. The effectiveness of the program was evaluated by ascertaining the number of mechanisms of action (MOA) of the antiseizure drugs revealed by the ASP screening techniques. Considering the MOA of drugs marketed before and after 1975—and the MOA of investigational compounds not yet marketed—the ASP has contributed to the characterization of antiseizure drugs that possess 16 distinctly different MOA.

Conclusion: The ever-evolving screening approach of the ASP has many characteristics of a final common pathway for antiseizure drug discovery.

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TRPV4 mediates changes in microglial morphology and process dynamics during treatment with hypotonic stimuli

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Severe disturbances in homeostatic ionic gradients contribute to reactive gliosis and neuronal hyperexcitability. It is unclear, however, how osmotic changes are transduced within glia to initiate either protective or pathological mechanisms. Microglia, the canonical immune cell of the central nervous system, have processes that dynamically assess the surrounding environment and are capable of sensing and responding to a wide variety of stimuli. Evidence from our lab suggests microglia respond to aniosmotic stimuli by retracting their processes and adopting a more reactive phenotype. Furthermore, we characterized a novel mechanism that may mediate this surveillance motility and active state in retinal microglia.

Acutely isolated retinal microglial cells from CX3CR1GFP/+ mice loaded with calcium indicator dyes were stimulated with aniosmotic solutions and pharmacological agonists and antagonists of TRPV4. TRPV4 (transient receptor potential isoform 4) serves as the main osmosensor for Müller cells, the primary macroglia in the retina, and thus may be a candidate for microglial osmosensing. We found robust calcium responses to hypotonic stimuli (HTS), which could be inhibited by the selective TRPV4 antagonist, HC-067047. Likewise, CX3CR1GFP/+ microglia in retinal explants exhibited an initial outward current followed by sustained inward current during whole-cell patch clamp electrophysiology. The inward current could be partially blocked with HC-067047. Flat-mounted retinal explants allow microglia to maintain complex branching morphology without triggering conversion to reactive macrophages. Using 2-Photon microscopy on these explants we clearly demonstrate that exposure to HTS elicits fast retraction of microglial processes and decreases the total area surveyed by each microglia. Furthermore, blocking TRPV4 with HC-067047 resulted in restoration of complex morphology as well as restoring process motility and the total area surveyed. Overall, our results suggest that microglia may be primed or activated by osmotic changes in the extracellular environment. Future studies may also elucidate whether changes in microglia morphology or dynamics affect neuronal hyperexcitability and/or survival.

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5-HT$_6$ receptor ligands modulate seizure thresholds and inhibitory synaptic transmission in the dentate gyrus

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5-HT$_6$ receptor antagonists improve memory in rodents and patients with Alzheimer’s disease (AD). These compounds also exhibit anticonvulsant effects in the rat maximal electroshock test. However, 5-HT$_6$ receptor antagonists have never been tested in an animal model of AD, and their effects in multiple seizure tests are lacking. Moreover, how 5-HT$_6$ receptor antagonists produce their procognitive and anticonvulsant effects remains unclear. We tested the hypothesis that 5-HT$_6$ receptor activation enhances inhibitory synaptic transmission in the DG, and that 5-HT$_6$ receptor antagonists improve DG-dependent memory in a transgenic model of AD while having paradoxically anticonvulsant activity in naïve mice.

Male J20 mice and their non-transgenic littermates were injected with the 5-HT$_6$ receptor antagonist SB-399885 or methyl cellulose and tested in the metric task, which relies on spatial memory. Recognition index (RID) was calculated by dividing total object exploration time during the last 5 min test by object exploration time in the last 5 min of the 15 min learning phase. For minimal clonic seizure testing, mice were stimulated at a previously determined strength that caused seizures in half of the male C57Bl6 or CF1 mice (6.5 or 6.8 mA, respectively, at 60 Hz for 0.2 sec.). To test the effects of 5-HT$_6$ receptor activation on synaptic transmission, coronal brain slices containing dorsal hippocampus were prepared from naïve rats. Field potential recordings and patch-clamp electrophysiology were used to test the effects of a 5-HT$_6$ receptor agonist WAY-208466 (1µM) on baseline field excitatory postsynaptic potentials (fEPSPs), evoked and spontaneous inhibitory postsynaptic currents (eIPSCs, sIPSCs). Drug effects on behavior, seizure thresholds and neurotransmission were compared with the Fisher exact test, Student’s t-test or one-way ANOVA.

Preliminary results suggest that SB-399885 (10mg/kg, 30 min, i.p.) did not reverse spatial memory impairments in J20 mice (vehicle RID: -0.01; drug RID: 0.28, N=6, p=0.28). Additionally, SB-399885 (10mg/kg, 30 min, i.p.) was proconvulsant in C57Bl6 mice (vehicle: 11/18 seized; drug: 16/16 seized) but had no effect in CF1 mice (vehicle 5/14; drug: 5/15). Another antagonist SB-271046 (10mg/kg, 30 min, i.p.) had no effect on seizures in either CF1 for C57 mice. Additionally, the 5-HT$_6$ receptor agonist WAY-208466 significantly decreased the amplitude of fEPSPs in the inner molecular layer of the DG (90±4.5%, N=12, p<0.05) in brain slices from naïve rats. WAY-208466 also increased the amplitude of eIPSC (151.8±13.2%, N=9, p<0.01) onto DG granule cells without affecting paired-pulse plasticity.

