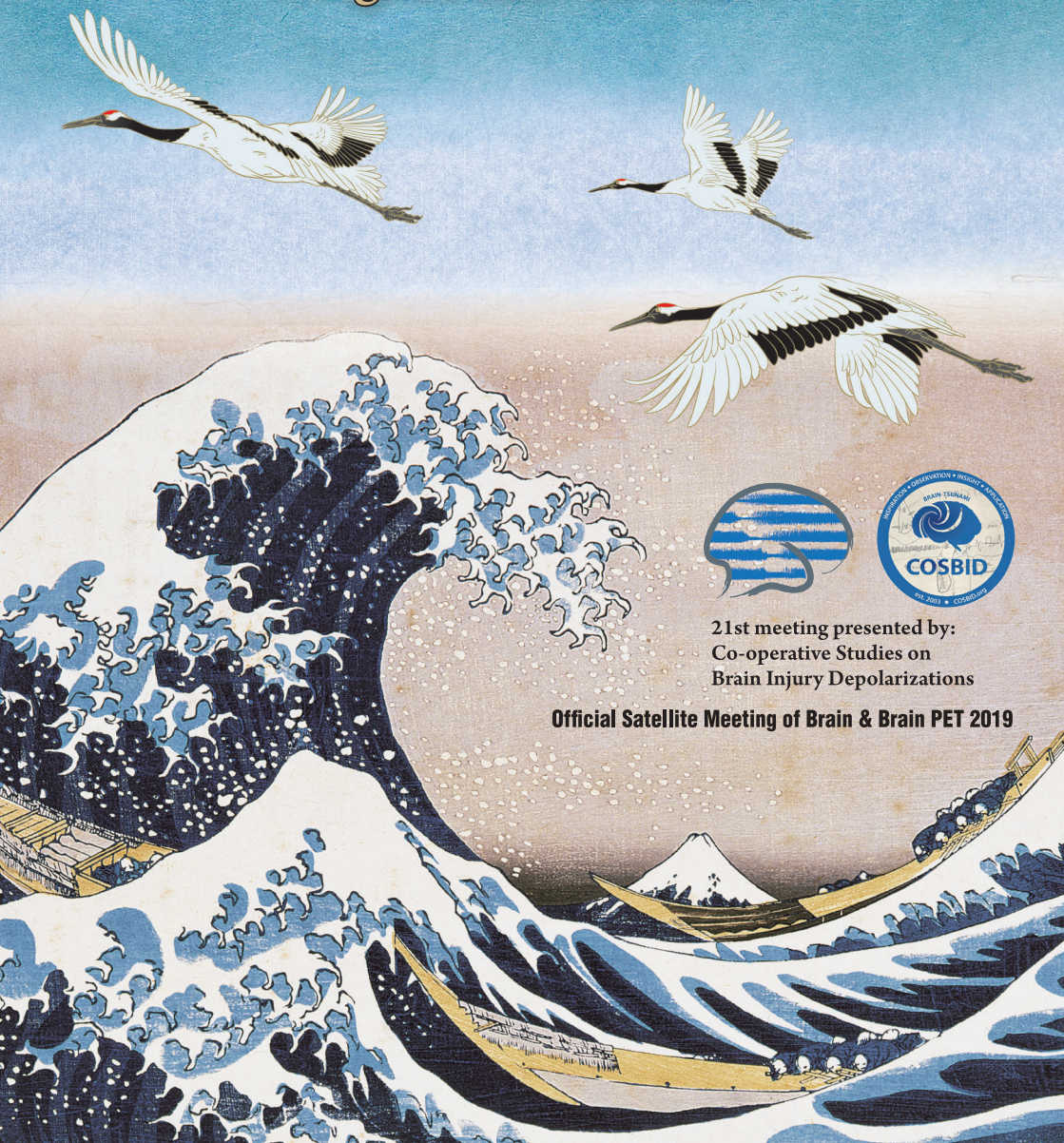
iCSD 2019

International Conference on Spreading Depolarizations

July 1 - 3, 2019 Yokohama, Japan

Program and Abstracts



21st meeting presented by:
Co-operative Studies on
Brain Injury Depolarizations

Official Satellite Meeting of Brain & Brain PET 2019

iCSD2019 Yokohama

Welcomes attendees from:

Program Committee

Cenk Ayata, MD.	Massachusetts General Hospital
Jens P. Dreier, MD, PhD.	Center for Stroke Research Berlin & Neurological Hospital
Eszter Farkas, PhD.	University of Szeged
Jed A. Hartings, PhD.	University of Cincinnati
Raimund Helbok, MD.	Medical University of Innsbruck
Bill Shuttleworth, PhD.	University of New Mexico
Johannes Woitzik, MD, PhD.	University of Oldenburg

Local Organizing Committee

Michiyasu Suzuki	Hisaharu Goto	Koki Okazaki
Fumiaki Oka	Nobuhiro Tanaka	Takuma Nishimoto
Sadahiro Nomura	Hirokazu Sadahiro	Naomasa Mori
Hideyuki Ishihara	Kazutaka Sugimoto	Kohei Haji
Eiichi Suehiro	Yuichi Fujiyama	Mahapatra Arindam Gajendra
Hirochika Imoto	Miwa Kiyohira	Mayumi Matsugaki

Department of Neurosurgery, Yamaguchi University Graduate School of Medicine

A special thanks to both committees for all their efforts!

Welcome to iCSD2019

Welcome to Japan for the 3rd International Conference on Spreading Depolarization (iCSD) !
We are all very pleased and honored to organize this exiting conference.

Since the COSBID group held its first annual meeting in 2003, this is the first time to be held outside of Europe or the United States. Just like a cortical spreading depolarizations, the enthusiasm of the COSBID group has been propagating slowly but steadily and finally arrived in Asia!

One of the special events of this conference is "preconference educational seminar". Thanks to Dr. Cenk Ayata and Dr. Jens Dreier, this is the first time to have basic educational seminars during iCSD. Dr. Ayata will talk about "Minimum knowledge to start the basic research on CSD" and Dr. Dreier will talk about "Monitoring of spreading depolarizations on the ICU". Both will definitely help the young investigators to start their research carrier in this field.
We received 38 abstracts from 11 different countries and all abstracts will be oral presentations. We planned 2 Open Discussions on the controversial issues. The themes of Open Discussions will be "Is SD really harmful?" and "SD, Seizures and Epilepsy". Both are fundamental and important issues and hope everybody will enjoy and have enough time to debate. Finally, there will be 4 keynote lectures. All speakers are experts from the different fields, and we are confident that they will bring us new insights.

Finally, we would like to express our gratitude to the members of program committee for their enormous cooperation for preparing this conference.

We hope all of you get new idea through a lively discussion and spend a fruitful time during the conference!
Have a great stay!!

Michi Suzuki

Michiyasu Suzuki, M.D., Ph.D.
(Professor and Chairman, Department of Neurosurgery
Yamaguchi University Graduate School of Medicine)

Sponsors

Ajisu Dohjin Hospital
Anno Heart Clinic
Astellas Pharma Inc.
Bristol-Myers Squibb K.K.
CSL Behring K.K.
GlaxoSmithKline K.K.
Hikari Municipal Hikari General Hospital
Idorsia Pharmaceuticals Japan
Integra Japan K.K.
KAKU Neurosurgery Clinic
Kanaya Medical Instruments Co., Ltd.
Kenwakai Ohtemachi Hospital
Masuda Red Cross Hospital
Nagato General Hospital
Nakamura Memorial Hospital
Nihon Medi-Physics Co.,Ltd.
Ogori Daiichi General Hospital
Ohirakai Medical Corporation (Minei Daiichi Hospital)
Okada Hospital
S&Brain Corporation
Saney Seiko Inc.
Shunan City Shinnanyo Hospital
Stryker Japan K.K.
The Federation of Pharmaceutical Manufacturers' Associations of JAPAN
The Mine-shi Hospital business station
Tsudumigaura Medical Center for Children with Disabilities
TSUMURA & CO.
Tsushimi Hospital
UCB Japan Co. Ltd.
Unimedic, Inc.
Unique Medical Co.,Ltd.
Yamaguchi-ken Saiseikai Shimonoseki General Hospital
Yoshikawa Neurosurgery Clinic

(Alphabetical Order)

General Information

Venue:
Hamagin Hall "VIA MARE"
TEL:+81-45-225-2173 FAX:+81-45-225-2183
3-1-1, Minatomirai, Nishi-ku, Yokohama, Kanagawa 220-8611 JAPAN
(Bank of Yokohama head office, 1F)
http://www.yokohama-viamare.or.jp/english.html

Registration: At Foyer
Monday, July 1 12:00-16:30
Tuesday, July 2 8:00-17:00
Wednesday, July 3 8:00-16:00

Social Program: All attendees are invited.
Welcome Reception Monday, July 1, 18:30-20:30 at Lobby, 1F of the venue

iCSD Dinner Tuesday, July 2, 19:30-21:30
at Sky Banquet Room "Rainbow", 70F of Hotel Bldg., Yokohama Royal Park Hotel

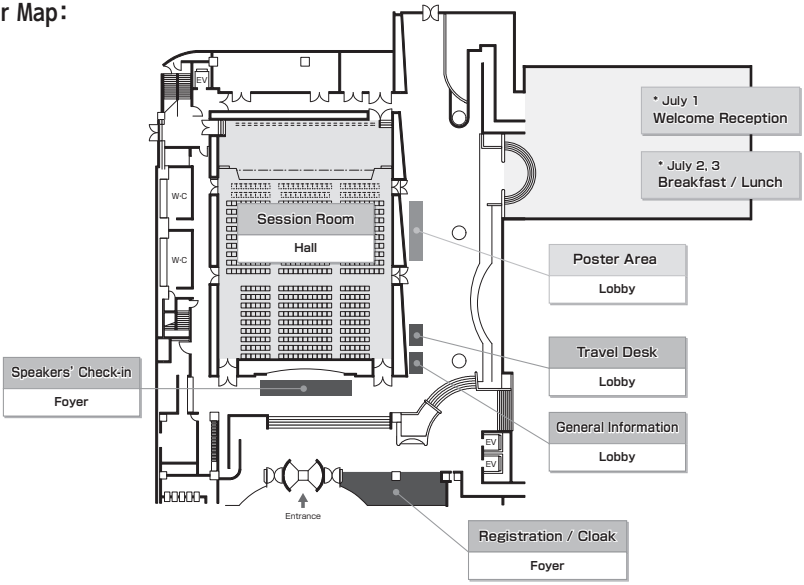
Breakfast
Tuesday, July 2 8:30-9:00
Wednesday, July 3 8:30-9:00
Lunch Break
Tuesday, July 2 12:50-14:00
Wednesday, July 3 11:50-13:00

Time Allocation for Oral Presentations:

Session	Presentation	Q & A	Total
1, 2, 3, 5, 6, 7	15 min.	5 min.	20 min.
4	10 min.	5 min.	15 min.

