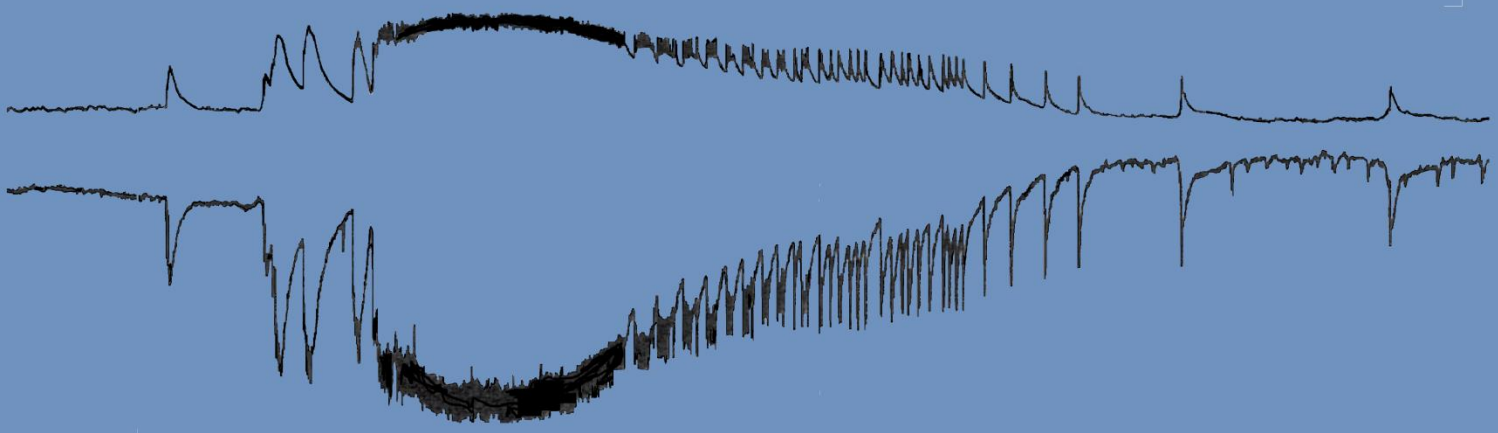
International conference on

# Excitatory-Inhibitory Signaling Balance as Therapeutic Target in Epilepsy

August 26-27, 2016

Montreal Neurological Institute
Montréal, Qc,
Canada



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Montreal Neurological Institute and Hospital

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## Welcome Address

I am happy to welcome you to this Conference that will focus on the roles played by ligand-gated signaling in epileptiform synchronization and epileptogenesis. Epilepsy has been a major topic of study at the Montreal Neurological Institute starting with the pioneering work of his founder, Wilder Penfield, and then continuing with the seminal discoveries made by scientists such as Herbert Jasper, Pierre Gloor and Brenda Milner.

I am indeed privileged and honored to have a Faculty of speakers from Canada, Europe and USA who are renowned leaders in their field of research and I anticipate that all participants, from basic neuroscientists to clinical neurologists, will greatly benefit from learning the most updated scientific evidence on topics such as genetics, neuronal plasticity and neuropharmacology in relation to epileptic disorders.

I would also like to warmly welcome the junior investigators (i.e., graduate students or postdoctoral fellows), and in particular those who will present their own scientific data at the poster session and platform presentations that are scheduled during the first day of this event.

Finally, I want to express my gratitude to our sponsors that have made this Conference happen.

Massimo Avoli

## Faculty

M. Avoli (Montreal, Qc, Canada)

M. Bazhenov (San Diego, CA, USA)

C. Bernard (Marseille, France)

G. Biagini (Modena, Italy)

A. Bragin (Los Angeles, CA, USA)

P. Carlen (Toronto, ON, Canada)

L. Carmant (Montreal, Qc, Canada)

S. Cash (Boston, MA, USA)

P. Cossette (Montreal, Qc, Canada)

V. Crunelli (Cardiff, UK)

M. de Curtis (Milano, Italy)

A. Delgado-Escueta (Los Angeles, CA, USA)

G. Di Cristo (Montreal, Qc, Canada)

P. Federico (Calgary, AL, Canada)

E. Fon (Montreal, Qc, Canada)

J. Gotman (Montreal, Qc, Canada)

J. Jefferys (Oxford, UK)

R. Köhling, (Rodstock, Germany)

M. Lévesque (Montreal, Qc, Canada)

K. Moxon (Philadelphia, PA, USA)

J. Noebels (Houston, TX, USA)

R. Olsen (Los Angeles, CA, USA)

M. Rogawski (Davis, CA, USA)

E. Rossignol (Montreal, Qc, Canada)

I. Timofeev (Quebec, Qc, Canada)

S. Williams (Montreal, Qc, Canada)

# Program

## Friday, August 26

08.30-08.45 **Welcome and Introduction**

Edward Fon, Scientific Director, Montreal Neurological Institute & Hospital

Massimo Avoli, McGill University

08.45-12.00 **Session 1 - Ligand-gated signaling, genes and generalized epilepsies**

Chair: Graziella Di Cristo

08.45-09.15 Next generation sequencing as applied to epilepsy

Patrick Cossette; University of Montreal, Montreal, Qc, Canada

09.15-09.45 EFHC1, ICK and IPO8 genes share same disease mechanism in JME

Antonio Delgado-Escueta; University of California Los Angeles, Los Angeles, CA, USA

09.45-10.15 Absence epilepsy: defining a critical balance in thalamocortical circuitry

Jeffrey Noebels; Baylor College of Medicine, Houston, TX, USA

10.15-11.00 *Coffee break*

11.00-11.30 Cortical inhibition in generalized epilepsies

Elsa Rossignol; University of Montreal, Montreal, Qc, Canada

11.30-12.00 Excitation-inhibition balance in thalamic neuronal ensembles during natural absence seizures

Vincenzo Crunelli; Cardiff University, Cardiff, United Kingdom

12.00-13.30 *Lunch and poster presentation (Jeanne Timmins Foyer)*

13.30-15.30 Platform discussion of posters

15.30-16.00 *Coffee break*

16.00-18.00 **Session 2 - EEG and metabolic patterns in patients with focal epileptic disorders**

Chair: Peter Carlen

16.00-16.30 The role of inhibition in sculpting human seizure dynamics

Sydney Cash; Harvard University, Cambridge, MA, USA

16.30-17.00 High Frequency Oscillations in Focal Epilepsy

Jean Gotman; McGill University, Montreal, Qc, Canada

17.00-17.30 Prolonged post-ictal vascular changes following focal seizures in humans

Paolo Federico; University of Calgary, AB, Canada

17.30-18.00 Influence of EEG synchronization and desynchronization on human epileptic activity during sleep