Contrary to earlier reports, the 5-HT$_6$ receptor antagonist SB-399885 exhibited proconvulsant effects in the minimal clonic seizure test in C57BL6 mice; future studies will test the pharmacokinetic-, pharmacodynamic-, and species-dependence of this effect. The 5-HT$_6$ receptor agonist WAY-208466 attenuated the amplitude of fEPSPs and increased the amplitude of eIPSCs onto DG GCs. Additionally, 5-HT6 receptor antagonists did not significantly reverse memory impairments, but may have been tending towards an improvement. Together, these results suggest that 5-HT$_6$ receptor antagonists, which are in clinical trials in AD, have complex effects on the balance of excitation and inhibition in the DG and may affect seizure liability and cognition in AD patients.
Systemic delivery of antagomir-134 produces long-lasting seizure-suppressive effects

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Epilepsy is a serious neurological disease characterized by recurrent seizures. Acquired epilepsy is associated with large-scale changes in gene expression which underlie the cell and network-level changes during epileptogenesis. Despite various efforts we still have no treatments to prevent the emergence of epilepsy following brain injury. Evidence has emerged that microRNAs, a family of small non-coding RNAs, are important regulators of gene expression in epilepsy. Recent work showed that miRNA-134 is overexpressed in the temporal lobe of patients with pharmacoresistant seizures and in experimental models of epilepsy. Silencing miR-134 using intracerebroventricular injections of antagomirs (Ant) potently suppressed evoked and spontaneous seizures in mice. Here we explored a more clinically relevant route of delivery of these macromolecules, timing injection of antagomirs with blood-brain barrier (BBB) opening after status epilepticus (SE) in mice.

SE was induced in C57BL/6 adult mice by an intra-amygdala microinjection of kainic acid. Timing of BBB opening was assessed by Evans blue and FITC-dextran injections, and confirmed by extravasation of serum albumin and mouse IgG levels into the brain parenchyma. Antagomirs were locked nucleic acid- and cholesterol-modified. Injections were then timed accordingly and mice subject to continuous long-term video-telemetry EEG recording.

BBB opening in this model was apparent 2 h after SE. Systemic injection of Ant-134 at this time point did not alter the duration or severity of status epilepticus in mice but significantly reduced the number of spontaneous seizures recorded in mice compared with scrambled-sequence and vehicle-injected status epilepticus controls. These seizure-suppressive effects persisted at 1 and 2 months after the SE.

The present study provides evidence that macromolecule targeting of an epilepsy-associated microRNA is effective using a clinically-relevant delivery route, supporting the potential translation of this anti-epileptogenic treatment for epilepsy.

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Design and validation of a rodent model of human organophosphate exposure producing status epilepticus and neuropathology

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Exposure to organophosphates (OP) often results in seizures and/or status epilepticus (SE) that may produce neural damage within the central nervous system (CNS). Early control of seizure activity could minimize potential seizure-related CNS neuropathology. Although standard therapies exist, there remains a need to develop more effective agents to reduce OP-induced SE. To evaluate novel anticonvulsant compounds, it is imperative to develop an animal model simulating the CNS effects of OP exposure observed in patients. Therefore, we characterized the effects of an OP (diisopropyl fluorophosphate; DFP) dose (2-7 mg/kg), and route of administration (IM vs. IP) of therapeutic agents (pyridostigmine, 2-PAM, and atropine methyl nitrate), on SE, brain neuropathology, and mortality in rats. All doses of DFP induced peripheral tremors, and the animals that were administered 3-7 mg/kg exhibited electrographic (EEG) seizures. The percentage of animals exhibiting EEG seizure activity, the latency to seizure initiation, seizure intensity, and mortality were dose related. In addition, all doses of DFP resulting in EEG seizures (3-7 mg/kg) produced similar degrees of brain neuropathology, which were not dose related. Finally, mortality was significantly lower in rats administered therapeutic agents IM compared to IP, while all other pathological effects were similar. In conclusion, these studies developed a rodent model of OP poisoning that demonstrates pathological characteristics similar to those observed in patients, and thus validates this model for investigating potential new therapeutic approaches for treatment.

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Progressive, seizure-Like, spike-wave discharges are common in both injured and uninjured Sprague-Dawley rats: Implications for the fluid percussion injury model of post-traumatic epilepsy

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Variable-duration oscillations and repetitive, high-voltage spikes have been recorded in the electrocorticogram (ECoG) of rats weeks and months after fluid percussion injury (FPI), a model of traumatic brain injury (TBI). These ECoG events, which have many similarities to spike-and-wave discharges (SWDs) and absence seizures, have been proposed to represent non-convulsive seizures characteristic of post-traumatic epilepsy (PTE). The present study quantified features of SWD episodes in rats at different time points after moderate to severe FPI, and compared them to age-matched “control” rats. Control and FPI-injured rats at 1 year of age displayed large-amplitude and frequent SWD events at frontal and parietal recording sites. At 3-6 months, SWDs were shorter in duration and less frequent; extremely brief SWDs (i.e., “larval”) were detected as early as 1 month. The onset of the SWDs was nearly always synchronous across electrodes and of larger amplitude in frontal regions. A sensory stimulus, such as a click, immediately and consistently stopped the occurrence of the SWDs. SWDs were consistently accompanied by “behavioral arrest”. All features of SWDs in control and experimental (FPI) rats were indistinguishable. None of the FPI-treated rats developed non-convulsive or convulsive seizures that could be distinguished electrographically or behaviorally from SWDs. Because SWDs have features similar to genetic absence seizures, these results challenge the hypothesis that SWDs after FPI reflect PTE.

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Effects of Dual Orexin Receptor Antagonism on Sleep and Seizure Profile in Kcna1-Null Model of Epilepsy

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Purpose: This study was designed to test the effects of almorexant, a dual orexin receptor antagonist (DORA), on the sleep and seizure profile of the Kcna1-null (KO) mouse. Orexin producing neurons in the lateral hypothalamus (LH) are upstream of the ascending reticular activating system and promote arousal and alertness, acting through the orexin 1 and 2 receptors. The Kcna1-null mouse is a model for temporal lobe epilepsy and co-morbid sleep disorder with signs of pathophysiology in the orexin-rich area of the LH.

Methods and Materials: KO and wild-type (WT) mice were implanted with subdural EEG electrodes and EMG electrodes under isoflurane anesthesia at P35 (subdural EEG coordinates: 1.5 mm anterior and 1 mm lateral of Bregma, and 1.2 mm posterior and 1 mm lateral of Bregma; EMG electrodes implanted in nuchal muscles). After recovery, mice were given vehicle (25% DMSO in sterile saline, i.p.) for 3 days followed by almorexant (100 mg/kg in vehicle, i.p.) for 3 days. Injections occurred at the start of lights on (Zeitgeber = 0). Analysis was constrained to the first 6 hours after the third injection of vehicle or almorexant (Z= 0 to 6).