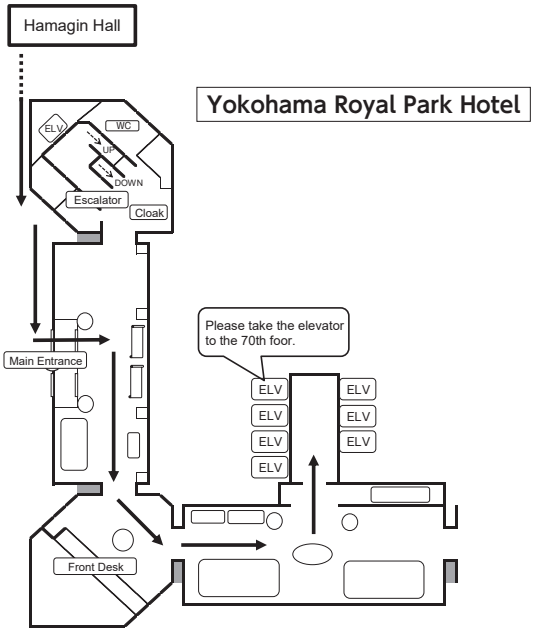
WIFI:
SSID: iCSD2019
Password: icsd2019

Floor Map:

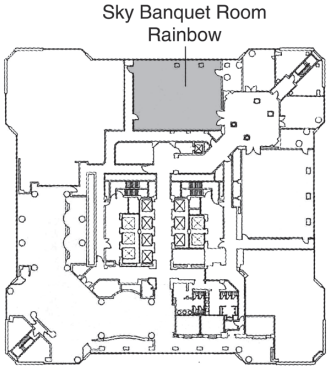


iCSD Dinner: Tuesday, July 2, 19:30–21:30
at Sky Banquet Room "Rainbow", 70F of Hotel Bldg., Yokohama Royal Park Hotel

1F

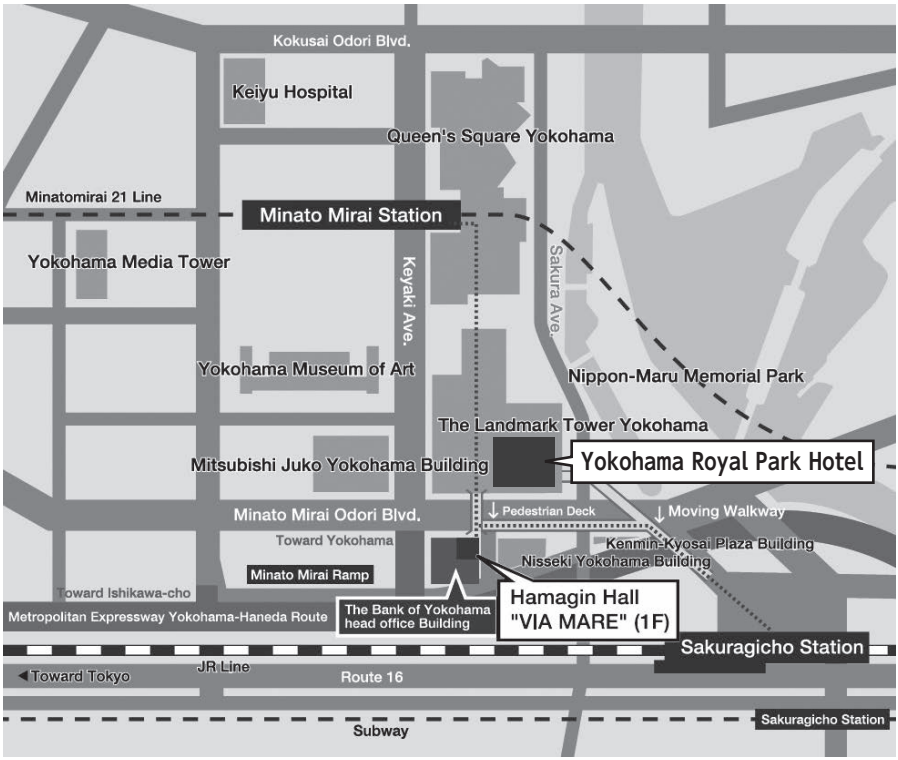


70F



Venue Map

VIA MARE is located in the Bank of Yokohama head office, 1F.
Address: 3-1-1 Minatomirai, Nishi-ku, Yokohama, Kanagawa, 220-8611 JAPAN



By Air
Narita Airport
JR Narita Express - JR Yokohama Sta. 90 min.
JR Yokohama Sta. [Change to JR Keihin Tohoku Line] - Sakuragicho Sta. (JR Keihin Tohoku Line) 3 min.
Sakuragicho Sta. (JR Keihin Tohoku Line) - Hamagin Hall Via Mare 5 min. on foot

Airport Limousine Bus - YCAT (Yokohama City Air Terminal,Yokohama Sta. east) 90 min.
YCAT - Hamagin Hall Via Mare 5 min. by Taxi or JR Yokohama Sta. - Sakuragicho Sta. (JR Keihin Tohoku Line) 3 min.
Sakuragicho Sta. (JR Keihin Tohoku Line) - Hamagin Hall Via Mare 5 min. on foot or Yokohama Sta. (Minatomirai Line) - Minatomirai Sta. 3 min.
Minatomirai Sta. (Minatomirai Line) - Hamagin Hall Via Mare 7 min. on foot

Haneda Airport
Haneda Airport Sta. (Keikyu Line) - Yokohama Sta. 24 min. by express
[Change to JR Keihin Tohoku Line] JR Yokohama Sta. - Sakuragicho Sta. (JR Keihin Tohoku Line) 3 min.
Sakuragicho Sta. (JR Keihin Tohoku Line) - Hamagin Hall Via Mare 5 min. on foot

Airport Limousion Bus - YCAT Yokohama City Air Terminal (Yokohama Sta. east) 30 min.
YCAT - Hamagin Hall Via Mare 5 min. by Taxi.
or JR Yokohama Sta. - Sakuragicho Sta. (JR Keihin Tohoku Line) 3 min.
Sakuragicho Sta. (JR Keihin Tohoku Line) - Hamagin Hall Via Mare 5 min. on foot or Yokohama Sta. (Minatomirai Line) - Minatomirai Sta. 3 min.
Minatomirai Sta. (Minatomirai Line) - Hamagin Hall Via Mare 7 min. on foot

By Train & Shinkansen
Shin Yokohama Sta. (Shinkansen Line) - Shin Yokohama Sta. (JR Yokohama Line) - Higashikanagawa Sta. [Change to JR Keihin Tohoku Line] - Sakuragicho Sta. (JR Keihin Tohoku Line) 15 min.
Sakuragicho Sta. (JR Keihin Tohoku Line) - Hamagin Hall Via Mare 5 min. on foot

Schedule At-A-Glance

Date	Time		Session
Monday July 1	9:00		Registration
	10:00	11:00	**Preconference Educational Seminar (Basic)**
	11:00	12:00	**Preconference Educational Seminar (Clinical)**
	12:15	13:45	COSBID steering committee meeting
	14:00	14:10	Opening
	14:10	15:50	**Session 1:** SD in Migraine
	15:50	16:20	Break
	16:20	16:50	**Keynote 1:** Jun Sakurai, M.D. Ph.D. Investigator-initiated Clinical Trial for Medical Devices and Regulatory Points to Consider
	16:50	18:30	**Open Discussion 1:** Current Controversies, Is SD really harmful?
	18:30	20:30	Welcome Reception
Tuesday July 2	8:30	9:00	Breakfast
	9:00	10:40	**Session 2:** Mechanisms of SD
	10:40	11:10	Break
	11:10	12:50	**Session 3:** SD in Injured Brain
	12:50	14:00	Lunch Break
	14:00	14:40	**Keynote 2:** Akio Ikeda, M.D., Ph.D, FACNS. Active ictal DC shifts and red slow in epilepsy patients : other slows among pathological DC brain potentials
	14:40	16:10	**Session 4:** Clinical Monitoring of SD
	16:10	16:40	Break
	16:40	18:40	**Session 5:** Treatments of SD
	19:30	21:30	iCSD Dinner
Wednesday July 3	8:30	9:00	Breakfast
	9:00	10:40	**Session 6:** Novel Technologies for Monitoring SD)
	10:40	11:10	Break
	11:10	11:50	**Keynote 3:** Shinji Nishimoto, Ph.D. Brain decoding: concepts and potential applications
	11:50	13:00	Lunch Break
	13:00	13:50	**Keynote 4:** Edith Hamel, Ph.D, FRSC. Neurovascular coupling in health and diseases
	13:50	15:50	**Session 7:** Clinical Research on SD
	15:50	16:20	Break
	16:20	17:50	**Open Discussion 2:** SD, Seizures and Epilepsy
	17:50		Closing

Detailed Schedule: July 1 (Mon.)

Monday, July 1, 2019

9:00–16:30 **Registration**

10:00–11:00 **Preconference Educational Seminar (Basic)**
Chair: Michiyasu Suzuki

Minimum knowledge to start basic research on CSD
Cenk Ayata
Massachusetts General Hospital, USA

11:00–12:00 **Preconference Educational Seminar (Clinical)**
Chair: Michiyasu Suzuki

Monitoring of spreading depolarizations on the ICU
Jens P. Dreier
Center for Stroke Research Berlin, Germany

14:00–14:10 **Opening**

14:10–15:50 **Session 1 | SD in Migraine**
Chairs: Cenk Ayata, Fumiaki Oka

Intravascular administration of endothelin-1 does not trigger or increase susceptibility to spreading depolarizations
Kazutaka Sugimoto
Departments of Radiology, Massachusetts General Hospital, Harvard Medical School, USA

Cortical spreading depression and seizures: bidirectional interactions and relevance for migraine
Isra Tamim
Neurovascular Research Laboratory, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA

Responsiveness to potassium-induced cortical spreading depression and subsequent c-Fos expression in a mouse model of familial hemiplegic migraine 2
Chunhua Tang
Department of Neurology, Keio University School of Medicine, Tokyo, Japan

Detailed Schedule: July 1 (Mon.)

Enhanced susceptibility and wide distribution of c-Fos expression to cortical spreading depression in two-types of Na+,K+-ATPase α2 subunit-deficient mice as a model of familial hemiplegic migraine 2

Miyuki Unekawa

Department of Neurology, Keio University School of Medicine, Tokyo, Japan

TRPA1/CGRP signaling mediates cortical spreading depression

Minyan Wang

Centre for Neuroscience, Department of Biological Sciences, Xi'an Jiaotong-Liverpool University, China

15:50–16:20 **Break**

16:20–16:50 **Keynote 1**

Chair: Michiyasu Suzuki

Investigator-initiated Clinical Trial for Medical Devices and Regulatory Points to Consider

Jun Sakurai

Center for Innovative Clinical Medicine, Okayama, Japan

16:50–18:30 **Open Discussion 1 | Is SD really harmful?**

Chairs: Cenk Ayata, David Andrew

18:30–20:30 **Welcome Reception**

Detailed Schedule: July 2 (Tue.)

Tuesday, July 2, 2019

8:30–9:00 **Breakfast**

9:00–10:40 **Session 2 | Mechanisms of SD**

Chairs: Bill Shuttleworth, David Andrew

NMDA receptors are activated in the sustained but not the initial depolarization phase during spreading depolarization

Ning Zhou

Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan

Non-spreading anoxic/ischemic depolarization

Eszter Farkas

Group of Cerebral Blood Flow and Metabolism, Department of Medical Physics and Informatics, University of Szeged, Szeged, Hungary

Non-spreading anoxic/ischemic depolarization is linked to impaired astrocyte function

Ákos Menyhárt

Department of Medical Physics and Informatics, Faculty of Medicine and Faculty of Science and Informatics, University of Szeged; Szeged, Hungary

Precapillary sphincters exist in the brain and are involved in cortical spreading depolarization

Søren Grubb

University of Copenhagen, Copenhagen, Denmark

TEMPORAL DYNAMICS OF ARTERIOLAR DIAMETER AND CAPILLARY PERFUSION DURING CORTICAL SPREADING DEPOLARIZATION AN OPTICAL COHERENCE TOMOGRAPHY STUDY

Maryam Anzabi

Center of Functionally Integrative Neuroscience (CFIN) and MINDLab, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

10:40–11:10 **Break**

Detailed Schedule: July 2 (Tue.)