Brigitte Frauscher; Queen's University, Kingston, ON, Canada

## Saturday, August 27

08.30-10.30 **Session 3 - Ligand-gated mechanisms and focal seizures *in vivo***
Chair: Jean Gotman

08.30-09.00 Failure of local inhibition leads to increase of the size of PIN clusters and to seizure occurrence

Anatol Bragin; University of California Los Angeles, Los Angeles, CA, USA

09.00-09.30 Network activity underlying in vivo and in vitro seizure-onset patterns in the temporal lobe

Maxime Levesque; McGill University, Montreal, Qc, Canada

09.30-10.00 Role of hypersynchronous activity in the transition to seizure

Karen Moxon; Drexel University, Philadelphia, PA, USA

10.00-10.30 Neurosteroids in the treatment of epilepsy and status epilepticus

Michael A. Rogawski; University of California Davis, Davis, CA, USA

10.30 -11.00 *Coffee break*

11.00-13:00 **Session 4 - Ligand-gated mechanisms and focal seizures in vitro**

Chair: Christophe Bernard

11.00-11.30 The role of presynaptic glutamate release in the evolution and devolution of neocortical seizures

Peter Carlen; University of Toronto, Toronto, ON, Canada

11.30-12.00 Inhibitory networks at the onset of low-voltage fast activity focal seizures

Marco de Curtis; Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy

12.00-12.30 Optogenetic approaches to define the role of inhibition and excitation in seizure initiation and control

Sylvain Williams; McGill University, Montreal, Qc, Canada

12.30-13.00 Ionic and synaptic mechanisms of seizures

Maxim Bazhenov; University of California, San Diego, La Jolla, CA, USA

13.00-14.00 *Lunch break*

14.00-18.00 **Session 5 - Synaptic plasticity and epileptogenesis**

Chair: Michael Rogawski

14.00-14.30 The dynamic epileptic brain

Christophe Bernard; Institut de Neurosciences des Systèmes, Marseilles, France

14.30-15.00 Neurosteroids and epileptogenesis

Giuseppe Biagini; Universitá di Modena e Reggio Emilia, Modena, Italy

15.00-15.30 Role of inflammation in epileptogenesis

Lionel Carmant; University of Montreal, Montreal, Qc, Canada

15.30-16.00 Synaptic plasticity of the limbic system

John Jefferys; University of Oxford, Oxford, United Kingdom

16.00-16.30 *Coffee break*

16.30-17.00 Excitation/inhibition imbalance and seizures resulting from aberrant plasticity of GABA-A and glutamate receptors

Richard Olsen; University of California Los Angeles, Los Angeles, CA, USA

17.00-17.30 Trauma-induced epileptogenesis: sleep slow waves and neuronal silence

Igor Timofeev, Université Laval, Québec, Qc, Canada

17.30-18.00 Becoming juvenile again: ligand-gated functional changes in a model of temporal lobe epilepsy recapitulate juvenile ontogenetic states

Rüdiger Köhling, Universitätsmedizin Rostock, Rostock, Germany

18.00-19.00 Discussion and closing remarks

20.00-22.30 ***Gala Dinner***

## Free Communications (Index)

Note that free communications will be presented at the poster session held during lunch break on Friday August 26, and they will be discussed during the following platform session.

**01. Cross Frequency Coupling (CFC) During Sleep in Patients with Focal Epilepsy.** M. Amiri, B. Frauscher, J. Gotman

**02. Reducing Premature KCC2 Expression Rescues Seizure Susceptibility and Spine Morphology in Atypical Febrile Seizures.** Patricia N. Awad, Nathalie T. Sanon, Bidisha Chattopadhyaya, Josianne Nunes Carriço, Mohamed Ouardouz, Jonathan Gagné, Sandra Duss, Daniele Wolf, Sébastien Desgent, Laura Cancedda, Lionel Carmant, Graziella Di Cristo

**03. Netrin-1 Promotes Phosphorylation and Membrane Insertion of GluA1 in Hippocampal Neurons.** Ian V. Beamish, Stephen D. Glasgow, Simon Labrecque, R. Anne McKinney, Paul De Koninck, Philippe Séguéla, Edward S. Ruthazer, Timothy E. Kennedy.

**04. Nocortical Networks Dynamics in a Novel Model of Seizures Induced by Peripheral Somatosensory Stimulation.** Aleksandra Bortel, Roland Pilgram, Amir Shmuel

**05. Optogenetic Kindling of Neocortex Elicits Seizures.** Elvis Cela, Andrew J Chung, Taiji Wang, P. Jesper Sjöström

**06. Single-cell Activity During Carbachol-induced Oscillations in an *in vitro* Brain Slice Preparation.** Li-Yuan Chen, Maxime Lévesque, Nancy Duan, Leila Leclerc, Massimo Avoli

**07. Netrin-1 Regulates Synaptic Transmission in CA1 Pyramidal Neurons of the Adult Mouse Hippocampus.** Stephen D. Glasgow, Ian V. Beamish, Edwin Wong, Lianne J. Trigiani, Julien Gibon, Edith Hamel, R. Anne McKinney, Philippe Séguéla, Edward S. Ruthazer, Timothy E. Kennedy.

**08. Age-dependent Plasticity of Cortical GABAergic Innervation Lessens Seizure Severity in Cacna1a Conditional Mutant Mice.** Xiao Jiang, Elena Samarova, Alexis Lupien-Meilleur, Sabrina Tazerart, Mathieu Lachance, Roberto Araya, Jean-Claude Lacaille, Elsa Rossignol

**09. Towards Understanding Epileptic Seizures in the Human Brain: A Computational Approach to Origins.** Riaz A. Khan, Vikas Rai

**10. Adult Hippocampal CA3 Interictal Discharges After Immature Status Epilepticus (SE): Two Different *in vitro* Models Reveal Aspects of SE-induced Plasticity, with Focus on Cholinergic Effects and Oscillatory Properties.** Lisgaras C., Mikroulis A., and Psarropoulou C.

**11. Investigating the Roleof *MYO9B* In Cortical Gabaergic Interneuron Migration in Epileptic Encephalopathies.** Praveen K Raju, Lydia Marcoux, Jade Falardeau, Mathieu Lachance, Elsa Rossignol

**12. High Frequency Oscillations Can Pinpoint Seizures Progressing to Status Epilepticus.** Salami P, Lévesque M, Avoli M

**13. Interneuron Activity Leads to the Initiation of Low Voltage Fast Onset Seizures.** Zahra Shiri, Frederic Manseau, Maxime Levesque, Sylvain Williams, Massimo Avoli

**14. Inverse Relationship Between EEG Desynchronization and Interictal Epileptic Activity in a Depth EEG Study of Human Sleep.** Nicolás von Ellenrieder, Birgit Frauscher, François Dubeau, Jean Gotman

## Free Communications (Abstracts)

### 01. Reducing Premature KCC2 Expression Rescues Seizure Susceptibility and Spine Morphology in Atypical Febrile Seizures

**Patricia N. Awad[1,2], Nathalie T. Sanon[2], Bidisha Chattopadhyaya[2], Josianne Nunes Carriço[2], Mohamed Ouardouz[2], Jonathan Gagné[1,2], Sandra Duss[2], Daniele Wolf[1,2], Sébastien Desgent[1,2], Laura Cancedda[3], Lionel Carmant[1,2], Graziella Di Cristo[1,2.]**

[1]Neurosciences Department, Université de Montréal, Montréal, Québec, Canada. [2]CHU Sainte-Justine Research Center, Montréal, Québec, Canada. [3]Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, Genoa, Italy.