10 second epochs were categorized into NREM, REM, and wake states based on EEG power in the Delta band (0.5 to 4 Hz), EMG power in the 10-50 Hz range, and behavior. All states were analyzed for total number of epochs and duration of bouts (periods of consecutive epochs), as well as transitions between states. Seizures were scored during the same period by an investigator blinded to condition. Number, duration, and severity on a Racine scale of seizures were all recorded based on EEG and behavior.

Results: KO mice spent significantly less time in NREM and REM, and more time awake, than WT mice on vehicle. Almorexant significantly increases NREM sleep and decreases wake times for KO mice. Almorexant had no significant effect on increasing REM in KO mice, but did decrease the latency to first REM epoch in most KO mice. Interestingly, the same dose of almorexant did not have a significant effect on wild-type mice. Finally, almorexant had no effect on the incidence of seizures in KO mice, but significantly decreased the severity of seizures in a majority of mice.

Conclusions: Approximately 30 percent of epileptic patients suffer from insufficient sleep with symptoms similar to those seen in non-epileptic patients with orexin dysregulation. Orexin receptor antagonism has been suggested as an alternative to current pharmacological sleep therapies due to a decreased likelihood of side-effects such as cognitive impairment and daytime drowsiness. In this study, we demonstrate that almorexant is capable of improving sleep in the Kcna1-null mouse, a clinically relevant model of epilepsy with co-morbid sleep disorder as well as reduce seizure burden in a majority of cases. This is likely an indirect effect of improved sleep, but requires further investigation.

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Efficacy of flupirtine given after hypoxia-ischemia in controlling neonatal seizures in rats

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Seizures are common in human neonates and are frequently associated with hypoxic-ischemic encephalopathy. Unfortunately, current first-line drugs such as phenobarbital, a g-aminobutyric acid (GABA) agonist, have limited efficacy in controlling neonatal seizures. Research studies suggest that uncontrolled seizures may contribute to brain injury and adverse neurological outcome. Hence, it is desirable to develop a safer and more effective treatment. In early-life, when the GABA inhibitory system is not fully developed, potassium channels play an important role in controlling excitability. Our earlier studies demonstrated that flupirtine, a KCNQ potassium channel opener, is more efficacious than diazepam and phenobarbital for the treatment of chemoconvulsant-induced neonatal seizures. In the current study, we compare the efficacy of flupirtine with phenobarbital in the treatment of hypoxia-ischemia (HI)–induced neonatal seizures. HI was induced by ligating the carotid artery of 7-day old rats and then exposing them to hypoxia for 2 hours. Five minutes after the rats were exposed to room air (immediate reperfusion period), they were treated with either 25mg/Kg of flupirtine, vehicle, or 25mg/Kg of phenobarbital. A synchronized video-EEG monitoring technique was used to determine the occurrence of electroclinical seizures, electrographic seizures and EEG abnormalities. Compared to vehicle treated rats (90%; n = 10), significantly fewer flupirtine treated rats (30%; n=10) developed electroclinical seizures during the immediate reperfusion period following hypoxia. Further, in the 30% of flupirtine rats that did experience seizures, the number of electroclinical seizures were significantly lower than the vehicle treated group of rats. However, at 24 hours after HI, seizures were observed in all of the flupirtine treated rats. The frequency and duration of these seizures were similar to vehicle treated rats. Phenobarbital treatment reduced the percentage of rats developing seizures (50%; n = 10) vs. vehicle, but they experienced seizures at a similar frequency and duration as the vehicle treated rats. Moreover, the phenobarbital treated rats that experienced seizures during the reperfusion period, showed a trend towards higher seizure frequency (mean = 4.9 seizures/hour) than flupirtine treated rats (mean = 0.7 seizures/hour). These results suggest that there is a trend toward flupirtine being more efficacious than phenobarbital in treating HI-induced neonatal seizures.

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An *in vitro* screen for antiepileptogenic compounds utilizing organotypic hippocampal slice cultures

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Traumatic brain injury is a major cause of medically intractable acquired epilepsy. There is an urgent need to identify new therapeutic interventions. Significant limitations are associated with studying seizures induced by acute exposure to convulsants in otherwise normal *in vitro* and *in vivo* preparations. The slicing preparation of organotypic hippocampal slice cultures parallels traumatic axonal shear injury and subsequently slices develop a dense recurrent connectivity that results in the development of spontaneous seizures after 1 week *in vitro*. Culture media components were determined not to be responsible for epileptic activity. The organotypic hippocampal slice cultures represent an *in vitro* model of severe post-traumatic epilepsy with a reproducible, accessible and accelerated course of epileptogenesis. We utilized this model to conduct a blind screen of over 500 drug-concentration combinations for anticonvulsant, antiepileptic and neuroprotective effects at speeds that are orders of magnitude faster than any other therapeutic testing strategy for chronic epilepsy. Drugs, primarily FDA-approved, were typically tested at 3 concentrations: the most likely effective concentration as well as 1 log above and below this concentration. The culture medium was collected and changed every 3-4 days with drugs, dissolved in DMSO (final concentration 0.1%), added to the media starting on DIV 3. All experiments included DMSO control slice cultures derived from the same animal. Lactate and LDH levels were assayed in spent culture media as biomarkers of seizure activity and ictal neuronal death, respectively, with the latter being correlated with seizure burden. Positive screens were repeated to confirm the findings. Compounds exhibiting significant anticonvulsant activity in chronic-application experiments advanced to a second stage consisting of wash-out experiments to differentiate anticonvulsant from antiepileptogenic effects as well as *in vitro* electrophysiological confirmation. When anticonvulsant/proconvulsant effects as well as neuroprotective/neurotoxic effects were expressed as ratios of lactate production and LDH release, respectively, in the presence of drug vs. control conditions, the effects were normally distributed about a mean of zero effect. Both distributions exhibited a small skew toward therapeutic efficacy, with approximately 5% of all tested drug-concentration combinations demonstrating therapeutic effects on seizure activity and/or cell death rates that were > 3 standard deviations from the mean. These *in vitro* assays for anticonvulsant and neuroprotective effects in a model of severe pediatric post-traumatic epilepsy demonstrated that, just as in human trials, clinically-available anticonvulsants had modest therapeutic effects and their distribution of the effects was not different from that of all tested drugs, with a mean of zero effect. Several compounds demonstrated unexpectedly robust anticonvulsant and neuroprotective effects, and these beneficial effects were strongly correlated, which we interpret to represent reductions in ictal cell death. A third and final stage will be comprised of double-blind, crossover-controlled, *in vivo* EEG testing in the kainite model of chronic epilepsy to confirm the anticonvulsant effect of a lead compound. This technology comprises a promising strategy for the rapid investigation of drug efficacy in pediatric post-traumatic epileptogenesis, and could be further scaled with available robotic technologies.