11:10–12:50 **Session 3 | SD in Injured Brain**

Chairs: Eszter Farkas, Laura Ngwenya

The impact of cortical depolarization on early brain injury after subarachnoid hemorrhage in rats

Satoshi Murai

Department of Neurological Surgery, Okayama University Graduate School, Okayama, Japan

Subarachnoid hemorrhage leads to early and persistent functional connectivity and behavioral changes in mice

David Y. Chung

Neurovascular Research Laboratory, Department of Radiology, Massachusetts General Hospital, Charlestown, MA, USA

Spreading Depolarizations and Mild Traumatic Brain Injuries: Behavior, Cognition, and Attention

Russell A. Morton

Department of Neurosciences, Center for Brain Recovery and Repair University of New Mexico HSC, Albuquerque, NM, USA

Shock wave-induced spreading depolarization and concomitant hemodynamic abnormalities in the rat brain

Shunichi Sato

Division of Bioinformation and Therapeutic Systems, National Defense Medical College Research Institute, Tokorozawa, Saitama, Japan

Effects of Intracranial Hypertension on the Cortical Spreading Depolarization

Takuma Nishimoto

Department of Neurosurgery, Yamaguchi University Graduate School of Medicine, Ube, Yamaguchi, Japan

12:50–14:00 **Lunch Break**

Detailed Schedule: July 2 (Tue.)

14:00–14:40 **Keynote 2**

Chair: Michiyasu Suzuki

Active ictal DC shifts and red slow in epilepsy patients: other slows among pathological DC brain potentials

Akio Ikeda

Department of Epilepsy, Movement Disorders and Physiology, Kyoto University Graduate School of Medicine, Kyoto, Japan

14:40–16:10 **Session 4 | Clinical Monitoring of SD**

Chairs: Michiyasu Suzuki, Johannes Woitzik

Perfusion-dependent impairment of cerebral autoregulation in malignant hemispheric stroke

Nils Hecht

Department of Neurosurgery, Charité – Universitätsmedizin Berlin, Berlin, Germany

Does the shape of a spreading depolarization matter?

Tomas Watanabe

Vagalume LLC, Palo Alto, California, USA

Scalp EEG could record cortical spreading depolarizations (CSDs) even with time constant 2 seconds: A case report of acute traumatic brain injury and hemorrhage

Takahito Tsukamoto

Department of Neurology, Kyoto University Graduate School of Medicine, Kyoto, Japan

Non-invasive detection of spreading depolarization: Initial results

Stephen C. Jones

CerebroScope, Pittsburgh, PA, USA

A reliable method for non-invasive detection of Spreading Depolarisations using Electroencephalography (EEG)

Sharon L. Jewell

Department of Basic and Clinical Neuroscience, King's College London, UK

Feasibility of a wireless, endovascular stent-electrode in the superior sagittal sinus for monitoring SD

Michael Ayad

New York Presbyterian Hospital, Brooklyn, USA

Detailed Schedule: July 2 (Tue.)

16:10–16:40 **Break**

16:40–18:40 **Session 5 | Treatments of SD**

Chairs: Andrew Carlson, Sharon Jewell

Therapies that influence spreading depolarizations

Edgar Santos

Department of Neurosurgery, University Hospital Heidelberg, Ruprecht-Karls-University Heidelberg, Germany

Ketamine reduces excitotoxic consequences of spreading depolarization after stroke

K. M. Reinhart

Department of Neurosciences, University of New Mexico School of Medicine, Albuquerque, NW, USA

A Single Spreading Depolarization can Induce Synaptic Strengthening and BDNF Upregulation

J. E. Weisend

Department of Neurosciences, University of New Mexico School of Medicine, Albuquerque, NM, USA

Trigeminal Nerve Stimulation To Modulate Cortical Spreading Depolarizations After Brain Injury

Chunyan Li

Department of Neurosurgery, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Hempstead/Northwell, Hempstead, NY, USA

The beneficial effect of FP receptor antagonism on spreading depolarization in cerebral ischemia

Dániel P. Varga

Department of Medical Physics and Informatics, University of Szeged, Szeged, Hungary

Surviving neurons in the ischemic core following focal stroke in mice

Rasha H. Mehder

Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Canada

19:30–21:30 **iCSD Dinner**

at Yokohama Royal Park Hotel

Detailed Schedule: July 3 (Wed.)

Wednesday, July 3, 2019

8:30–9:00 **Breakfast**

9:00–10:40 **Session 6 | Novel Technologies for monitoring SD**

Chairs: Stéphane Marinesco, Alan Urban

Whole brain functional ultrasound imaging (fUSi) for detection and tracking of spreading depolarization during stroke in anesthetized rats

Alan Urban

NeuroElectronics Research Flanders, IMEC, VIB, KU Leuven, Leuven, Belgium

Multispectral photoacoustic imaging of hemodynamics during spreading depolarization

Thomas Kirchner

Division of Computer Assisted Medical Interventions, German Cancer Research Center (DKFZ), Heidelberg, Germany

Near-infrared reflectance imaging for monitoring spreading depolarizations and accompanying lesion progression in a rat focal cerebral ischemia model

Satoko Kawauchi

Division of Bioinformation and Therapeutic Systems, National Defense Medical College Research Institute, Tokorozawa, Saitama, Japan

Real time nitric oxide changes during spreading depolarization, ischemia and reperfusion.

Baptiste Balança

Lyon Neuroscience Research Center, Lyon, France

Monitoring Rat Cortical Spreading Depolarizations Using Minimally-invasive microelectrode biosensors Based on Platinized Carbon Fibers

Stéphane Marinesco

Lyon Neuroscience Research Center, Team TIGER Faculty of Medicine, Lyon, France

10:40–11:10 **Break**

Detailed Schedule: July 3 (Wed.)

11:10–11:50 **Keynote 3**

Chair: Michiyasu Suzuki

Brain decoding: concepts and potential applications

Shinji Nishimoto

CiNet, National Institute of Information and Communications Technology, Osaka, Japan

11:50–13:00 **Lunch Break**

13:00–13:50 **Keynote 4**

Chair: Jens P. Dreier

Neurovascular coupling in health and diseases

Edith Hamel

Montreal Neurological Institute, McGill University, Montreal, Canada

13:50–15:50 **Session 7 | Clinical Research on SD**

Chairs: Jed Hartings, Baptiste Balanca

Spreading Depolarizations Are Associated with Worsening Pathologic Anatomy in Computed Tomographic Imaging after Severe Brain Trauma

Laura B. Ngwenya

Department of Neurosurgery, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

The Impact of Physiological Variables on Spreading Depolarization and Neurovascular Coupling after Malignant Hemispheric Stroke

Leonie Schumm

Department of Neurosurgery, Charité Universitätsmedizin Berlin, Germany

Preliminary evidence that clinical manipulations are associated with increased cortical spreading depolarization in patients with acute neurologic injury

Andrew P. Carlson

Department of Neurosurgery, University of New Mexico School of Medicine, Albuquerque, NM, USA

Detailed Schedule: July 3 (Wed.)

Effect of locally delivered nimodipine microparticles on spreading depolarization after aneurysmal subarachnoid hemorrhage.

Andrew P. Carlson

Department of Neurosurgery, University of New Mexico School of Medicine, Albuquerque, NM, USA

Characteristics of ictal DC shifts, another infraslow EEG, recorded by scalp EEG in epilepsy patients: Comparison between scalp- and subdural recording

Shunsuke Kajikawa

Department of Neurology, Kyoto University Graduate School, Japan

Correlates of spreading depolarization, spreading depression and negative ultraslow potential in human epidural versus subdural electrocorticography

Jens P. Dreier

Center for Stroke Research Berlin, Germany

15:50–16:20 **Break**

16:20–17:50 **Open Discussion 2 | SD, Seizures and Epilepsy**

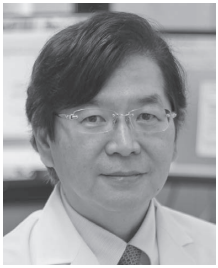
Chairs: Jens P. Dreier, Ákos Menyhárt

17:50– **Closing**

Keynote Speakers

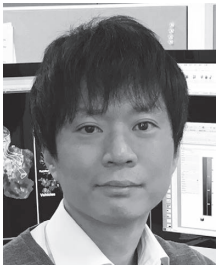
Jun Sakurai, M.D., Ph.D.
Center for Innovative Clinical Medicine, Okayama, Japan

Jun Sakurai, MD, PhD, is a radiologist and now working as Associate Professor in the Center for Innovative Clinical Medicine in Okayama University Hospital from 2015. He worked as a medical device reviewer in Pharmaceuticals and Medical Devices Agency (PMDA) from 2013 to 2015, and reviewed the applications for the new medical device approval especially for the implanted intravascular devices. Then, he promotes several investigator-initiated clinical trials and supports the collaboration between industries and academic researchers to create new medical devices and facilitate open innovation and regulatory application.



Akio Ikeda, M.D., Ph.D, FACNS.
Department of Epilepsy, Movement Disorders and Physiology,
Kyoto University Graduate School of Medicine, Kyoto, Japan

Akio Ikeda, MD,PhD,FACNS, is the president of Japan Epilepsy Society (JES), the Chair of Commission on Asian and Oceanian Affairs (2017-2021) of the ILAE, and the exco-members of Japanese Society of Clinical Neurophysiology (JSCN).
Besides his teaching and clinical roles, he is involved with numerous professional bodies: he is the incumbent Council Member and Chair of the Advanced EEG Seminar of the Japanese Society of Clinical Neurophysiology, and holds various Council and Committee Member positions in the Japanese Neurological Society.
Professor Ikeda has been working on the clinical epilepsy, wide-band EEG (ictal DC shifts) and the role of glia in epileptogenicity, and earned his PhD from Kyoto University in 1993, with his thesis on movement-related potentials recorded from the supplementary and primary motor areas of the brain (Brain,1992). He has published extensively in the literature having authored 300 original articles in English, 290 review articles, 160 book chapters and 7 books in both English and Japanese. He was an Associate Editor of Epilepsia, and Neurology & Clinical Neuroscience, and currently sits on the editorial boards of many journals.



Shinji Nishimoto, Ph.D.
CiNet, National Institute of Information and Communications Technology, Osaka, Japan

Shinji Nishimoto received his Ph.D. in Neurophysiology from Osaka University in 2005. He worked as a Postdoctoral Fellow and an Associate Specialist at Helen Wills Neuroscience Institute at the University of California, Berkeley, from 2005 to 2013. Then, he joined the Center for Information and Neural Networks (CiNet) at the National Institute of Information and Communications Technology (NICT) as a Senior Researcher. He has also been affiliated as a Guest Professor with Osaka University Graduate School of Medicine and Frontier Biosciences. His primary research interest is the quantitative understanding of neural information processing and representations.

Edith Hamel, Ph.D, FRSC.
Professor
Montreal Neurological Institute
McGill University, Montreal, Canada

Edith Hamel is director of the Laboratory of Cerebrovascular Research at the Montreal Neurological Institute at McGill University. She obtained her PhD degree from l'Université de Montréal, and performed post-doctoral training in cerebrovascular pharmacology and physiology (USA and France), and in electron microscopy (Canada). She was President of the International Society of Cerebral Blood Flow and Metabolism (2013-2015). She received several awards including her nomination as a Fellow of the Royal Society of Canada (2017). Her research focuses on the interactions between neurons, astrocytes and blood vessels that assure a proper blood supply to activated brain areas, a phenomenon referred to "neurovascular coupling", in both normal and disease conditions like Alzheimer's disease and vascular cognitive impairment and dementia. She has published 156 original articles.