Febrile seizures affect about 5% of children during the first year of life. Atypical febrile seizures, particularly febrile status epilepticus, correlate with a higher risk of developing cognitive deficits and temporal lobe epilepsy as adults, suggesting that they may permanently change the developmental trajectory of neuronal circuits. In fact, the presence of a cerebral malformation predisposes to the development of atypical febrile seizures and temporal lobe epilepsy. The mechanisms underlying these effects are not clear. Cation-chloride cotransporter KCC2 decreases intracellular $Cl^-$ levels and renders GABA responses hyperpolarizing. Recent data suggest that KCC2 also modulates excitatory synapse development. Here, we demonstrated that KCC2 expression is altered by early-life febrile status epilepticus and investigated the functional impact of this alteration on subsequent synapse formation.

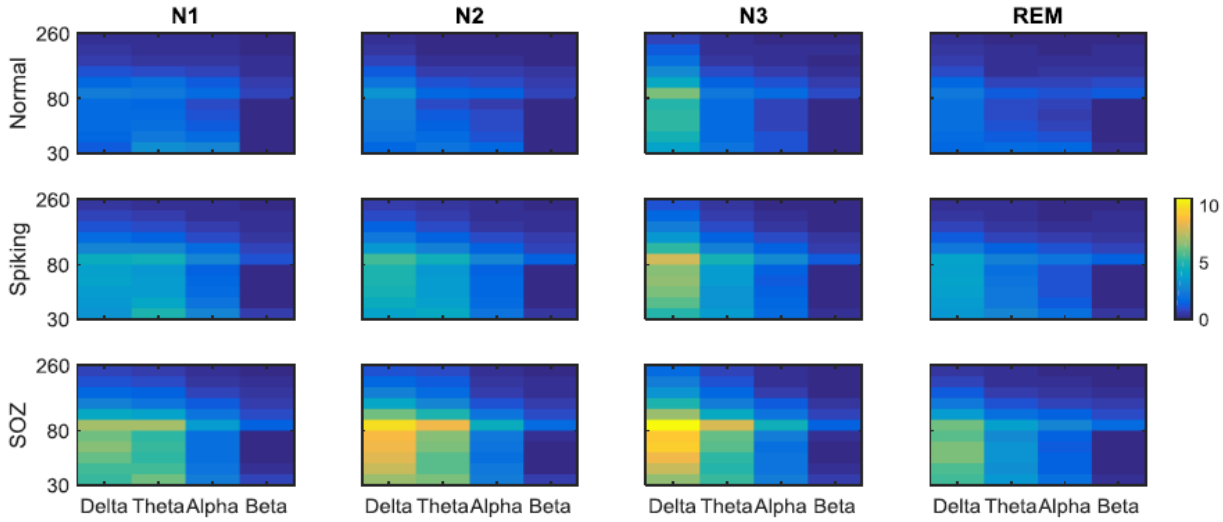
We analyzed KCC2 expression and spine density in the hippocampus of a well-established rodent model of atypical febrile seizures, combining a cortical freeze lesion at post-natal day 1 (P1) and hyperthermia-induced seizure at P10 (LHS rats). 86% of these LHS males develop epilepsy and learning and memory deficits in adulthood. At P20, we found a precocious increase in KCC2 protein levels, accompanied by a negative shift of $E_{GABA}$ by whole-cell recording and gramicidin-perforated patch. In parallel, we observed a reduction in dendritic spine size by DiI labelling, a reduction of mEPSC amplitude in CA1 pyramidal neurons, as well as impaired spatial memory. To investigate whether the premature expression of KCC2 plays a role in the alterations observed in the LHS model, and on seizure susceptibility, we reduced KCC2 *in vivo* by *in utero* electroporation of shRNA. This manipulation lead to reduced febrile seizure susceptibility, and rescued spine size shrinkage in LHS rats. Our results show that an increase of KCC2 levels induced by early-life insults affect seizure susceptibility and spine development and may be a contributing factor to the occurrence of hippocampal atrophy and associated cognitive deficits in LHS rats.

## 02. Cross Frequency Coupling (CFC) during Sleep in Patients with Focal Epilepsy

**M. Amiri, B. Frauscher, J. Gotman**

Montreal Neurological Institute, McGill University, Montreal, Canada

**Rationale**: It has been shown that the amplitude of EEG higher frequency oscillations is often modulated by the phase of lower frequency activities. During sleep, this modulation facilitates the communication among specific brain regions. In this study we investigate the variation of CFC during different sleep stages, and also in epileptic, and normal regions.
**Methods:** We studied interictal data of 25 patients with focal epilepsy. During the first sleep cycle, the first 4 minutes of each stage (stage N1, N2, N3 and REM) was selected. The signal was band-pass filtered into low (delta, theta, alpha, and beta) and high (gamma and ripple) frequency bands. The Modulation Index was calculated for each epoch, and each pair of low and high frequencies. Higher values of this index represent stronger coupling between two frequency bands. Sharp transients were discarded allowing the comparison of sections free of epileptic discharges.
**Results:** The average modulation index in all bands except beta was higher in N3 and N2 compared to REM ($p<0.05$). The average coupling in delta, theta and alpha band in all stages was significantly higher in the seizure onset zone (SOZ) compared to normal channels ($p<0.05$). The coupling of delta and theta in all stages was higher in SOZ compared to spiking channels outside SOZ.
**Discussion:** Considerable differences were seen between the SOZ and normal channels. Stronger coupling in the epileptic regions, and during deep sleep may be explained by increased neuronal synchrony. CFC may lead to an index for localizing epileptic brain regions.