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Age-Dependent Seizure Severity and Neuronal Damage in Response to the Organophosphate DFP

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Exposure to organophosphates (OPs) that inhibit acetylcholinesterase can initiate seizures and/or status epilepticus (SE) resulting in neurological damage. Electrographic and neuropathological sequelae of SE have been characterized in adult rodent models, but they have not been adequately investigated in immature animals, due to lack of validated, age-appropriate experimental procedures. We developed protocols to expose postnatal day (PND) 7, 14, 21 and 28 rats to DFP, then evaluated subsequent behavior and the electrographic (EEG) correlates of seizure activity. In addition, brains were collected and sectioned, and then stained with Fluoro-Jade B to determine neuropathology. Immature rats exhibited both behavioral and EEG activity in response to DFP. However, the incidence, duration, and severity of both behavioral and electrographic indicators were age-dependent, being minimal in PND7 rats and progressively increasing in severity in PND14, 21, and 28 animals. No behavioral indicators correlated with the initiation of EEG activity characteristic of seizures at any age. Fluoro-Jade B staining was observed in the hippocampus, amygdala, thalamus, piriform cortex, and cortex. Neuronal injury was more widespread in the PND21 and PND28 groups compared to PND14 animals, and was absent in brains of PND7 rats. In conclusion, using novel evaluation procedures, we found DFP provoked robust SE and neuronal injury in PND21 and PND28, but not PND7 and PND14, rat pups. These differential responses should be considered when evaluating the efficacy of medical countermeasures for NA and OP exposure in pediatric populations.

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Albumin-induced reactive gliosis following blood-brain barrier disruption: A novel therapeutic target and a model for age-related seizure susceptibility

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Post-traumatic epilepsy (PTE), occurring after brain insult, is one of the most common epilepsies, affecting millions of people worldwide. The progression of PTE is marked by a period of neuronal network reorganization in which post-injury inflammatory responses are thought to contribute to a hyperexcitable neural environment, ultimately leading to chronic and spontaneous seizures. Since PTE is often resistant to current anti-seizure medications, we targeted the early mechanisms of epileptogenesis to develop new therapeutic strategies for preventing PTE before it starts. Our previous research led us to target breakdown of the blood-brain barrier (BBB), as occurs during injury, as a key step causing epileptogenesis. We found that BBB disruption allows the serum protein albumin to enter the brain, where it binds to transforming growth factor beta receptors (TGF-βR) on astrocytes and induces inflammatory TGF-β signaling, reactive astrocytosis, increased neuronal excitability, and epileptogenesis. Furthermore, pharmacologically blocking the TGF-βR prevents activation of TGF-β signaling and onset of seizure activity in rodents exposed to albumin.

Interestingly, an increase in reactive astrocytosis, pro-inflammatory TGF-β signaling and BBB permeability is found in the aging brain, suggesting that the elderly will be more susceptible to epileptogenesis mediated by BBB injury. Indeed, the incidence and prevalence of seizures significantly increases in the elderly, but the causes and mechanisms of age-related vulnerability are poorly studied and remain elusive. Therefore, we investigated whether aged mice show impairments in BBB integrity and leakage of albumin into the brain, reactive gliosis, and increased vulnerability to seizure induction. Furthermore, we tested whether therapeutic intervention during aging can reduce seizure vulnerability.

We show that the aged brain has high amounts of albumin present, heightened reactive gliosis (both astrocytes and NG2 glia), and increased vulnerability to induced seizures. We characterized the astrogial response to albumin in both the young adult and aged mouse brain. We also quantified albumin signal transduction in both age groups, used specific transgenic and pharmacological approaches to analyze the spatio-temporal patterns of reactive gliosis and block astrocytic TGF-β signaling, and assessed the effects of reactive cell signaling and age on seizure threshold. These experiments explore the potential for preventative treatments targeted at astrocyte reactivity before the onset of epilepsy. Moreover, these findings have broader implications, given that the chronic presence of albumin in the aged brain, and accompanying inflammatory load, could be a major source of cognitive decline across a variety of aging-related dementias due to a similar vascular pathology.

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Celastrol acutely suppresses spasm frequency in the multiple-hit rat model of infantile spasms

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**Background:** Infantile spasms (IS) are the typical seizures of West syndrome, an infantile epileptic encephalopathy with poor prognosis. The multiple-hit rat model of IS is a chronic model of refractory IS due to structural lesions, which is resistant to adrenocorticotropic hormone (ACTH) and partially responds to vigabatrin. This model has been used to identify new candidate therapies with acute efficacy on spasms.

**Objective:** To determine whether a single injection of Celastrol (quinone methide triterpene), a potent antioxidant and anti-inflammatory drug, given after spasms manifest, can suppress spasms acutely at doses that do not impair the neurodevelopmental milestones in the multiple-hit rat model of IS.