ABSTRACTS

Intravascular administration of endothelin-1 does not trigger or increase susceptibility to spreading depolarizations

Kazutaka Sugimoto[1,2], Andreia Morais[1], Homa Sadeghian[1], Tao Qin[1], Tsubasa Takizawa[1], David Y. Chung[1,3], Isra Tamim[1], Anders Hougaard[4], Messoud Ashina[4], Cenk Ayata[1,3]

[1]Departments of Radiology, Massachusetts General Hospital, Harvard Medical School, USA
[2]Department of Neurosurgery, Yamaguchi University School of Medicine, Japan
[3]Departments of Neurology, Massachusetts General Hospital, Harvard Medical School, USA
[4]Danish Headache Center, Glostrup Hospital, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Objectives : How spreading depolarizations (SD) are triggered in migraineurs is unknown. Current evidence suggests a vascular trigger. Direct carotid puncture has been reported to trigger migraine with aura in patients. In rats, topical endothelin-1 (ET-1) causes SD. Furthermore, a recent large GWAS found that migraine is significantly associated with a gene variant that results in higher expression of the ET-1 gene and increased binding of ET-1 to ETA receptors on vascular smooth muscle cells. Here, we tested whether intraarterial (carotid) ET-1 infusion can trigger SD, and whether systemic ET-1 infusion increases the susceptibility to SD.

Methods : All experiments were carried out under isoflurane anesthesia. For carotid infusions, we inserted a catheter retrogradely into the external carotid artery in C57BL/6 mice (n=32, ~10week, ~25g). Surgical approach preserved common carotid artery flow with the catheter tip at the bifurcation. Blood pressure was monitored via the femoral artery. After saline infusions at baseline, ET-1 (1.25 nmol/ml) was infused through the carotid catheter at 2 μl/min (n=2), 8 μl/min (n=8), or ramp up (2, 4, 8, 16 μl/min) (n=2). In another cohort, we infused ET-1 at 8 μl/min (n=7) or 16 μl/min (n=1) with heparin in the catheter. SDs were detected using glass microelectrodes placed on both hemispheres and a laser Doppler on the ipsilateral hemisphere. ET-1 dose-rates were chosen based on the literature. We also tested vehicle (PBS with 0.01% acetic acid) infusion (n=7) and studied sham-operated mice (n=5). For systemic infusions, we administrated ET-1 (1 nmol/kg, n=7) or vehicle (n=7) from tail vein in male SD rats (~275g) in a blinded manner. SD susceptibility (electrical SD threshold and KCl induced SD frequency) was evaluated 5 mins after the infusion.

Results : Four out of 19 mice (21%) developed SD during saline infusion. During vehicle infusion, one out of 7 mouse had SD (14%), while ET-1 infusion triggered SD in 6 out of 12 mice (50%, p=0.1733 vs vehicle, Fisher's exact test). SD occurrence even during saline or vehicle infusions raised the possibility of carotid microemboli as the trigger. When we pre-heparinized the carotid catheter, we did not observe any SD either during baseline infusion or after ET-1 infusion (n=8), suggesting that carotid cannulation caused microembolization during the infusions. We next tested the effects of systemic ET-1 elevation and did not find any change in the electrical SD threshold or in the frequency of KCl-induced recurrent SDs.

Conclusions : Intravascular ET-1 does not trigger or increase susceptibility to SD. Microembolization was the likely trigger for migraine auras in patients during carotid puncture.

Cortical spreading depression and seizures: bidirectional interactions and relevance for migraine

Isra Tamim[1,2], David Chung, MD, PhD[1,3], Andreia Lopes de Morais[1] and Cenk Ayata[1,3]

[1]Neurovascular Research Laboratory, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA
[2]Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin Institute of Health, Klinik und Hochschulambulanz für Neurologie und Centrum für Schlaganfallforschung Berlin (CSB), Berlin, Germany
[3]Stroke Service and Neuroscience Intensive Care Unit, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

Introduction: Migraine is a common multifactorial neurovascular disorder often linked to neuronal hyperexcitability. Indeed, migraine and epilepsy are comorbid conditions. Cortical spreading depression (CSD) is the electrophysiological substrate of migraine aura and can trigger migraine headache. We tested this hypothesis.
Methods: Seizure foci were induced in male CD1 mice by topical application of 4-aminopyridine (100mM and 30mM), bicuculline methiodide (5mM) and penicillin G (188434IU/ml) on to parietal cortex. Subdural ECoG recordings at the seizure focus and remote frontal cortex two point were used to capture seizure activity, spread and CSD occurrence. Hyperemia in seizing brain tissue was identified via intrinsic optical imaging (530nm wavelength ; the isosbestic point of haemoglobin) to determine the spatial extent of seizures. Continuous measurement of mean arterial blood pressure was performed via femoral artery catheter to identify changes during status epilepticus and effects after CSDs. In a subset of experiments CSDs were exogenously triggered by topical KCl.
Results: 14 out of 18 animals developed CSDs during seizures generated by 4-AP (2.9 ± 2.3/animal). In 57% of these animals multiple CSDs could be observed (9 maximum) showing first appearance between 8 and 193 min. Out of 40 spontaneous SDs during ictal periods or epileptiform activity 15 CSDs occurring between 8 and 114 min were capable of penetrating the seizure focus showing a time-time-dependent resistance towards CSD. Three out of 8 animals showed spontaneous CSDs spreading from seizure foci generated by Penicillin G ; 5 of the 6 spontaneous SDs were capable of stopping or interrupting epileptiform activity. No CSDs could be observed in animals treated with BMI (n=6). Severity of seizures interpreted by spatial extent, duration, amplitude and power was associated with the occurrence of spontaneous CSDs. Remote CSDs induced at 10 or 30 min after seizure onset (n=6), further suppressed seizures compared to spontaneous CSDs (mean seizure duration 53 ± 14 min vs 170 ± 87 min). Induced SDs were more likely to penetrate the seizure focus and suppress or stop epileptiform activity.
Conclusions: Our data show that epileptic activity can trigger CSDs (i.e. experimental surrogate for migraine aura), and that CSDs in turn can curb seizure activity. We propose that cortical "microseizure" foci may be a trigger for migraine aura, and aura may serve as the brain's endogenous anti-epileptic defense, providing a teleological basis for the evolutionary persistence of CSD.

Responsiveness to potassium-induced cortical spreading depression and subsequent c-Fos expression in a mouse model of familial hemiplegic migraine 2

Chunhua Tang[1], Miyuki Unekawa[1], Mamoru Shibata[1], Yutaka Tomita[1], Yoshikane Izawa[1], Hiroki Sugimoto[2], Keiko Ikeda[2,3], Kiyoshi Kawakami[2], Norihiro Suzuki[4], Jin Nakahara[1]

[1]Department of Neurology, Keio University School of Medicine, Tokyo, Japan
[2]Division of Biology, Center for Molecular Medicine, Jichi Medical School, Tochigi, Japan
[3]Division of physiology, International University of Health and Welfare, Chiba, Japan
[4]Department of Neurology, Shonan Keiiku Hospital, Kanagawa, Japan

Introduction: Familial hemiplegic migraine (FHM) is an autosomal dominant subtype of migraine with aura, and FHM2 is identified by mutations in the *ATP1A2* gene, which encodes the α2 subunit of the Na^+, K^+-ATPase. The E700K mutation in *ATP1A2* exon 15 was found in three migrainous individuals with typical FHM2. Cortical spreading depression (CSD) has been hypothesized to be the underlying mechanism of the migraine aura, and we demonstrated enhanced susceptibility to CSD and difference depending upon the knockout strategy for the gene disruption in two types of *Atp1a2*-deficient mice [1].

Objective : To clarify the pathogenesis of FHM2 by comparing the characteristics of CSD and the expression of c-Fos in the brain after CSD in adult wild-type mice and mice harboring the E700K mutation in *Atp1a2* gene

Methods : CSD was elicited by application of a gradient KCl over the occipital cortex surface in transgenic mice, C57BL/6J-Tg (Atp1a2*E700K) 9151Kwk (Tg, N=30, both males and females) and their wild-type littermates (WT, N=38) under urethane anesthesia and artificially ventilated, and the responsiveness and sensitivity to CSD were examined. 2h after CSD induction, mice were transcardially perfused and brains were cut into 30μm-thick coronal sections for enhanced immunohistochemistry using anti-c-Fos antibody.

Results : In total, Tg mice exhibited faster propagation velocity (Tg, 4.76±0.94 mm/min ; WT, 3.89±0.99 mm/min ; P=0.001) and a longer full-width-at-half-maximum (Tg, 87.1±34.6 s ; WT, 61.5±29.7 s ; P=0.003), reflecting slower recovery from DC deflection, compared to WT mice. The threshold for initiating CSD tended to be lower in the Tg group, especially in female mice. The initial decrease of regional cerebral blood flow (rCBF) elicited by the first CSD seemed to be similar in each group, but the subsequent transient increase of rCBF in the Tg group was slightly but significantly larger than that in WT in the case of males. For the immunohistochemistry, the CSD-induced ipsilateral side showed robust increase of c-Fos positive cells in the somatosensory cortex, the piriform cortex, and the amygdala, and a small increase in the striatum. More c-Fos positive cells were observed in ipsilateral side of amygdala in Tg group.

Conclusion : Although E700K mutant mice showed a similar threshold to WT for KCl-induced CSD, the effect of CSD might be greater. Significant c-Fos expression in ipsilateral amygdala of Tg group may indicate altered neurolimbic system in the Tg mice, implying an enhanced functional connectivity between amygdala and visceroceptive cortex in migraine patients.

[1] Unekawa M, et al. Cephalalgia. 2018 (38) :1515-1524

Enhanced susceptibility and wide distribution of c-Fos expression to cortical spreading depression in two-types of Na^+,K^+-ATPase α2 subunit-deficient mice as a model of familial hemiplegic migraine 2

Miyuki Unekawa[1], Keiko Ikeda[2,3], Yutaka Tomita[1], Yoshikane Izawa[1], Kiyoshi Kawakami[3], Norihiro Suzuki[4], Jin Nakahara[1]

[1]Department of Neurology, Keio University School of Medicine, Tokyo, Japan
[2]Division of Biology, Center for Molecular Medicine, Jichi Medical School, Shimotsuke, Japan
[3]Department of Physiology, International University of Health and Welfare, School of Medicine, Narita, Japan
[4]Department of Neurology, Shonan Keiiku Hospital, Fujisawa, Japan

Introduction: Patients with familial hemiplegic migraine type 2 (FHM2) have a mutated *ATP1A2* gene (encoding Na^+,K^+-ATPase α2 subunit, mainly expressed in astrocytes) and show prolonged migraine aura, which may be profoundly related to cortical spreading depression (CSD).
Objectives: We examined sensitivity and responsiveness to CSD and distribution of c-Fos expression in two types of *Atp1a2*-defective heterozygous mice, *Atp1a2*^{tm1Kwk} (C-KO) and *Atp1a2*^{tm2Kwk} (N-KO), in order to elucidate the mechanisms involved in the pathogenesis of FHM2.
Methods: Mutant and wild-type mice were examined under urethane anesthesia with mechanical ventilation (male, n=65 in total). Threshold to CSD (the minimum concentration of KCl required to elicit CSD), propagation velocity (calculated from the time-lag and distance between the proximal and distal electrodes for DC potential), full width at half maximum (FWHM ; determined from the DC potential curve), the change of electroencephalogram (EEG ; root-mean-square of digital filtered DC potential) were evaluated as an electrophysiological effect. Regional cerebral blood flow (rCBF) was simultaneously recorded by laser-Doppler flowmetry. Next, after 2 hours from 5 to 6 times of CSD induction in left hemisphere, distribution of c-Fos expression was immunohistochemically evaluated
Results: Heterozygotes of N-KO exhibited a low threshold KCl concentration for induction of CSD (0.12±0.04 vs 0.15±0.04 M, $p<0.05$), faster propagation velocity (4.2±1.0 vs 3.4±0.5 mm/min, $p<0.05$), slower recovery from DC deflection (FWHM ; 52.0±14.2 vs 41.0±8.6 s, $p<0.05$), and profound suppression of the EEG (-43.1±14.7 vs -31.9±12.7 %, $p<0.05$), compared to wild-type mice. The difference of every endpoint was slightly greater in N-KO than in C-KO. Change of rCBF in response to CSD showed no significant difference between the heterozygotes and wild-type mice. Expression of c-Fos was widely enhanced in ipsilateral hemisphere, not only in cortex but also in amygdala and piriform cortex. Significant difference was not found between genotypes in both deficient mice.
Conclusion: Heterozygotes of *Atp1a2*-defective mice exhibited high susceptibility to CSD rather than cortical vasoreactivity. The precise effects may differ depending upon the knockout strategy for gene disruption. These results indicated that *Atp1a2*-defective mice simulated FHM2, and suggest that patients with FHM2 may exhibit high susceptibility to migraine.
Reference: Unekawa M. *et al.*, Cephalalgia, 38:1515-24:2018.