The average of modulation index for all channels in different sleep stages, and in different regions

## 03. Netrin-1 Promotes Phosphorylation and Membrane Insertion of GluA1 in Hippocampal Neurons

**Ian V. Beamish, Stephen D. Glasgow, Simon Labrecque, R. Anne McKinney, Paul De Koninck, Philippe Séguéla, Edward S. Ruthazer, Timothy E. Kennedy.**

Montreal Neurological Institute, McGill University, Montreal, Canada

Netrin-1 plays an important role in the establishment of neural circuits during development; however, the functional relevance of netrin-1 in the mature central nervous system remains unclear. Selective removal of the netrin receptor Deleted in Colorectal Cancer (DCC) from adult forebrain neurons results in significant impairments in long-term potentiation (LTP), a form of activity-dependent synaptic plasticity, suggesting a role for netrin-1 in learning and memory. Using electrophysiological recordings in adult hippocampal slices, our lab has recently found that netrin-1 potentiates synaptic responses via activation of DCC. LTP induction has been shown to trigger post-translational modifications of glutamate receptor subunits of the AMPA subtype, which regulate their subcellular trafficking and are correlated with alterations in dendritic spine volume. Here, we show that application of exogenous netrin-1 results in increased levels of CaMKII phosphorylation, as well as phosphorylation of critical serine residues on the GluA1 AMPAR subunit. We also show that application of netrin-1 increases fluorescence intensity of super ecliptic pHluorin-tagged GluA1 (SEP-GluA1) at synaptic sites. Additionally, we demonstrate that brief application of netrin-1 leads to long-term increases in the volume of thin type dendritic spines. Finally, depolarization of hippocampal neurons increases extracellular netrin-1, consistent with an activity-dependent release of netrin-1. These results point to a critical role for netrin-1 in the regulation of synaptic transmission and long-term plasticity via DCC-mediated modulation of the phosphorylation status of GluA1.

## 04. Nocortical Networks Dynamics in a Novel Model of Seizures Induced by Peripheral Somatosensory Stimulation

**Aleksandra Bortel, Roland Pilgram, Amir Shmuel**

Montreal Neurological Institute, McGill University, Montreal, Canada

One major limitation in epilepsy research is that we have insufficient knowledge about the mechanism of initiation, propagation and termination of seizures. Animal models of seizures and epilepsy play a fundamental role in our understanding of the complex mechanisms underlying ictogenesis and epileptogenesis. Animal models also serve a variety of pharmacological purposes such as testing the efficacy of new antiepileptic drugs or other therapeutic interventions. Experimentally, seizure-like activity can be elicited by different type of chemoconvulsants or electrical stimulation. However, these techniques bear few disadvantages. Electrical stimulation used to induce seizures, disable studying the spatiotemporal dynamics of neuronal activity because of the impairment of electrophysiological recordings by electrical artifacts. Chemical injections into the brain lack spatiotemporal precision. Moreover, deep brain stimulations may synchronize or desynchronize networks participating in seizure generation. The cortical function during seizure episodes remains largely unknown and seizure symptoms of epilepsy are often associated with highly modified cortical activity. A diverse allocation of excitatory and inhibitory connections in the neocortex leads to laminar differences in seizure susceptibility. Likewise, the seizure onset and maintenance is highly determined by different cell types in each cortical layer, dendritic and axonal arborization of neurons and their intra- and inter-laminar connections. Hence, spontaneous discharges can be evoked in all superficial, middle and deep layers, indicating that each laminar network contains cell assemblies that may work as a discharge initiator. In this study, we seek to present an electrical artifact-free model of seizures in adult rats evoked by peripheral somatosensory stimulation to the rat digits that provides the particular scope to evaluate the spatiotemporal mechanisms underlying ictogenesis in a layer-specific manner. To this aim, we performed continuous neurophysiological recordings and we characterized laminar local field potentials of the primary somatosensory cortex S1FL after stimulating each of two digits separately. Four major conclusions can be drawn from the experiment presented here. First, peripherally applied somatosensory stimulation to the rat digits D3 or D5 produces epileptiform discharges. Second, the evoked seizures induced by the somatosensory stimulation represents an artifact-free model of electrical seizures and may resemble a reflex seizures observed in humans. Third, the seizure susceptibility depends on animal age. Fourth, ripples and fast ripples coincide with all epileptiform discharges evoked by peripheral somatosensory stimulation and they predict specific seizure onset zones. We postulate that our electrical artifact-free model of seizures provides a unique opportunity to study the spatiotemporal mechanisms underlying onset, propagation, and termination of seizures in a layer-specific manner.

## 05. Optogenetic Kindling of Neocortex Elicits Seizures

**Elvis Cela[1,2], Andrew J Chung[1], Taiji Wang[1], P. Jesper Sjöström[1]**

[1] Centre for Research in Neuroscience, Department of Neurology and Neurosurgery, The Research Institute of the McGill University Health Centre, Montreal General Hospital, Montreal, Qc
[2] Integrated Program in Neuroscience, McGill University, Montreal, Qc,Canada