**Methods:** Male Sprague-Dawley rats received right intracerebroventricular injection of doxorubicin and right intracortical injection of lipopolysaccharide on postnatal day (PN) 3. Intraperitoneal injection (i.p.) of p-chlorophenylalanine was given on PN5. From PN3 daily neurodevelopmental studies conducted. Video monitoring was done on PN4 (1 hour pre-drug injection and 5 hours post-drug injection) and on PN5 (2 two-hour sessions). Celastrol (1, 2, or 4mg/kg) or vehicle were administered i.p. on PN4, after spasms onset. This is a randomized, blinded, vehicle-controlled, dose-response study. Linear mixed model analysis was used to analyze raw or normalized log-transformed spasms rates, considering the repeated observations on each animal. 10-14 rats per group were studied.

**Results:** After spasms onset, a single i.p. injection of administered Celastrol produced dose-dependent spasm suppression within 3 hours and was well tolerated. Neurodevelopmental outcomes were unaffected.

**Discussion:** We determined that single injection of Celastrol causes effective spasm suppression in the multiple-hit rat model of IS that are refractory to ACTH. Studies are ongoing to determine the Celastrol effect on the electroclinical spasms and interictal EEG. Future studies will evaluate the effects of repeated Celastrol administration on spasms and long-term neurodevelopmental and cognitive outcomes in the multiple-hit rat model of IS.

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A critical developmental window for 17B-estradiol anti-epileptogenic effect in a mouse model of x-linked infantile spasms

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X-linked Infantile Spasms Syndrome (ISSX) is a catastrophic childhood epilepsy disorder characterized by motor spasms that arise in the first year of life and often develop epilepsy later in life. Currently, there are few treatments available that effectively reduce spasms, prevent epilepsy later in life and improve developmental outcomes in children with ISSX. The most common genetic cause of ISSX is a triplet repeat expansion in the Aristaless-related homeobox (ARX) gene. Our lab generated a mouse model with this mutation (Arx⁽GCC⁾¹⁰⁺⁷). Arx⁽GCC⁾¹⁰⁺⁷ mice recapitulate many phenotypic features seen in patients with ISSX, including epilepsy and motor spasms in early life. ARX is a crucial transcription factor for the development of cortical GABAergic interneurons, a population responsible for synaptic inhibition in the CNS. Arx⁽GCC⁾¹⁰⁺⁷ mutant mice have significantly reduced numbers of cortical GABAergic interneuron subtypes. Recently, our lab demonstrated that a one-week treatment of 17beta-Estradiol (E2) (40ng/g/day) administered to neonatal (P3-10) Arx⁽GCC⁾¹⁰⁺⁷ mice reduced spasms in neonates and seizures in adults. Interestingly, E2 treatment in neonatal Arx⁽GCC⁾¹⁰⁺⁷ mice also restored numbers of GABAergic interneuron subtypes in adults. This effect was age-dependent, as treatment of adult mice (P33-40) with E2 had no effect on these phenotypes. These results indicate that only early administration of E2 may have an antiepileptogenic effect in the Arx⁽GCC⁾¹⁰⁺⁷ model and in order to effectively translate this therapy to the clinic, we need to further define this critical developmental window. In this work, we aimed to define the temporal boundary for effective E2 treatment by delaying treatment initiation from P3 until P7. Delaying E2 treatment until the second postnatal week (P7-13), using either 40ng/g/day or 80ng/g/day, did not reduce seizures, interictal spikes (a second measure of cortical hyperexcitability), or social behavioral deficits in adult Arx⁽GCC⁾¹⁰⁺⁷ mice. These results indicate that there is a critical developmental window for antiepileptogenic effect of E2 in the Arx model of ISSX. Little is understood about the molecular mechanism of the antiepileptogenic effects of E2 and the basis for the critical therapeutic window. As estrogen receptor beta (ERβ) is expressed in developing interneurons, we began by exploring whether activation of this single estrogen receptor subtype is sufficient to reproduce the effect seen with E2. We found that selective activation of ERβ using LY500307 (Eli Lilly & Co.) at 200ng/g/day from P3-10 was sufficient to produce a partial antiepileptogenic effect in Arx⁽GCC⁾¹⁰⁺⁷. Treatment with LY500307 significantly reduced seizures but not neonatal spasms or interictal spikes. Full effect may require activation of other ERs or a higher dosage of LY500307. We aim to further elucidate the cellular and molecular mechanisms underlying the antiepileptogenic and neuroprotective effects of E2 in the Arx⁽GCC⁾¹⁰⁺⁷ model of infantile spasms.

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Low-dose methamphetamine reduces seizure incidence and susceptibility after severe TBI

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Traumatic brain injury (TBI) induced epilepsy is universally recognized as one of the most common forms of acquired epilepsy and the likelihood of developing epilepsy after a severe TBI can be as high as 50%. It is estimated that TBI causes 20% of symptomatic epilepsies, and individuals that sustain a severe TBI have a 3 times greater risk of developing epilepsy. Chronic, recurrent seizures that occur as a consequence of TBI are typically pharmacoresistant to currently available AEDs. The development of effective strategies for treating or preventing Post-traumatic epilepsy (PTE) is desperately needed. Although it is believed that PTE represents one of the most amenable human epilepsy syndromes for application of new therapies, clinical trials of AEDs have consistently failed to control TBI-induced seizures. A possible explanation for these failures is that most AEDs are discovered using animal models of seizures induced by maximal electroshock or chemo-convulsants. In contrast, PTE may involve different mechanisms relative to these models. Therefore, the successful development of effective PTE therapies will likely require the use of TBI models that closely replicate the conditions associated with human PTE. Using the lateral fluid percussion injury model, we have demonstrated that low dose methamphetamine significantly improves cognition and functional behavior following severe TBI. In addition, methamphetamine treatment significantly reduced pro-inflammatory signals and enhances granule cell neurogenesis. We therefore hypothesized that low dose methamphetamine might reduce seizures or prevent PTE. Here we demonstrate that treatment with low dose methamphetamine within 8 hours after severe TBI, significantly reduces susceptibility to PTZ-induced seizures. In addition methamphetamine treatment significantly reduces the incidence of TBI-induced spontaneous tonic-clonic seizures. Interestingly, we did not observe a strong correlation between animals that were highly susceptible to PTZ and exhibited spontaneous tonic-clonic seizures. These data strongly suggest that PTZ and TBI induce seizures via different mechanisms but that methamphetamine can potentially impact both mechanisms.