TRPA1/CGRP signaling mediates cortical spreading depression

Liwen Jiang, Minyan Wang

Centre for Neuroscience, Department of Biological Sciences, Xi'an Jiaotong-Liverpool University, China

Objectives : Cortical spreading depression is a well-known concept as the underlying cause of migraine aura. The transient receptor potential ankyrin A 1 (TRPA1) is a sensor of oxidative stress and is a nonselective transmembrane cation channel that is located in both peripheral and central nervous system. Peripheral TRPA1 study proposed it as a potential target for drug development against migraine. However, little is known about its role in central mechanism of migraine. This study aimed to understand the role of TRPA1 in cortical spreading depression (CSD) and explore how TRPA1 signaling in in regulating cortical susceptibility to CSD involving calcitonin gene-related peptide (CGRP), the key target of migraine therapy.
Methods : Immunohistochemistry was used for detecting TRPA1 expression. CSD was induced by K^+ on the cerebral cortex, monitored using electrophysiology in rats, and intrinsic optical imaging in mouse brain slices, respectively. Antibodies were perfused into contralateral ventricle of rats.
Results : The results show that TRPA1 was expressed in both cortical neurons and astrocytes of rats and mice. TRPA1 activation facilitated sub-maximal CSD propagation in the mouse brain slice ; consistently, deactivation of TRPA1 by the anti-TRPA1 antibody and two selective TRPA1 antagonists, A967079 and HC-030031, prolonged the CSD latency and reduced magnitude. The suppression of CSD by HC-030031 was reversed by TRPA1 agonist, allyl-isothiocyanate (AITC). Furthermore, TRPA1 deactivation by an anti-TRPA1 antibody also reduced cortical susceptibility to CSD in rats. Interestingly, the inhibitory action of A967079 on CSD was reversed by exogenous CGRP ; whilst blockade of CGRP prolonged CSD latency, which was reversed by the TRPA1 agonist, AITC.
Conclusions : TRPA1/CGRP signaling plays a critical role in regulating cortical susceptibility to CSD. Deactivation of TRPA1 channels may have therapeutic benefits in preventing migraine aura via CGRP.

NMDA receptors are activated in the sustained but not the initial depolarization phase during spreading depolarization

Yu-Ying Mei[1,2], Ting-Chun Cheng[1,2], I-Han Hsiao[3], Dong Chuan Wu[1,2], Ning Zhou[1,2]

[1]Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan
[2]Translational Medicine Research Center, China Medical University Hospital, Taichung, Taiwan
[3]Department of Neurosurgery, China Medical University Hospital, Taichung, Taiwan

Introduction : At the level of individual neurons, the early phase of spreading depolarization (SD) is characterized by a rapid membrane potential depolarization resulting from a sharp opening of large cation conductance. Although NMDA receptors (NMDARs) are critically involved in the ignition, propagation, and maintenance of SD, it remains debated whether NMDAR activation underlies the early phase of the rapid depolarization. Most previous studies used the NMDAR blockers by either *in vivo* systemic administration or by bath application to the brain slice ; however, by this means it was difficult to differentiate between blockade of the ignition, propagation, or depolarization mechanisms of SD. In the present study, we intracellularly delivered MK-801 from the patch pipette into individual neurons without influencing SD propagation. This strategy could help us to answer the question whether NMDAR currents mediate the major depolarizing component at the early phase of SD.
Methods : SD was induced by ejection of a small amount of KCl solution from a glass pipette onto the surface of mouse hippocampal slices. Whole-cell patch clamp recordings were applied to assess neuronal membrane potential changes and whole-cell currents.
Results : By using intracellular MK-801 delivery to block NMDARs in the recorded cells, we found that NMDARs had little functional contribution to the initial neuronal depolarization. Instead, NMDARs are activated during the sustained depolarization phase, and their inhibition suppressed the late-phase amplitude and reduced the duration of SD. Analysis of neuronal input resistance (R_N) revealed a sharp decline at the beginning of SD. Intracellular MK-801 did not prevent the maximum reduction of R_N but facilitated its recovery. Furthermore, bath application of MK-801 produced different effects from the intracellular infusion : it dose-dependently decreased not only the sustained phase but also the initial depolarization.
Conclusion : Our results indicate that although activation of NMDARs plays a minor role the initial depolarization phase of SD, the contribution of NMDARs to the sustained depolarization is a pivotal step in the self-sustaining propagation of the SD wave.

Non-spreading anoxic/ischemic depolarization

Eszter Farkas, Ákos Menyhárt

Group of Cerebral Blood Flow and Metabolism, Department of Medical Physics and Informatics, University of Szeged, Szeged, Hungary

Introduction : Spreading depolarization (SD) and the associated cerebral blood flow (CBF) response display great variations in duration and magnitude, and have been accepted to define a spectrum or continuum. Here we set out to explore specific propagation properties of SD and the associated CBF response in the rodent cortex exposed to hypoxia/ischemia of various severity.
Methods : Spontaneous spreading depolarization occurred in response to forebrain ischemia (iSD), which was initiated by the bilateral occlusion of the common carotid arteries in isoflurane-anesthetized, old (18 months) male Sprague-Dawley rats (n=16). Thirty minutes later, the complete withdrawal of O_2 from the anesthetic gas mixture, maintained for 4-5 min (arterial blood pO_2=35±10 mmHg) gave rise to reversible anoxic/ischemic depolarization (AiD). The depolarization events and the associated CBF response were recorded either with two glass capillary microelectrodes and adjacent laser-Doppler probes positioned over the parietal cortex 3-4 mm apart (n=15), or visualized with multi modal imaging involving a voltage-sensitive dye (RH-1838), and laser speckle contrast analysis (LASCA) (n=1). Mean arterial blood pressure (MABP) was continuously monitored through a femoral artery catheter. MABP and the CBF traces were used for the calculation of the cerebrovascular autoregulatory index (rCBFx).
Results : As expected, iSDs evolved as a propagating wave, characterized by a transient negative DC shift that occurred with a delay at the recording site more distant to the origin of the event, and as a wave of increased RH-1838 fluorescence intensity travelling across the field of view at a rate of 2.31 mm/min. In contrast, AiD, which was reversible on the restoration of oxygen supply, was detected at both electrodes synchronously, and emerged as a rapid increase of RH-1838 fluorescence intensity involving the entire field of view simultaneously. The CBF response to both iSD and AiD was typically a transient drop of CBF (to 10.5±3.0 and 8.4±4.0 %, iSD and AiD, respectively). The CBF response to iSD was coupled by spreading ischemia independent of any variations in MABP (rCBFx remained below 0.3), whereas AiD caused a non-spreading reduction of CBF tightly coupled to the drop of MABP (rCBFx=0.56±0.31).
Discussion : This study has identified – in the context of asphyxia under ongoing ischemia – a novel, non-spreading AiD in the spectrum of mass depolarization events. Non-spreading AiD emerged in the face of dysfunctional cerebrovascular autoregulation and related hypoperfusion, rather than being coupled by spreading ischemia.

Funding: The National Research, Development and Innovation Office of Hungary (nr. PD128821 and K111923) and EFOP-3.6.1-16-2016-00008.

Non-spreading anoxic/ischemic depolarization is linked to impaired astrocyte function

Ákos Menyhárt[1], Rita Frank[1], Viktória Varga[1], Ádám Nyúl-Tóth[2], Anne Meiller[3], Stephane Marincesco[3], István Krizbai[2], Ferenc Bari[1], Eszter Farkas[1]

[1]Department of Medical Physics and Informatics, Faculty of Medicine and Faculty of Science and Informatics, University of Szeged; Szeged, Hungary
[2]Physiology and Pathology of the Blood-Brain Barrier Research Group, Molecular Neurobiology Research Unit, Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences; Szeged, Hungary
[3]Lyon Neuroscience Research Center, University Claude Bernard Lyon I, Lyon, France

Introduction : Cellular ATP depletion is the pivotal event that causes rapid anoxic depolarization (AD) of neurons in cerebral ischemia. Astrocytes, which are less vulnerable to ischemic injury than neurons recycle excess K^+ and glutamate, regulate AD velocity and support the neuronal recovery after AD. We have recently observed AD events in the anoxic/ischemic rat cerebral cortex, which involved the monitored cortical tissue virtually simultaneously (non-spreading anoxic/ischemic depolarization, AiD). We hypothesized that the novel non-spreading nature (or extreme high propagation speed) of AiD could be directly linked to impaired astrocyte K^+ and glutamate clearance.

Methods: Cerebral anoxia/ischemia was created in isoflurane-anesthetized, old, male Sprague-Dawley rats (18 months, n=20) by the short (45 min) withdrawal of O_2 from the anesthetic gas mixture 30 min after the bilateral occlusion of the common carotid arteries (2VO). Local field potential (LFP), extracellular potassium, extra-synaptic glutamate and tissue oxygen saturation were measured using ion sensitive electrophysiology and amperometry. Cleaved caspase-3 (CC3) was co-localized with astrocytes (GFAP) and neurons (NeuN) in the cortex, hippocampus and striatum relying on immunocytochemistry Pharmacological manipulations (NMDA receptor antagonism with MK801, 100 µM ; blockade of astrocyte metabolism with fluorocitrate - FC, 0.5 M ; gap junction inhibition with carbenoxolone - CBX, 200 µM) were conducted on live coronal rat brain slices (350 µm thickness, n=50), exposed to oxygen-glucose deprivation.

Results : Non-spreading AiD was associated with impaired K^+ (231±36 vs. 33±15 s, AiD vs. iSD) and glutamate clearance (846±170 vs. 31±16 s, AiD vs. iSD) when compared to ischemic spreading depolarization (iSD) that occurred spontaneously after 2VO. Approximately 50 % of all GFAP-labeled astrocytes were CC3 positive, whereas only 4-12 % of NeuN-labeled neurons expressed CC3. NMDA receptor antagonism did not reduce the peak of glutamate concentration (95.1±78.7 vs. 71.4±40.7 µM, MK801 vs. control) with AiD in brain slices. Both FC and CBX treatment delayed AiD onset (317±75 or 381±60 vs. 465±121 s, FC or CBX vs. control) and markedly accelerated AiD propagation speed (3.23±0.73 or 2.88±1.12 vs. 1.19±0.43 mm/min, FC or CBX vs. control).