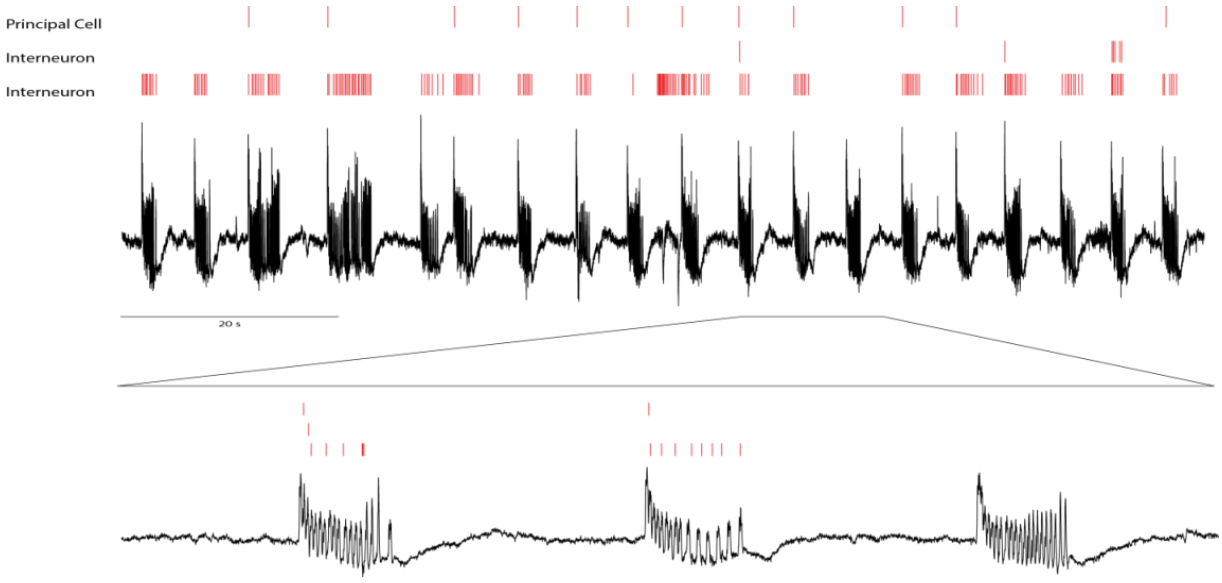
How seizures arise in the otherwise healthy brain remains poorly understood. One way of addressing this is to unravel the circuit changes that are associated with seizure initiation. To this end, we sought to test the hypothesis that seizures can eventually be initiated in healthy mice solely by repeatedly driving pathological activity in a genetically defined subset of neocortical pyramidal cells. We developed a novel kindling paradigm based on repeated optogenetic stimulation of primary motor cortex (M1). Channelrhodopsin-2 (ChR2) was expressed in M1 of male C57BL/6J mice by stereotactically injecting AAV-CaMKIIa-hChR2-E123T/T159C-p2A-EYFP bihemispherically. After 21 days of recovery and ChR2 expression, animals were kindled by repeatedly illuminating M1 in 3-second-long 50-Hz burst every 48 hours using a 445-nm laser. Animals were monitored by EEG and video during each session. We observed seizures eventually occurring in 6 out of 6 animals after 13 sessions. Seizures were defined as EEG power exceeding background levels by two standard deviations for longer than 3 seconds. We quantified their duration using EEG recordings, and severity using a modified Racine scale. We found that seizure duration ($r=0.52$, $p<0.001$, $n=4$), severity ($r=0.59$, $p<0.001$, $n=4$), as well as the number of seizures ($r=0.48$, $p<0.001$, $n=6$) increased with session, while seizure threshold was decreased ($r=-0.59$, $p<0.001$, $n=4$). We next examined if animals retained their seizure susceptibility after being unstimulated for 36 days. Indeed, after pausing stimulation, seizures had higher Racine scores ($p<0.05$, $n=5$) and lasted longer ($p<0.01$, $n=4$) than sessions preceding the stimulation hiatus. The seizure threshold ($p<0.01$, $n=4$) as well as the number of sessions prior to the first seizure was reduced ($p<0.05$, $n=4$). Finally, preliminary immunohistology for NeuN and GFAP indicated that there was no gross neuronal damage nor appreciable glial activation near the injection site. In summary, our results show that optogenetic stimulation of a small part of neocortex is sufficient to evoke seizure activity in healthy animals. In line with classical kindling findings, we found an elevated retention of seizure susceptibility in kindled animals, a decrease in threshold for seizure activity, and worsening seizure severity, as well as increased seizure duration over time. These results appear to have arisen in the absence of gross brain damage. We anticipate that optogenetic kindling may be incorporated as a new means to selectively examine the contributions of specific cell populations to epileptogenesis.

## 06. Single-cell Activity During Carbachol-induced Oscillations in an *in vitro* Brain Slice preparation

**Li-Yuan Chen, Maxime Lévesque, Nancy Duan, Leila Leclerc, Massimo Avoli**

Department of Neurology and Neurosurgery, Montreal Neurological Institute and McGill University

In mesial temporal lobe epilepsy (MTLE), one of the most refractory forms of epilepsy, seizures originate from the hippocampal formation, which is known for generating theta rhythm in the EEG. Data from both patients and animal models have shown that theta rhythm can precede ictal discharge, suggesting that this rhythm may play a role in ictogenesis, possibly through synchronizing excitatory and inhibitory neurons before ictal discharge onset. We investigated single-unit activity patterns during theta oscillations induced *in vitro* by bath application of carbamylcholine chloride (carbachol, 100 µM) for 4 hours. Brain slices (450 µm) were obtained from adult male Sprague-Dawley rats (250-275 g). Single-unit activity was recorded for 10 min. after which the tetrode wires were moved to another region of the entorhinal cortex (EC). Data were acquired at a sampling rate of 20 kHz, filtered between 300-3000 Hz to facilitate the identification of single units and between 1-70 Hz for visualising "slow" field potential signals. Single-units were classified as interneurons or principal cells with WaveClus. We found that carbachol application induced theta oscillations in the EC. These theta oscillations were dependent on fast glutamatergic transmission since they were abolished by the application of 10 uM 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione and 10 uM 3-(2-Carboxypiperazin-4-yl) propyl-1-phosphonic acid. Analysis of single-unit activity revealed an increase in firing rates of both interneurons and principal cell at the onset of theta oscillations. Interneurons fired during the entire duration of theta oscillations. Our data support the view that carbachol-induced theta oscillations are modulated by GABAergic interneuronal networks. We propose that excessive synchronization during theta rhythms, due to an enhanced interneuronal activity, could contribute to ictogenesis and perhaps to the development of an epileptic focus.




*Example of single-unit activity and carbachol-Induced theta oscillations (Black).*

## 07. Netrin-1 Regulates Synaptic Transmission in CA1 Pyramidal Neurons of the Adult Mouse Hippocampus

**Stephen D. Glasgow, Ian V. Beamish, Edwin Wong, Lianne J. Trigiani, Julien Gibon, Edith Hamel, R. Anne McKinney, Philippe Séguéla, Edward S. Ruthazer, Timothy E. Kennedy.**

Montreal Neurological Institute, McGill University, Montreal, Canada

Netrin-1 is a secreted protein that has been implicated in axon guidance during development; however little is known about its role in adulthood. Netrin-1 protein is readily detectable in the adult mouse hippocampus, suggesting potential roles in synaptic plasticity and learning and memory. Here, we report that brief application of netrin-1 increases thin-type dendritic spine volume, and leads to a significant increase in the frequency of miniature excitatory postsynaptic currents with no detected change in presynaptic function, indicating that netrin-1 can increase the number of excitatory synapses on CA1 pyramidal neurons. Moreover, transient application of netrin-1 induces a long-lasting potentiation of Schaffer collateral-evoked excitatory AMPA-mediated postsynaptic currents in CA1 pyramidal neurons in acute brain slices. We provide evidence that his potentiation is due to an increase in GluA1 AMPA receptor insertion and requires postsynaptic netrin receptor deleted-in-colorectal-cancer (DCC). DCC-mediated GluA1 insertion is dependent on PLC, intracellular calcium, PKC, and CaMKII, but not on NMDARs or mTOR. We also show that genetic deletion of netrin-1 from forebrain excitatory principal neurons results in attenuation of long-term potentiation (LTP), as well as deficits in spatial memory. Together, these findings identify a novel function for netrin-1 in the regulation of synaptic plasticity in the adult brain.

## 08. Age-dependent Plasticity of Cortical GABAergic Innervation Lessens Seizure Severity in Cacna1a Conditional Mutant Mice

**Xiao Jiang[1,2], Elena Samarova[1,2], Alexis Lupien-Meilleur[1], Sabrina Tazerart[2], Mathieu Lachance[1], Roberto Araya[2], Jean-Claude Lacaille[2], Elsa Rossignol[1,2]**

[1] CHU Ste-Justine Research Center, Montréal, Canada; [2] Department of Neurosciences and GRSNC, Université de Montréal, Montreal, Canada.