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Selective, unilateral ablation of hippocampal interneurons causes acute seizures.

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The loss of GABAergic interneurons has long been hypothesized to be an important mechanism of epilepsy. Pharmacological studies that non-specifically block one or more mechanisms of GABA-mediated inhibition have been shown to produce profound seizures in normal brain, and abundant evidence from animal models and human tissue suggests loss of interneurons can occur in acquired epilepsy. However, relatively little is known about whether selective interneuron lesions cause seizures, or the quantitative relationship between interneuron loss and seizure susceptibility. To test the hypothesis that selective lesions of GABAergic interneurons induce seizures, GAD2-IRES-CRE mice were injected unilaterally in the dorsal CA1 area with an adeno-associated virus containing the diphtheria toxin receptor (DTR), under cre-dependent control of expression to target interneurons for DTR-mediated ablation. At the same time, an electrode connected to a miniature-telemetry recording device was positioned at the injection site for continuous chronic recordings of local field potentials from the CA1 area of the hippocampus. Focal ablation of interneurons was achieved via intraperitoneal injection of diphtheria toxin (DT) 2-3 weeks after transfection. In a separate group of animals, the effect of the interneuron ablation procedure was examined with whole-cell recordings from pyramidal cells and immunohistochemical staining of GABAergic interneurons. Immunohistochemistry for GAD67 and GFP confirmed that the transfected cells were ablated by 6 days after DT administration, and whole cell recordings from CA1 pyramidal cells in ex vivo slices from the experimental animals confirmed that ablation did in fact specifically reduce GABA-mediated inhibition (i.e., the frequency of miniature inhibitory post-synaptic currents). Likewise, animals in the experimental group (n = 5) were observed to have seizures during the acute and sub-acute periods after the ablative procedure. Seizure onset was between 2 and 6 days after DT treatment. Three animals had seizures only during the week after the ablation procedure, but were seizure-free for the remainder of the recording period (18-68 days). One animal had seizures that stopped in the second week (8 days seizure free at end of recording). And one animal was observed to develop epilepsy; that is, spontaneous recurring seizures were observed for 5 weeks in only one of the mice subjected to the ablative procedure. Therefore, unilateral CA1 interneuron ablation is sufficient to produce acute/sub-acute convulsive seizures. However, in 4 of the 5 interneuron-ablated mice, seizures did not persist. Therefore, the chronic condition of spontaneous recurrent seizures (i.e., epilepsy) may require either a more extensive loss of interneurons, more time since the interneuron lesion, or additional changes to the neural network.

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Mutations in epilepsy and intellectual disability genes in patients with Rett-like features

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Our goal is to identify and evaluate mutations in known or new candidate genes of Rett syndrome or neurodevelopmental disorders with Rett-like features. Rett syndrome is a clinically defined neurogenetic syndrome characterized by developmental regression followed by stabilization and most often epilepsy. Patients typically have partial or complete loss of acquired purposeful hand skills and spoken language, gait abnormalities, and stereotypic hand movements. Rett syndrome almost exclusively affects females although the disorder has been seen in males. The most common cause of Rett syndrome is mutations in the gene methyl-CpG-binding protein, or MECP2. The MECP2 gene is located on the X chromosome and encodes a transcriptional regulator that is important in chromatin modification, synapse function in the brain, and messenger RNA processing. While mutations in MECP2 account for almost 95% of cases of classical Rett syndrome, there are also patients with features suggestive of Rett syndrome for whom MECP2 sequencing and deletion/duplication testing is unrevealing of a causative mutation. We ascertained a cohort of 11 patients with Rett-like features and negative clinical testing for mutations in MECP2. Using massively parallel (“next generation”) sequencing of patient exomes, we identified de novo mutations in most of the patients, including a somatic mosaic mutation in one case. The genes include MECP2, FOXG1, SCN8A and IQSEC2. These findings provide important information for genetic counseling and development of gene panel assays. Future study of the interactions between these genes’ products should lead to a deeper understanding of the biology of Rett syndrome and related disorders.
Modeling SCN8A mutant epilepsy in patient-derived cortical and autonomic neurons

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De novo mutations are major contributors to the development of childhood epileptic encephalopathies (CEEs) such as Dravet syndrome, infantile spasms, and Lennox-Gastaut syndrome (LGS) (Carvill et al 2013; O'Brien et al 2013; Epi4K Consortium 2013). CEEs comprise some of the most severe and pharmaco-resistant classes of epilepsy (Chiron and Dulac 2011). Mutations in the SCN8A gene have recently been shown to be causative for cases of infantile spasm, LGS, and CEE (Veeramah et al 2012; O'Brien et al 2013). CEEs often lead to intellectual and physical disabilities in later life as well as high rates of SUDEP (Sudden Unexpected Death in Epilepsy). These mutations have been shown to result in both gain-of-function and loss-of-function mutations (Veeramah et al 2012; Blanchard et al 2015). The goal of this research project is to use patient-derived cells to characterize electrophysiology alterations caused by SCN8A mutations associated with early-onset epilepsy and develop a novel platform for identifying effective pharmacological agents for this debilitating disease.

SCN8A, which encodes for the voltage-gated sodium channel is expressed in both the PNS and CNS, where it is the most abundant Nav channel. We have identified two CEE patients with missense mutations in SCN8A (Arg1872>Leu and Val1592>Leu). Fibroblasts from the patients’ skin biopsies were reprogrammed into induced pluripotent stem cells (iPSCs). These iPSCs have subsequently been differentiated by two different protocols into neurons expressing cortical excitatory and autonomic markers respectively. These cells are being used to first deduce the effect the Nav1.6 mutations have on electrophysiological properties of the neurons, particularly sodium current density and persistent sodium current. We hypothesize that these mutations will increase both of these measures leading to hyperexcitability of the neurons as has been shown in the majority of other SCN8A mutations in mouse models and heterologous systems.