Discussion : On the basis of our data, compromised astrocyte network function is proposed as a pathomechanism of impaired metabolite clearance, and the unique non-spreading nature of the AiD event identified. These data accentuate the key role of astrocyte-linked clearance mechanisms in tissue survival under ischemic conditions.

Funding: The National Research, Development and Innovation Office of Hungary (nr. PD128821 and K111923) and EFOP-3.6.1-16-2016-00008.

Precapillary sphincters exist in the brain and are involved in cortical spreading depolarization

Søren Grubb[1], Changsi Cai[1], Lila Khennouf[1,2], Aske G. K. Jensen[1,3], Reena Murmu[1], Bjørn Hald[1], Stefan Zambach[1] and Martin Lauritzen[1]

[1]University of Copenhagen, Copenhagen, Denmark
[2]University City London, London, England
[3]UC San Diego, California, USA.

Introduction : In the brain, mural cells on the first order branches of penetrating arterioles (PA) regulate blood flow to the capillary bed. We wish to investigate whether precapillary sphincters (PS) exist in the brain. PS are mural cells encircling the initial segment of the PA branch and are known from most textbook examples of microcirculation, but their presence and function in the brain has not been convincingly demonstrated[1]. We provide evidence that they do exist in the mouse brain, and that they have a significant role in the hemodynamic response of cortical spreading depolarization.

Methods : We performed *in vivo* experiments in anaesthetized NG2-dsRed mice, by whisker pad stimulation and consecutive two-photon imaging. The mice were administered FITC-dextran i.v. allowing us to identify and image branch points of PAs in layer 1-3 of the right barrel cortex. We investigated the PS function by hyperstack imaging of whisker pad stimulation, line scanning to measure red blood cell (RBC) velocity through the PS or during cortical spreading depolarization.

Results: We found that PS's do exist in the mouse brain, as NG2-positive mural cells encircling the proximal PA branches. The PS lumen (~4µm) formed a bottleneck because the diameter was narrower the rest of the 1st order capillary (~6µm). Upon whisker stimulation, the PS lumen dilated (26±3%) significantly more than the rest of the 1. order capillary (16±2%) (N=14, LME, Tukey post hoc test). Resonance line scanning revealed that the red blood cells (RBC) pass quickly through the PS but when they enter the 1st order capillary they slow down. During whisker pad stimulation the RBC flux and velocity through the PS increased significantly, but not for the 1st order capillary, indicating that the sphincter works as a safety mechanism ensuring steady state RBC velocity in the capillary bed (N=5, LME, Tukey post hoc test). During the early and late constriction phase of cortical spreading depression, the PS lumen narrowed to a diameter where the RBC flux in the capillary was either slowed or blocked, indicating that the PS is also a bottleneck in cortical spreading depolarization.

Conclusions : Precapillary sphincters do exist in the mouse brain, they are contractile, and they function as the first encounter of vascular resistance the RBC's meet in the capillary bed and can therefore actively regulate blood flow at baseline and during cortical spreading depolarization.

References : 1. Sakai, T. & Hosoyamada, Y. Are the precapillary sphincters and metarterioles 'universal components of the microcirculation? An historical review. *J. Physiol. Sci.* **63**, 319–331 (2013).

TEMPORAL DYNAMICS OF ARTERIOLAR DIAMETER AND CAPILLARY PERFUSION DURING CORTICAL SPREADING DEPOLARIZATION AN OPTICAL COHERENCE TOMOGRAPHY STUDY

Maryam Anzabi[1,2], Baoqiang Li[2], Hui Wang[2], Sreekanth Kura[2], Sava Sakadžić[2], David Boas[2], Leif Østergaaard[1], and Cenk Ayata[3,4]

[1]Center of Functionally Integrative Neuroscience (CFIN) and MINDLab, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[2]Optics Division, MGH/MIT/HMS Athinoula A Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA
[3]Department of Radiology, Neurovascular Research Laboratory, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA
[4]Stroke Service and Neuroscience Intensive Care Unit, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

Introduction : Cortical spreading depolarization (CSD) is considered a key pathophysiological mechanism involved in migraine aura and headache [1-3]. In animal studies of CSD, hypoperfusion and profound capillary flow disturbances accompany poor tissue oxygenation [4-6]. In the injured brain, spontaneous and recurrent CSDs seemingly emerges as a result of tissue hypoxia [7], and the phenomenon has been linked to the progression of tissue injury in animal studies of subarachnoid hemorrhage [8], traumatic brain injury [9], and cortical infarction [10]. In the current study, we examined whether arteriolar constriction or maldistribution of capillary flow is the primary hemodynamic event in relation to CSD and whether the degree of capillary flow disturbances affects CSD-related changes in upstream arteriolar diameter.

Methods : Two consecutive CSDs were induced by focal application of KCl in mice. High-resolution optical coherence tomography was employed to investigate the temporal characteristics of the vascular events following CSDs. Thereafter, arteriolar diameter changes in response to the CSD waves and vessel density at the capillary level were estimated in the pre-defined regions of interest.

Results : We observed that the initial drop in the number of erythrocyte-filled capillaries, as well as the subsequent recovery of capillary filling, started *prior* to the changes in upstream vessel diameter. Furthermore, we observed severe, long-term capillary flow disturbances in relation to CSD, where flow disturbances persisted, remaining at the time of the second CSD. The temporal profile of the arteriolar diameter changes differed between the first and second CSD passage.

Discussion : Our results show that poorly perfused capillaries emerge several seconds before any changes in arteriolar diameter, confirming the notion that primary regulation of cerebral blood flow (CBF) is initiated at the capillary level [11], possibly to adjust CBF according to the lower oxygen extraction efficacy permitted by heterogeneous capillary flow pattern [6]. Our serial arteriolar diameter measurements corroborate earlier observations of differential CBF-responses to consecutive CSDs in mice [5]. We have hypothesized that this phenomenon may owe to residual capillary flow disturbances, and thereby reduced oxygen extraction efficacy, at the time of the second CSD [6]. Although our experiments did not allow us to monitor tissue metabolism during and after the two consecutive CSDs, our data are consistent with the notion that capillary flow disturbances modulate flow-metabolism coupling, and thus the arteriolar diameter response to the second CSD.

References:
1. Chen, S.P. and C. Ayata, *Spreading Depression in Primary and Secondary Headache Disorders.* Curr Pain Headache Rep, 2016. **20**(7): p. 44.
2. Charles, A.C. and S.M. Baca, *Cortical spreading depression and migraine.* Nat Rev Neurol, 2013. **9**(11): p. 637-44.
3. Hadjikhani, N., et al., *Mechanisms of migraine aura revealed by functional MRI in human visual cortex.* Proc Natl Acad Sci U S A, 2001. **98**(8): p. 4687-92.
4. Yuzawa, I., et al., *Cortical spreading depression impairs oxygen delivery and metabolism in mice.* J Cereb Blood Flow Metab, 2012. **32**(2): p. 376-86.
5. Ostergaard, L., et al., *Neurovascular coupling during cortical spreading depolarization and -depression.* Stroke, 2015. **46**(5): p. 1392-401.
6. Dreier, J.P., et al., *Products of hemolysis in the subarachnoid space inducing spreading ischemia in the cortex and focal necrosis in rats: a model for delayed ischemic neurological deficits after subarachnoid hemorrhage?* J Neurosurg, 2000. **93**(4): p. 658-66.
7. von Bornstadt, D., et al., *Supply-demand mismatch transients in susceptible peri-infarct hot zones explain the origins of spreading injury depolarizations.* Neuron, 2015. **85** (5): p. 1117-31.
8. Dreier, J.P., et al., *Nitric oxide scavenging by hemoglobin or nitric oxide synthase inhibition by N-nitro-L-arginine induces cortical spreading ischemia when K+ is increased in the subarachnoid space.* J Cereb Blood Flow Metab, 1998. **18**(9): p. 978-90.
9. Nilsson, P., et al., *Regional changes in interstitial K+ and Ca2+ levels following cortical compression contusion trauma in rats.* J Cereb Blood Flow Metab, 1993. **13**(2): p. 183-92.
10. Nedergaard, M. and J. Astrup, *Infarct rim: effect of hyperglycemia on direct current potential and [14C]2-deoxyglucose phosphorylation.* J Cereb Blood Flow Metab, 1986. **6** (5): p. 607-15.
11. Hall, C.N., et al., *Capillary pericytes regulate cerebral blood flow in health and disease.* Nature, 2014. **508**(7494): p. 55-60.

The impact of cortical depolarization on early brain injury after subarachnoid hemorrhage in rats

Satoshi Murai[1], Tomohito Hishikawa[1], Masafumi Hiramatsu[1], Kenji Sugiu[1], Yoshimasa Takeda[2], Hiroshi Morimatsu[2], Isao Date[1]

[1]Department of Neurological Surgery, Okayama University Graduate School
[2]Department of Anesthesiology and Resuscitology, Okayama University Graduate School

Early brain injury (EBI) of the acute phase of subarachnoid hemorrhage (SAH) aggravates the neurological outcome. Cortical spreading depolarization (CSD) has received attention as one of the important factors of EBI. First, we tried to visualize the dynamics of CSD, and evaluate the effects of cortical depolarization on neuronal damage in EBI using in the perforation model. We successfully visualized CSD using NADH fluorescence, and showed that the duration of depolarization associated with the severity of neuronal damage (Shimizu T. J Neurosurg 128 ; 137-143, 2018). Second, we evaluated of the impact of CSD on electroencephalogram in the single injection model of SAH. We found that the duration of suppression of electroencephalogram correlated with neuronal damage. Non-invasive monitoring of EEG can be useful to predict neurological outcome after SAH. Third, we evaluated the impact of cortical depolarization and extracellular glutamate level on EBI after SAH in the single injection model. In this study, we showed dynamic changes of physiological parameters in the acute phase of SAH. The duration of cortical depolarization, cerebral blood flow and the peak concentration of extracellular glutamate level significantly correlated with neurological outcome. Investigation of mechanisms of EBI will contribute to development of a novel therapy. Therapeutic hypothermia is a potential treatment targeting EBI, which can improve cerebral perfusion and metabolism, and reduces release of glutamate after SAH.

Subarachnoid hemorrhage leads to early and persistent functional connectivity and behavioral changes in mice

David Y. Chung[1,2], Gina Jin[3], Andrea Harriott[1,4], William J Edmiston[3], Sreekanth Kura[5], Fumiaki Oka[1,6], Sanem Aykan[1], Tao Qin[1], Hang Lee[7], Mohammad A Yaseen[8], Sava Sakadžić[8], David A Boas[5], Michael J Whalen[3], Cenk Ayata[1,4]

[1]Neurovascular Research Laboratory, Department of Radiology, Massachusetts General Hospital, Charlestown, MA, USA
[2]Division of Neurocritical Care, Department of Neurology, Massachusetts General Hospital, Boston, MA, USA
[3]Department of Pediatrics, Massachusetts General Hospital, Boston, MA, USA
[4]Stroke Service, Department of Neurology, Massachusetts General Hospital, Boston, MA, USA
[5]Neurophotonics Center, Department of Biomedical Engineering, Boston University, Boston, MA, USA
[6]Department of Neurosurgery, Yamaguchi University School of Medicine, Ube, Japan
[7]Department of Medicine, Massachusetts General Hospital, Boston, MA, USA
[8]Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Charlestown, MA, USA

Introduction : Subarachnoid hemorrhage (SAH) is a severe form of stroke resulting in significant mortality and long-term cognitive deficits. Recent evidence in humans using functional neuroimaging suggests that cognitive deficits following SAH are associated with alterations in resting state functional connectivity (RSFC). However, common functional connectivity measures determined from modalities such as fMRI are challenging in small rodents. Therefore, we used a novel, non-invasive optical approach to determine RSFC in the mouse.