CACNA1A loss-of-function mutations result in cerebellar ataxia and epilepsy in humans. We previously showed that the pre-natal deletion of Cacna1a in the Nkx2.1Cre; Cacna1ac/c mutant mice, causing the ablation of voltage-gated CaV2.1 Ca2+ channels in forebrain GABAergic interneurons (IN), results in synaptic impairment of parvalbumin (PV) fast-spiking basket cells and induces generalized epilepsy. As CACNA1A mutation-associated epilepsy improves with age in patients, we propose that specific compensatory mechanisms may occur that, in the face of cortical disinhibition, re-establish the inhibition/excitation balance with time. We generated conditional mutant mice carrying a post-natal deletion of Cacna1a in PV+ neuronal populations (PVCre;Cacna1ac/c). PVCre;Cacna1ac/c mutant mice develop cerebellar ataxia and a mild epileptic phenotype with spike-wave seizures after postnatal day 45 (P45). We show a comparable reduction of GABAergic perisomatic boutons on cortical PC and a similar impairment of synaptic release from PV-INs in paired-recordings in both pre-natal (Nkx2.1Cre) and post-natal (PVCre) mutants, suggesting that both mutant lines develop a significant impairment of perisomatic inhibition. However, surprisingly, PVCre;Cacna1ac/c mutants displayed a two-fold increase in the frequency of miniature inhibitory synaptic currents (mIPSC) in cortical pyramidal cells (PC) at P60, whereas these events were unchanged in Nkx2.1Cre; Cacna1ac/c mutants at P20, suggesting that age-dependent compensatory plasticity changes in GABAergic circuits occur in the PVCre;Cacna1ac/c mutant mice. In particular, we demonstrate a significant increase of functional dendrite-targeting GABAergic projections from somatostatin (SOM) INs in PVCre;Cacna1ac/c mutants, using a combination of paired-recordings, immunostaining and two-photon imaging. Therefore, we propose that, in the face of altered PV-INs synaptic function, progressive reorganization of dendritic inhibition, including synaptic connectivity of SOM-INs, restricts cortical excitability and lessens seizure severity in PVCre; Cacna1ac/c mutants. A similar phenomenon has recently been described in chronic post-status epilepticus epilepsy models suggesting that this is a common phenomenon in genetic and non-genetic forms of chronic epilepsy.

## 09. Towards Understanding Epileptic Seizures in the Human Brain: A Computational Approach to Origins

**Riaz A. Khan, Vikas Rai**

MRIU, National Capital Region, Faridabad, HR 121 001; India

**Rationale:** The deterministic chaotic brain looks for pathological order. This order is represented by 'transient periodicity' in the brain dynamics and when it finds such an order, the synchronization of various chaotic saddles takes place. The epileptic seizures manifest themselves when synchronization of chaotic saddles in the hippocampus sub-regions takes place.

**Method:** The transient periodicity observed in EEG recordings obtained from patients suffering from epilepsy form the basis for analysis. We have applied a computational approach by simulating the dynamics of mean membrane potential of a pyramidal cell in the hippocampus.

**Results:** The presences of rare bursts of periodicity caused by unstable invariant sets (chaotic saddles) were detected in a subnetwork model. This model represents the dynamics of mean membrane potential of pyramidal cell of sub-regions of hippocampus. This periodicity is responsible for occurrences of epileptic seizures. The synchronization and desynchronization of chaotic saddles are spontaneous events.

**Conclusions:** The transient periodicity observed in the analysis of electro encephalograph recordings, obtained from patients, forms the basis of pathological condition of epilepsy. The rare bursts in neuronal signals (transient periodicity) is caused by unstable invariant sets present in the reconstructed phase space of neuronal dynamics in sub-regions of hippocampus, e.g., CA1, CA2, CA3. Modelling studies on electro encephalograph recordings indicate that the neural behaviour responsible for this brain disorder is due to the presence of certain chemical marker, their electro - physiological and molecular properties, whereby the inherent chaos of a healthy brain is lost. In this meta-stable state of transient periodicity, synchronization of various chaotic invariant sets occur leading to epileptic seizures. The epileptic condition appears and disappears as the spontaneous dynamical shifts from chaos to periodicity take place.

## 10. Adult Hippocampal CA3 Interictal Discharges After Immature Status Epilepticus (SE): Two Different *in vitro* Models Reveal Aspects of SE-induced Plasticity, with Focus on Cholinergic Effects and Oscillatory Properties

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Sustained generalized seizures in immature life have been associated to adult cognitive and behavioral abnormalities and to changes in adult seizure threshold. Previously, we have demonstrated that a Status Epilepticus (SE)-like seizure during CNS development provokes adult CNS changes, detectable both *in vitro* (Meilleur et al., 2000 and 2003; Potier et al., 2005; Mikroulis & Psarropoulou 2012;) and *in vivo* (Kouis et al., 2014). In this work, we investigated whether epileptiform discharges of different etiology would reveal different aspects of the SE-induced plasticity, by employing the 4-Aminopyridine (4-AP) and the $Mg^{2+}$-free ACSF models, generating spontaneous Interictal epileptiform discharges (IEDs) by blocking $I_A$ or by activating NMDA receptors respectively. In this setting, we further focused on cholinergic effects on IED rates of recurrence and high-frequency oscillation (HFO) content in the Ripple (R) and Fast Ripple (FR) frequencies. Septal (S) and Temporal (T) hippocampal slices were obtained from 104 adult (>P60) Sprague Dawley rats, >40 days after an SE-like Pentylenetetrazole-induced seizure (SE-slices) or from their normal littermates (N-slices). 50μM 4-AP or $Mg^{2+}$-free ACSF perfusion induced spontaneous hippocampal CA3 IEDs, whose rates (Hz) and HFO content were detected and analyzed prior to and after eserine (10μM, anti-AChE), CCh (1μM, non-hydrolysable ACh analog) or atropine (10μM, muscarinic antagonist) perfusion. T slice IED frequencies were higher than those of S slices in both models ($p<0.0001$), while SE-slice IEDs displayed significantly higher frequencies in 4-AP *vs* $Mg^{2+}$-free ACSF ($p=0.01$; N slices did not differ between the two models); N *vs* SE slice IED frequency differed (was higher) in the $Mg^{2+}$-free ACSF, not in 4-AP. CCh increased IED rates more than eserine in $Mg^{2+}$-free ACSF and equally to eserine in 4-AP. Atropine depressed IED rates more in 4-AP than in $Mg^{2+}$-free ACSF; Atropine-induced depression was more pronounced in T *vs* S N-slices, a gradient that was reversed in SE-slices, in both models (2-way ANOVA $p=0.023$). HFOs in the R and FR frequencies were detected in and coincided with IEDs. The computed FR/R ratio depended on anatomical origin (T or S) and conditioning (N or SE); In 4-AP, CCh decreased the IED percent R content in N-T slices and increased it in N-S slices ($p<0.05$ in both cases); it had no such effect in SE slices. CCh appeared to affect differently the IED percent FR content, depending on the anatomical origin of slices (T or S; trend). Our findings demonstrate that the plastic changes induced by a single early-life SE may influence *differently* epileptiform discharges of varying etiology and anatomical origin. Endogenous ACh affects temporal and septal hippocampal excitability to varying degrees, a relationship that changes following an immature SE-like seizure; this change may affect (adult) hippocampal information processing, especially in behavioral conditions of rising extracellular ACh levels. In conclusion, the manifestations of the "long term effects" of immature seizures may vary depending on the stimuli that the adult brain faces. *Funded in part by NSRF Research Funding Program Thales MIS 380342*