We have also used a multielectrode array platform (Axion Biosystems) to measure spontaneous activity. Cortical neurons from three patient lines all show increased spontaneous activity and burst activity compared to one human iPSC-derived neuronal control line. Autonomic neurons from at least one SCN8A IEE patient show abnormal patterns of synchronous bursting on the MEAs, which may provide insight into potential SUDEP mechanisms. Future studies will involve culturing cortical and autonomic neurons, as well as cardiac myocytes, on MEAs to test known antiepileptic drugs and screen drug libraries for amelioration of hyperexcitable phenotypes. This work will shed light on SCN8A CEE seizure and SUDEP mechanisms and should provide lead compounds for clinical testing.
KCNQ2 variants causing epileptic encephalopathy display variable complexity of disruption of localization and function at the axon initial segment

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The genes KCNQ2 and KCNQ3 encode voltage-gated potassium channel subunits. Heteromeric KCNQ2/3 channels underlie the M-current (IM), which has an important role regulating neuronal excitability. Mutations in KCNQ2 and (more rarely, KCNQ3) may lead to mild and transient symptoms (Benign Familial Neonatal or Benign Familial Infantile Seizures, BFNS/BFIS) or severe and persistent impairment (epileptic encephalopathy). The reasons why some genetic variants cause self-limiting symptoms and others cause profound disability are not known. Potential factors include effects on channel gating, ion conductance and selectivity, trafficking, neurotransmitter modulation, and protein stability. In adult rodents, localization and concentration of KCNQ2/3 channels at the axon initial segment (AIS) tightly regulates normal firing patterns. However, the human KCNQ2/3 phenotypes are conspicuous very early in life, and changes in distribution of KCNQ2/3 channels throughout development may play a role in severity of mutations of the KCNQ2 gene. In order examine these issues, I performed immunofluorescence labeling in rodent tissue and cultured rat hippocampal neurons, and perform surface biotinylation assays in CHO cells expressing mutant KCNQ2 cDNAs. Immunofluorescence microscopy performed on tissue sections from transgenic mice overexpressing the dominant-negative mutant G279S revealed an aberrant labeling pattern: KCNQ2 was completely absent at the AIS and was retained at intracellular puncta in the soma and dendrites. KCNQ3 was partially redistributed to these puncta. In expression studies of 4 different epileptic encephalopathy pore domain mutations, surface biotinylation assays in CHO cells show no changes in total or surface protein levels. However, a variant located within the calmodulin binding region shows a proportional decrease in both total and surface protein, potential reflecting accelerated degradation of the mutant KCNQ2 protein. Additionally, in co-immunoprecipitation experiments, calmodulin binding with this variant is selectively decreased. In contrast to results obtain in the CHO cells, hippocampal neuron cultures show a disruption of KCNQ2 and KCNQ3 proteins detected at AISs in pore mutations, yet variants within the calmodulin binding region seem to target normally at the AIS. Further studies determining the half-life of this KCNQ2 variant is necessary as well as electrophysiological studies to determine whether or not activity is reduced. Some mutations may act by preventing surface trafficking and AIS concentration. Already, our experiments reveal that mutation effects can appear different in vitro, depending on the model system. Since some effects may not be easily revealed through heterologous expression, further development of in vivo models is warranted.
**PCDH19-Related Epilepsy**

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**PCDH19**-related epilepsy (formerly called Epilepsy in Females with Mental Retardation) is a monogenic epilepsy characterized by seizure onset in infancy or early childhood and cognitive impairment. The disorder is caused by mutations in the **PCDH19** gene located at Xq22.1. **PCDH19** encodes a 1,148 amino-acid protein belonging to the protocadherin δ2 subclass of the cadherin superfamily of cell-cell adhesion molecules. The **PCDH19** gene contains a signal sequence, 6 extracellular cadherin repeats (EC), a transmembrane domain, and a cytoplasmic region with two conserved domains (CM1 and CM2). The biological role of PCDH19 is not well understood. However, members of the protocadherin family are predominantly expressed in the nervous system and are postulated to be involved in the establishment of neuronal connections and signal transduction at the synaptic membrane. Disorders arising from mutations on the X chromosome are typically characterized by affected males and unaffected carrier females. In contrast, **PCDH19**-related epilepsy has a unique pattern of inheritance, in which female are affected and males transmit the disorder.

The phenotype of **PCDH19**-related epilepsy is highly variable. Patients present with different seizure types with a hallmark feature of seizure clustering in many cases, developmental delay/intellectual disability, and behavior problems. Over 100 pathogenic point mutations in **PCDH19** have been reported in the literature, most of them located in exon 1, which encodes the extracellular domain that appears to be crucial for the normal function of the protein.

We created a registry of patients with **PCDH19**-related epilepsy to better understand the full range of clinical features that are associated with **PCDH19**, to provide a resource of natural history data, and to inform the development of future animal models of **PCDH19**-related epilepsy.

We have enrolled 21 female patients and one male with 18 unique variants present in the heterozygous state. All but 3 were located in exon 1. Nine were missense variants (p.D121H, p.D124Y, p.D155N, p.T209P, p.V257L, p.R327W, p.D445E, p.V493M, p.S808N); four were nonsense variants (p.Y166X, p.K331X, p.R705X, p.R939X); three were frameshift deletions (p.K26fs, p.P216fs, p.Y366fs); and one was a deletion of the whole **PCDH19** gene (Xq21.33-p22.1del). Three mutations (p.D121H; Xq21.33-q22.1del; p.K26fs) were previously described in association with **PCDH19**-related epilepsy, and 12 variants are described for the first time.