Methods : We used functional hemodynamic optical intrinsic signal imaging to determine the effect of SAH on measures of RSFC in mice at early (day 4-5), intermediate (1 month), and late (3 months) time points. We also assessed longitudinal behavioral outcomes after SAH on the Morris water maze (MWM), open field test (OFT), Y-maze, and rotarod from approximately 2 weeks to 3 months from SAH induction.

Results : We found early qualitative differences in seed-based RSFC maps between sham and SAH mice. SAH also led to a quantifiable decrement in seed-based connectivity indices, which persisted in the posterior network at 3 months. Seed-to-seed connection matrices demonstrated an overall attenuation of correlation coefficients in SAH compared to sham mice, with persistence in predominantly posterior correlations at later time points. We also found decreased correlations between mirror pixels in contralateral hemisphere following SAH. When looking at the underlying hemodynamic fluctuations on which the correlation analysis is based, we found decreased globally regressed signal in post-SAH mice at only the early time point. Behavioral testing revealed deficits on early MWM hidden platform testing, early open field testing, and late Y-maze assessment for SAH mice. There was no difference detected between groups on rotarod testing.

Discussion : SAH leads to early and persistent alterations in hemodynamically-derived RSFC which are associated with long-term spatial memory deficits.

Spreading Depolarizations and Mild Traumatic Brain Injuries: Behavior, Cognition, and Attention

Johann. M. Pacheco, Claire Stratton, Ashlyn Hines-Lanham, Haikun Zhang, and Russell A. Morton

Department of Neurosciences, Center for Brain Recovery and Repair University of New Mexico HSC, Albuquerque, NM, USA

Introduction : It has been estimated that there are 3.8 million sport related concussions per year, in the United States. Dizziness, headaches, balance abnormalities, and/or difficulties concentrating are common short-term symptoms lasting a few days after a concussion. Most of these symptoms spontaneously resolve within a few days. However, 20 – 30% of patients suffering from these symptoms do not recover quickly and symptoms can last for months or even years. Proper diagnosis and treatment of concussions/mTBIs are severely lacking because we do not fully understand the cellular and molecular effects that occur immediately following a blow to the head. Our laboratory as well as others has shown that spreading depolarizations (SDs) are initiated by mTBI-like impacts (Bouley et al., 2018).
Methods : To model a mTBI-like injury, we used a closed skull electromagnetic impactor model in mice without head restriction. SDs were detected using silver ball electrodes placed on the brain surface in awake and behaving animals. Animal's behavior was monitored with a video tracking system for the first few hours following the impact. We utilized a touchscreen based operant task prior to, the day of an impact, and subsequent days to test for cognitive and attention deficits following a mTBI-like impact.
Results : Our preliminary data indicate that the initial state of immobility displayed by impacted animals is associated with a SD and recovery of movement coincides with the recovery of high frequency activity. Studies are currently underway using a touchscreen based 5-choice serial reaction time tasks paired with a go/no go paradigm testing cognitive and attention deficits 4 hours post-impact and subsequent days.
Discussion : Overall, our data suggests that the short-term behavioral, cognitive, and attention deficits are associated with mTBI induced SDs. Our data provide a critical link between SDs and the short-term behavioral, cognitive, and attention deficits associated with mTBIs, and indicate that SDs may be an underlying mechanism of mTBIs.

Support: Center for Brain Recovery and Repair (P20 GM109089-01)

Shock wave-induced spreading depolarization and concomitant hemodynamic abnormalities in the rat brain

Shunichi Sato[1], Masaki Inaba[2], Takuya Osawa[2], Takeshi Adachi[3], Izumi Nishidate[2], Satoko Kawauchi[1]

[1]Division of Bioinformation and Therapeutic Systems, National Defense Medical College Research Institute, Japan
[2]Graduate School of Bio-Application and Systems Engineering, Tokyo University of Agriculture and Technology, Japan
[3]Department of Internal Medicine, National Defense Medical College, Japan

Introduction : Due to frequent terrorist attacks using improvised explosive devices (IEDs) not only in regions of conflict but also in normal cities, the number of patients suffering from blast-induced traumatic brain injury (bTBI) has been growing worldwide. Most of the patients have been diagnosed as having mild bTBI by conventional imaging diagnosis (mainly CT), but many of them show higher brain dysfunction in the chronic phase, indicating that they are not actually mild. However, the mechanisms of such bTBI are unclear. To reveal the mechanisms, it would be important to observe what happens in the brain when exposed a shock wave. In our previous study, we performed real-time diagnosis of the rat brain exposed to a laser-induced shock wave (LISW) on the basis of diffuse reflectance spectroscopy and observed the occurrence of cortical spreading depolarization (CSD), which was followed by long-lasting oligemia/hypoxemia[1,2]. As described below, we hypothesized that nitric oxide (NO) is involved in the abnormal hemodynamic changes. In this study, we investigated this hypothesis by using an inhibitor of NO synthesis (NOS) for the same model.

Hypothesis: A shock wave causes calcium ion surge in the endothelial cells, by which eNOS is stimulated, producing NO. Calcium ion is also associated with production of super-oxide anion (O_2^-) in mitochondria. NO and O_2^- are efficiently combined to create peroxynitrite ($ONOO^-$). $ONOO^-$ is highly cytotoxic, causing vascular dysfunction. This process can be accelerated by the onset of CSD through stimulation of nNOS in neurons.

Experiments : Rats were divided into two groups : rats with application of an NOS inhibitor, L-NAME before LISW application and those without L-NAME application. A low-impulse LISW was topically applied to the rat brain through the skull, for which optical fiber-based diffuse reflectance spectroscopy was performed. We observed occurrence of CSD in the both groups of rats, but the levels of oligemia and hypoxemia were significantly reduced for the rats with L-NAME application, indicating that hemodynamic abnormalities were suppressed by NOS inhibition and hence supporting our hypothesis.

References:
1. Sato S et al., PLoS ONE 9(1) : e82891 (2014).
2. Kawauchi S et al., J Biomed Opt. 24(3) :1-17 (2019).

Effects of Intracranial Hypertension on the Cortical Spreading Depolarization

Takuma Nishimoto, Fumiaki Oka, Takao Inoue, Michiyasu Suzuki

Department of Neurosurgery, Yamaguchi University Graduate School of Medicine, Japan

Introduction : Spreading depolarization (SD) is a depolarization of the wave of neuron and glial cells that slowly propagate in the gray matter. SD develops after stroke and subarachnoid hemorrhage and traumatic brain injury. These diseases often accompanied by intracranial hypertension. Although cerebral perfusion pressure (CPP) has been reported as a critical factor of SD duration, the impact of intracranial pressure (ICP) which is one of the determinants of CPP, has not been studied yet. Here, we studied the effect of increase in ICP on recovery rate of SD.

Methods : ICP was monitored and manipulated by an artificial cerebrospinal fluid reservoir connected to the cisterna magna in anesthetized rats. CPP was altered by manipulation of ICP and/or arterial blood pressure. SD was triggered by application of 1M KCl on the brain surface and the electrocorticogram and direct current potential were recorded to detect SD. Cerebral blood flow was monitored by using laser Doppler flowmetry. In the first set of experiment, CPP was maintained at 70 mmHg with different ICP (10, 30 and 50 mmHg). In order to maintain CPP, mean arterial pressure (MAP) was also manipulated to 80, 100 and 120 mmHg, respectively, by intravenous administration of dopamine. In the second set, MAP was maintained at 80mmHg while ICP was increased from 10 to 30 and 50 mmHg and as the result CPP was decreased from 70mmHg to 50 and 30mmHg, respectively.

Results : Under the same CPP at 70 mmHg, increase in ICP did not significantly influence the number, duration, amplitude or spreading speed of the SD. In contrast, decrease in CPP by elevating ICP significantly prolonged SD duration (from 17.6 ± 2.4 seconds to 44.2 ± 10.2 seconds, $P < 0.01$). CBF responses to the SD were almost the same among the maintained CPP animals at 70mmHg even though ICP was increased up to 50mmHg, however, decrease in CPP by elevating ICP augmented the initial hypoperfusion and greatly diminished the subsequent hyperemic response.

Discussion : As reported previously, CPP was the determinant factor of the duration of SD even in the model of intracranial hypertension. And within the normal range of CPP, elevation of ICP itself seems not to affect SD duration nor CBF response to the SD.

Perfusion-dependent impairment of cerebral autoregulation in malignant hemispheric stroke

Nils Hecht[1,3], Max Schrammel[1,3], Jens Dreier[2,3], Peter Vajkoczy[1,3], Johannes Woitzik[2,3]

[1]Department of Neurosurgery, Charité – Universitätsmedizin Berlin, Berlin, Germany
[2]Department of Neurology, Charité – Universitätsmedizin Berlin, Berlin, Germany
[3]Center for Stroke Research Berlin (CSB), Charité – Universitätsmedizin Berlin, Berlin, Germany

Introduction: Regional loss of cerebral autoregulation (CA) has been shown to play a key role in secondary brain damage attributable to edema formation and infarct progression in malignant hemispheric stroke (MHS). However, it remains unclear to what extent CA impairment depends on the regional perfusion level within the affected hemisphere. Therefore, in the present study we performed a spatial-temporal assessment of CA in patients undergoing decompressive hemicraniectomy (DC) for treatment of MHS.
Methods: In 24 patients undergoing DC, autoregulation over the affected hemisphere was intraoperatively assessed by continuous cortical perfusion mapping by Laser Speckle Imaging (LSI) and mean arterial blood pressure (MAP) monitoring. Regions of interest (ROIs) were positioned within the color-coded LSI perfusion map and LSI-specific cortical perfusion was calculated within the infarcted and non-infarcted cortex. Cortical perfusion was normalized to 100% and six levels of cortical perfusion were defined (0-20%, 20-40%, 40-60%, 60-80%, 80-100% and >100%). Means, standard deviations and confidence intervals (CI) of the cortical perfusion level, MAP and the interaction of both variables during a 20-minute monitoring period were estimated with a linear random slope model and Pearson correlation analysis.
Results: For each perfusion level, n=578 ROIs were analyzed across all hemispheres. Pearson correlation analysis showed significantly less autoregulation impairment in non-infarcted tissue (>100%: r=0.36) than in tissue with critically reduced cortical perfusion (20-40%: r=0.67; 40-60%: r=0.68; 60-80%: r=0.68; *p<0.05). Further, linear cortical perfusion changes per mmHg MAP were significantly greater in areas with critically reduced perfusion (40-60%: 0.42% per mmHg and 60-80%: 0.46% per mmHg) compared to non-infarcted (>100%: 0.22% per mmHg) or infarcted (0-20%: 0.29% per mmHg) areas (*p<0.001).
Conclusion: Spatial identification of regions with penumbral perfusion patterns may be critical for effectively targeting CA as a strategy to prevent secondary neurologic injury in patients suffering MHS.

Does the shape of a spreading depolarization matter?