## 11. Investigating the Role Of *MYO9B* in Cortical Gabaergic Interneuron Migration in Epileptic Encephalopathies

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**Abstract**

Epileptic encephalopathies (EEs) are severe, childhood early onset conditions characterized by refractory seizures, developmental delay and/or intellectual disability generally associated with poor prognosis. The underlying causes of EEs are heterogeneous. In a previous report of whole-exome sequencing (WES) study in 18 familial trios, our lab has identified a range of *de novo* mutations in several new putative candidate genes for EEs. A majority of these genes are proposed to be involved in the processes of cytoskeletal reorganization of the actin and microtubules or in the processes of cell migration. One such essential candidate gene is *MYO9B* in which a potentially deleterious *de novo* mutation was identified. A recent WES study in autism patients has also identified *de novo* LoF mutations in *MYO9B* (De Rubeis et al 2014). *MYO9B* encodes an unconventional myosin-IXb protein involved in maintenance of cell shape and motility mediated by its actin-based motor functions. *Myo9b* was shown to control dendritic patterning of cortical pyramidal cells, but its roles in INs are unknown. Given the importance of the integrity of GABAergic interneurons (INs) in the control of cortical excitability, we hypothesized that deficiency of *Myo9b* may possibly result in structural and functional disturbance of cortical INs by altering their migration and maturation leading to epilepsy. To study the role of *Myo9b* on the migration and development of INs, we have optimized an *ex vivo* gene repression strategy to selectively repress the expression of *Myo9b* in the medial ganglionic eminence (MGE) of E13.5 mice embryos by *in utero* electroporation of a plasmid expressing an anti-Myo9b shRNA under the control of the *Dlx5/6* promoter. Knockdown of *Myo9b* using this method revealed a significant reduction in the migration of cortical INs in E16 embryos in electroporated cells. Furthermore, we have noticed a significant alteration in the morphology of these INs with an increased branching of leading and trailing processes. Importantly, the morphological defects were rescued with a shRNA-resistant version of the wild-type cDNA, which suggests the specificity of the findings to the *Myo9b* gene function. Together, our preliminary results suggest that prenatal repression of *Myo9b* results in significant morphological alterations in migrating INs. These data provide insights into the molecular pathways controlling cortical GABAergic INs development and propose that human mutations in *MYO9B* might cause GABAergic network dysfunction that could play a crucial role in the pathogenesis of EEs.

## 12. High Frequency Oscillations Can Pinpoint Seizures Progressing to Status Epilepticus

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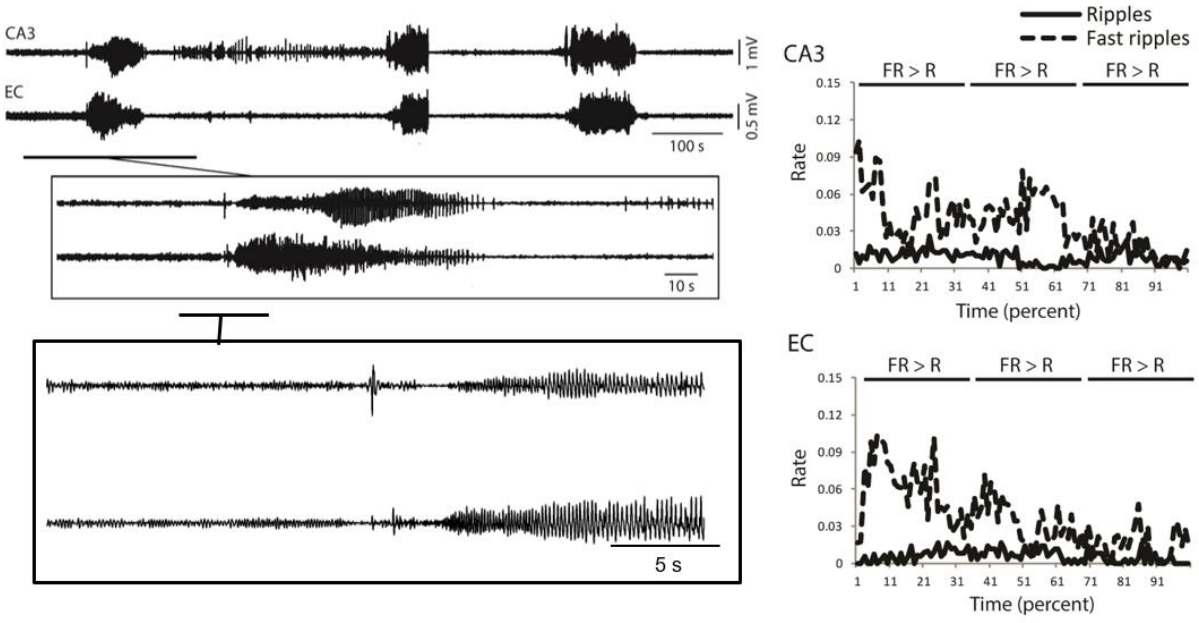
Status epilepticus (SE) is defined as a seizure lasting more than 5 min or a period of recurrent seizures without recovery between them. SE is a serious emergency condition that requires immediate intervention; therefore, identifying SE electrophysiological markers may translate in prompt care to stop it. Here, we analyzed the EEG signals recorded from the CA3 region of the hippocampus and the entorhinal cortex in rats that responded to systemic administration of 4-aminopyridine (4AP) by generating either isolated seizures or seizures progressing to SE. We found that high frequency oscillations (HFOs) - which can be categorized as ripples (80-200 Hz) and fast ripples (250-500 Hz) - had different patterns of occurrence in the two groups (n = 5 for each group). Specifically, fast ripples in CA3 and entorhinal cortex of the SE group occurred at higher rates than ripples, both during the ictal and post-ictal period when compared to the HFOs recorded from the isolated seizure group. Our data reveal that different patterns of HFO occurrence can pinpoint seizures progressing to SE, thus suggesting the involvement of different neuronal networks at the termination of seizure discharges.