Our future goals include understanding genotype-phenotype correlations in **PCDH19**-related epilepsy and to model these mutations—using CRISPR/Cas9 genome editing—in a zebrafish model of the disorder. Using this approach, we aim to use the zebrafish system to develop drug screening to identify novel therapeutics for **PCDH19**-related epilepsy.
Repeated low-dose kainate administration in C57BL/6J mice produces temporal lobe epilepsy pathology and spontaneous recurrent seizures

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Modeling acquired epilepsy following kainate or pilocarpine-induced status epilepticus has been invaluable for understanding the cellular and molecular underpinnings of epileptogenesis. However, in transitioning from rat models to mouse models of epilepsy, systemic kainate insults have failed to produce pathological features of status epilepticus in genetically tractable mouse strains. Further, the long-term impact of kainate in C57 mice has not been studied electrographically. Herein, we describe the acute and chronic response of C57BL/6J mice to repeated low-dose kainate injections (i.p.). Systemic injection of kainate, using any of four low-dosing paradigms, reliably produces behavioral and/or electrographic status without mortality. Three out of four dosing paradigms examined histologically produce pathological features indicative of temporal lobe epilepsy, including cell death, prevalent astrogliosis, and expression of astrocyte receptors commonly associated with pathology (mGluR5) seven days after kainate. Further analyses inducing status in animals expressing the genetically encoded calcium indicator, GCaMP5, indicate preliminarily that mGluR5 expressed on astrocytes is functional and transduces calcium signaling within the astrocyte, when investigated at 14 days. Only one low-dose paradigm tested to date produces spontaneous recurrent seizures, but at a low rate of induction and with infrequent seizures. However, the repeated low-dose kainate paradigm can serve as a reliable and effective means to model status epilepticus in the genetically tractable C57BL/6J mouse, or mice of a mixed C57 background.

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The anticonvulsant action of the galanin receptor agonist NAX-5055 involves modulation of both excitatory- and inhibitory neurotransmission

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The endogenous neuropeptide galanin is ubiquitously expressed throughout the mammalian brain. Through the galanin receptors GalR1-3, galanin has been demonstrated to modulate both glutamatergic and GABAergic neurotransmission, and this appears to be important in epilepsy and seizure activity. Accordingly, galanin analogues are likely to provide a new approach to seizure management. However, since peptides are generally poor candidates for therapeutic agents due to their poor metabolic stability and low brain bioavailability, a search for alternative strategies for the development of galanin-based anti-convulsant drugs was prompted. Based on this, a rationally designed GalR1 preferring galanin analogue, NAX-5055, was synthesized. This compound demonstrates anti-convulsant actions in several animal models of epilepsy. However, the cellular mechanism mediating these effects is not known. We have investigated possible mechanisms of action of NAX-5055 at the cellular level by determining its effects on excitatory and inhibitory neurotransmission, i.e. vesicular release of glutamate and GABA, respectively, in cerebellar, neocortical and hippocampal preparations. In addition, to evaluate potential cell toxicity mediated by NAX-5055, its effects on cell viability and neurotransmitter transporter capacity were examined. It was found that vesicular release of glutamate was reduced dose-dependently by NAX-5055 in the range from 0.1-1000 nM. Moreover, exposure to 1 µM NAX-5055 led to a reduction in the extracellular level of glutamate and an elevation of the extracellular level of GABA. Altogether these findings may at least partly explain the anti-convulsant effect of NAX-5055 observed in vivo.
Evaluating the therapeutic potential of Huperzine A in SCN1A-derived epilepsy

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Genetic factors play an important role in the etiology of epilepsy, and the neuronal voltage-gated sodium channels (VGSCs) have emerged as an important family of epilepsy genes. In particular, de novo loss-of-function mutations in the VGSC SCN1A (encoding Na⁺,1.1) are the main cause of Dravet syndrome (DS), a catastrophic early-life encephalopathy associated with prolonged and recurrent early-life febrile seizures (FSs), refractory afebrile epilepsy, cognitive and behavioral deficits, and a 15-20% mortality rate. SCN1A mutations also lead to genetic epilepsy with febrile seizures plus (GEFS+), which is an inherited disorder characterized by early-life FSs and the development of a wide range of adult epilepsy subtypes. Current anti-epilepsy drugs often fail to provide adequate protection against the severe seizures and neuropsychiatric comorbidities that occur in patients with SCN1A mutations. Furthermore, almost a third of epilepsy patients do not achieve adequate seizure control, highlighting the urgent need to develop multimodal treatments that can effectively mitigate the broad spectrum of clinical features associated with refractory epilepsies, while minimizing unwanted side effects.

In the proposed studies, we will test the hypothesis that Huperzine A (Hup A), a naturally occurring sesquiterpene Lycopodium alkaloid, will be efficacious in the treatment of DS and GEFS+. This hypothesis is based on the biological properties of Hup A, its demonstrated clinical safety, tolerability, ability to improve cognitive function, and our preliminary data. To determine a Hup A dose appropriate to use for subsequent experiments, we first conducted a Hup A dose response curve in wild-type (WT) CF1 male mice utilizing two seizure induction paradigms: 6 Hz and flurothyl. In the 6 Hz induction paradigm (44 mA), seizure score was based on a modified Racine scale: 0, no abnormal behavior; 1, staring, unresponsive for ≥ 5 seconds; 2, forelimb clonus; 3, rearing and falling. In the flurothyl seizure induction paradigm, latency to the myoclonic jerk, generalized tonic-clonic seizure (GTCS), and GTCS with hindlimb extension (HLE) was assessed. We found that a dose of 1 mg/kg Hup A provided the maximum seizure protection in the CF1 WT mice. Therefore, we used this dose of Hup A in heterozygous Scn1a knockout mice (Scn1a⁺⁻, a model of DS) and heterozygous Scn1a knock-in mice expressing the human SCN1A epilepsy mutation R1648H (RH mutants, a model of GEFS+) to evaluate the potential of Hup A to increase resistance to 6 Hz and flurothyl-induced seizures. Hup A (1 mg/kg) provided robust protection against 6 Hz-induced seizures in both mouse lines (N = 8-12/group). In response to flurothyl, Hup A significantly increased seizure latencies in the RH mutants and WT mice, but we did not observe protection in the Scn1a⁺⁻ mice.

In summary, Huperzine A might provide a novel therapeutic strategy for increasing seizure resistance in DS and GEFS+, and more broadly, in other forms of refractory epilepsy.

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