*Seizures recorded in a SE group rat (EEG traces in the left column) and temporal distribution of HFOs during seizures recorded from 5 rats in this group (graphs in the right column); note that the rate of occurrence of fast ripples is higher compared to ripples throughout the entire seizure.*

## 13. Interneuron Activity Leads to the Initiation of Low Voltage Fast Onset Seizures

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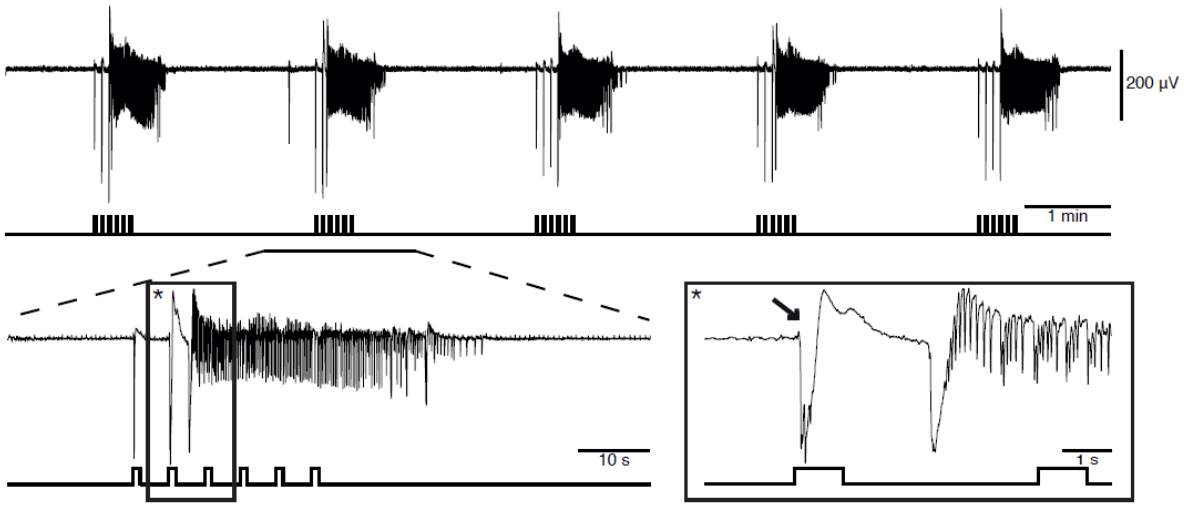
Seizures in temporal lobe epilepsy can be classified as hypersynchronous and low-voltage fast according to their onset patterns. Low-voltage fast onset seizures usually initiate with one or two interictal-like spikes followed by low amplitude, high frequency activity whereas hypersynchronous onset is characterized by a series of focal spikes occurring at approximately 2 Hz. Experimental evidence suggests that low-voltage fast-onset seizures mainly result from the synchronous activity of GABA releasing cells. In this study, we tested this hypothesis using the optogenetic control of parvalbumin-positive interneurons in the entorhinal cortex, in the in vitro 4-aminopyridine model. Local field potential recordings revealed that both spontaneous and optogenetically-induced seizures had similar low-voltage fast-onset patterns. Using simultaneous local field potential and whole-cell patch clamp recordings we also identified the contribution of GABAergic interneurons to low-voltage fast onset ictal discharges. Furthermore, pharmacological blockade of $GABA_A$ and $GABA_B$ receptors effectively blocked all ictal discharges as well as stimulus-induced responses. Finally, both spontaneous and stimulated seizure-like discharges presented with higher ripple than fast ripple rates at ictal onset. Our data thus demonstrate the involvement of interneuron networks in the initiation of low-voltage fast onset seizures.




*Ictal discharges evoked by 1 s light pulses delivered at 0.2 Hz during bath application of 4AP; one of these events is further expanded to reveal the low-voltage fast onset pattern*

## 14. Inverse Relationship Between EEG Desynchronization and Interictal Epileptic Activity in a Depth EEG Study of Human Sleep

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**Rationale:** It is well demonstrated that interictal epileptic discharges (IEDs) in focal epilepsy are influenced by sleep, and in particular that rapid eye movement (REM) sleep has a suppressing effect on epileptic activity. The decrease in epileptic activity is assumed to be due to EEG desynchronization which is mainly mediated by cholinergic neurotransmission. Based on these considerations we investigated the occurrence of IEDs and high frequency oscillations – a novel biomarker of the epileptogenic zone – across both phasic and tonic REM sleep. We hypothesized that interictal events are even more suppressed during phasic REM sleep because of additional intermittent cholinergic drive.

**Methods:** Twelve patients underwent polysomnography during long term intracerebral EEG recording. After sleep staging, segments of phasic REM sleep were identified and marked. An equal number of segments of matching duration was marked randomly during tonic REM sleep in the same sleep cycle. We computed the EEG power in frequencies up to 30 Hz and from 30 to 500 Hz in the tonic and phasic segments. In the same segments we marked IEDs and high frequency oscillations: ripples in the 80–250 Hz band and fast ripples in the 250–500 Hz band. We grouped the intracerebral channels into channels in the seizure onset zone (SOZ), channels in the irritative zone but outside the SOZ, and channels without any epileptic activity, presumably recording only physiologic activity. We compared the EEG power and the proportion of events in phasic and tonic segments, and tested whether the rate of ripples was significantly different in the three channel groups, as ripples might be of pathologic or physiologic origin.

**Results:** Power in frequencies below 30 Hz was lower during phasic than tonic REM sleep (phasic to tonic power ratio lower than one, $p<0.001$, Bonferroni corrected), possibly reflecting increased desynchronization. All types of interictal events were significantly less frequent during phasic REM sleep compared to tonic REM sleep (39% of 2767 spikes, 35% of 3162 ripples, and 18% of 708 fast ripples. In contrast to ripples in channels with epileptic activity, presumably physiologic ripples were more abundant during phasic REM (73% of 336 ripples in normal channels, 30% of 816 in channels in the irritative zone but outside the SOZ, 28% of 2010 in the SOZ – in all cases the differences are highly significant, Bonferroni corrected $p<0.001$). During tonic REM sleep the mean ripple rate was significantly different among the three groups of channels (Bonferroni corrected $p<0.001$). During phasic REM sleep the difference between mean ripple rate in normal channels and channels in the irritative zone outside the SOZ was not statistically significant.

**Conclusions:** Phasic REM sleep has an enhanced suppressive effect on epileptic activity compared with tonic REM sleep, corroborating the role of EEG desynchronization in the suppression of epileptic activity. In contrast, physiological ripples were increased during phasic REM sleep, possibly reflecting REM-related memory consolidation and dreaming.

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