Tomas Watanabe[1] and Jed A. Hartings[2-4]

[1]Vagalume LLC, Palo Alto, California, USA
[2]Department of Neurosurgery, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA
[3]Collaborative for Research on Acute Neurologic Injury (CRANI), University of Cincinnati (UC) College of Medicine, Cincinnati, Ohio, USA
[4]UC Gardner Neuroscience Institute, Cincinnati, Ohio, USA

Introduction: The negative DC shift (~2-3 min) of spreading depolarizations (SDs) in electrocorticographic (ECoG) recordings is thought to reflect the period of neuronal depolarization, and its duration is reported to correlate with the duration of spontaneous activity depression. Here, we examine the impact of DC shift morphology on these interpretations.
Methods: Analyses were based on 2,921 SDs identified in 91.5 days of 6-channel DC-coupled ECoG in 19 of 25 TBI patients. After detrending the ECoG, the main negative DC shift, the following positivity, and spontaneous activity power depressions of SDs were measured with semi-automated methods. Time-to- and time-from- minimum voltage were used as proxies for the depolarization and recovery slopes of the DC shift. Median comparisons were done via Wilcoxon rank sum tests, and interactions tested via ANOVA.
Results: A large proportion (67%; n=1960) of SDs had steeper recovery than depolarization slopes and were considered paradoxical, in contrast to typical SDs with steeper depolarizations (n=940). Paradoxical SDs had shorter depression durations (median 828 s, $p<0.001$, effect: -83, -158, -255 s versus Q1, Q2, and Q3 of the typical group) and larger amplitudes of the late positivity (median 1073 uV, $p < 0.001$, effect: +131, +263, and +568 uV). The same result was found within individual subjects, in 11 of 16 cases. DC shift durations were longer for paradoxical SDs (162 s, $p<0.001$, effect: +14, +21, +28 s), but this variable was multimodally distributed, making interpretation unclear. Differences in mean depression duration were observed in a 3-way comparison (subject x DC duration x SD type) with subject as main effect ($F=2.79$, $p<0.001$), and subject-by-DC duration ($F=2.1$, $p<0.005$) and subject-by-SD type ($F=2.27$, $p<0.002$) as significant interactions. Non-ordinal interactions between factors were seen, which warns against interpreting them independently.
Conclusion: The *duration* of the DC shift and its *shape* influence the duration of depression. The presence of strong intra-individual interactions precludes establishing direct correlations between ECoG features and biological processes. This is a major motivation to develop models that explain the heterogeneity seen in SDs.

Scalp EEG could record cortical spreading depolarizations (CSDs) even with time constant 2 seconds : A case report of acute traumatic brain injury and hemorrhage.

Takahito Tsukamoto[1,3], Shunsuke Kajikawa[1], Masayuki Honda[4], Takefumi Hitomi[4], Akihiro Shimotake[5], Takeshi Funaki[2], Kazumichi Yoshida[2], Makoto Urushitani[3], Ryosuke Takahashi[1], Akio Ikeda[5]

[1]Department of Neurology, Kyoto University Graduate School of Medicine, Kyoto, Japan
[2]Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto, Japan
[3]Department of Neurology, Shiga University of Medical Science, Otsu, Shiga, Japan
[4]Department of Clinical Laboratory Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan
[5]Department of Epilepsy, Movement Disorders and Physiology, Kyoto University Graduate School of Medicine, Kyoto, Japan

introduction : Previous intracranial electroencephalography (iEEG) study mentioned that CSDs can be a useful prognostic biomarker for acute brain insult such as trauma, ischemia and hemorrhage (Hartings et al, 2011). However, CSDs were seldom reported by scalp EEG at least because of many technical problems. Epileptic ictal DC shifts, i.e., another infraslow EEG similar to CSD from generator mechanism point of view, were reported by scalp EEG (Ikeda et al, 1999). We herein reported that CSDs were detected by scalp EEG with in a patient with traumatic brain injury.

Methods : The patient was an 82 year-old female who developed acute consciousness disturbance (JCS : 3) at 6 days after head trauma by falling. Head CT showed left acute subdural hematoma and right subcutaneous hematoma, and the patient underwent emergency hematoma evacuation and decompression. Her consciousness state worsen (JCS : 100-200) even after surgery. To investigate the presence of focal epileptic status, routine scalp EEG (time constant (TC) 2 sec) was examined 2 days after surgery.

Results: Scalp EEG demonstrated by TC of 2 sec showed continuous large negative infraslow (cycle of 40 to 65 sec and amplitude of 550 to 840 μV) at in the right posterior quadrant, and it was superimposed by continuous delta slow in the same region. The former EEG finding was judged as CSDs, and infrequent spike was also seen in the right hemisphere. Clinically consciousness impairment remained (JCS : 10) 1 month after surgery in spite of treatment by anti-epileptic drugs.

Discussion : In this patient, scalp EEG could detect CDSs and excluded NCSE for its diagnosis. CSD by means of scalp EEG has been little reported ; one study detected CSDs by continuous scalp EEG monitoring in patients with traumatic brain injury who underwent craniotomy (with full-band DC amplifier) (Hartings et al,2014), and not in patients without craniotomy (with full-band DC amplifier). (Hofmeijer et al, 2018). Epileptic ictal DC shifts, another infraslow was reported by means of TC of 10sec (Ikeda et al., 1996, 1999 : Kanazawa et al,2016), but recently EEG with TC of 2 sec could demonstrate at least partly ictal DC shifts (Kajikawa et al,2018).

Conclusion : In a patient of acute head injury and hemorrhage, CSDs was recorded on the scalp EEG with TC of 2sec, and it may explain the cause of unconsciousness state, especially to differentiate from focal status.

Non-invasive detection of spreading depolarization: Initial results

Stephen C. Jones, Samuel J. Hund, Eric Singer, and Benjamin R. Brown

CerebroScope, Pittsburgh, PA, USA

Introduction : Non-invasive detection of spreading depolarization (SD) has been a goal since SDs were first detected from the human brain surface in 2002 (Strong et al., Stroke). An attempt to provide non-invasive detection (Hartings et al., Ann Neurol, 2014) has been challenged (Hofmeijer et al., Front Neurol, 2018). Here, we present the initial results of our approach based on numerical simulations of the propagation of the known brain-surface SD potentials to the scalp. Our hypothesis is that DC-potentials recorded by closely-spaced scalpplaced electrodes are associated with ECoG-detected SDs and will provide non-invasive SD detection.

Methods : A 75 x 58 mm thermoplastic elastomer array with 29 embedded 6-mm diameter Ag/AgCl electrodes, the CerebroPatch, was adhesively placed on the forehead with an intervening electrode gel interface to record 2 hours to 12 days of full-band EEG in 5 normal subjects and 9 Neuro-Intensive Care Unit patients as approved by the human studies committee. Initial data analysis targeted 17 one-hour epochs from one patient with sub-arachnoid hemorrhage with the CerebroPatch positioned over an ECoG strip previously placed during a craniotomy. The signals from the CerebroPatch and the ECoG strip were collected by a Moberg CNS Advanced ICU Amplifier and processed with lab-designed algorithms to produce timecourse channel plots and heat-map voltage movies of CerebroPatch-recorded voltages. The movies were reviewed and the times and CerebroPatch electrode positions of suspected SDs were recorded. Finally, the presence of ECoG-detected SDs at the same times as the CerebroPatch-identified SDs was checked.

Results : Three out of 17 movies were excluded due to artifacts. Eight suspected SDs were identified in the remaining 14 CerebroPatch movies and 7 showed corresponding DC-shifts in the ECoG recordings. Of these 7, all were stationary isoelectric SDs. In this limited sample, we identified SDs in 50% of the good CerebroPatch movies. The wide spatial spread (~2 cm) in the CerebroPatch channels is consistent with our numerical simulation.

Discussion : These results suggest that non-invasive SD detection might be possible using skin DC-potential signals. The correspondence of DC-shifts from the scalp and brain-surface recordings is encouraging. The detection of only isoelectric SDs suggests that the 0.29 fractional incidence of isoelectric SDs in this patient increased their probability of detection relative to propagating CSDs or our image analysis procedures are optimal for isoelectric SDs.

Funding : USPHS NINDS NS092181

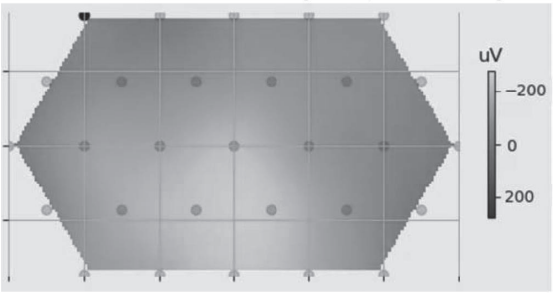


Figure: A DC-shift with a peak voltage of -220 µV recorded from the scalp that corresponds to an underlying ECoG detected SD.

A reliable method for non-invasive detection of Spreading Depolarisations using Electroencephalography (EEG)

Sharon L. Jewell[1,2], Jose-Pedro Lavrador[3], Clemens Pahl[4], Marytn G Boutelle[2] & Anthony J Strong[1]

[1]Department of Basic and Clinical Neuroscience, King's College London, UK
[2]Department of Bioengineering, Imperial College London, UK
[3]Department of Neurosurgery, King's College Hospital, London, UK
[4]Department of Anaesthesiology, King's College Hospital, London, UK

Background : In humans, SDs are appreciated on ECoG as a large, creeping, slow potential change (SPC) and concurrent depression of baseline activity. ECoG electrodes however, must be placed at the brain surface and are therefore restricted to patients requiring operation. To study SDs in patients not requiring surgery, non-invasive methods must be progressed. Three studies have previously explored EEG[1-3] by applying electrodes according to the 10-20 system. This standardised application system covers major brain regions and allows comparison between subjects. It takes little account however, of individual variations in the sites at which cortical generators evolve and propagate. This may account for the lack of propagating SPCs and negative results in 1 study. Placement of electrodes according to an individual's injury foci may facilitate more robust detection.
Objective : To test if EEG electrodes placed according to an individual's injury foci facilitates reliable detection of SD.
Methods : In 7 patients, an ECoG electrode (ADtech) was placed over tissue at risk. Ag/AgCl EEG electrodes were applied according to imaging & intraoperative findings. Signals were acquired in common reference at 512Hz-2kHz, DC-Nyquist, using CNS (Moberg) & Pegasus (Neuralynx). ECoG & EEG were recorded concurrently. Manual analysis was undertaken.
Results : 144 SDs were seen on ECoG in 4/7 subjects. In 2/4, in whom craniectomy was performed, 96/146 SDs were observed. Artifact excluded 2. Of the 93 remaining, 100% were identified on EEG. 90/93 displayed isolated, stereotyped SPCs of 0.3-1.8mV and depression of 8-30mins in 3-12 electrodes. Clear propagation was seen between at least 2 electrodes in 80/90. In 3/93, SD intervals were <30min and EEG SPCs merged, giving rise to SPC undulation and widespread 0.5-45Hz attenuation. In 1 patient in whom craniotomy was performed, 26 SDs were seen on ECoG. Artefact excluded 1. Of the 25 remaining, 24 (96%) were identified on EEG. 19/25 displayed isolated, simultaneous, SPCs of 0.15-0.5mV in 1-6 electrodes. Diffuse SPC undulation was observed for 4 SDs in a cluster. The remaining craniotomy patient developed a large subgaleal hygroma, bridging the electrode potentials. Nevertheless, even in this subject a widespread continuous depression down to 60% occurred during clustered SDs.
Conclusions : Our results support and add to those of[1,2] by demonstrating for the first time, propagating EEG SPCs. Our results substantiate EEG as a method to identify SDs non-invasively, but suggest that reliable detection may hinge on an individualised approach to electrode application. Future studies evaluating EEG will need to account for fluid collections and negative results should be treated with caution in this group.

1. Hartings JA, Wilson JA, Hinzman JM, Pollandt S, Dreier JP, DiNapoli V, Ficker DM, Shutter LA, Andaluz N. Spreading depression in continuous electroencephalography of brain trauma. Ann Neurol 2014;76(5):681-94.
2. Drenckhahn C, Winkler MK, Major S, Scheel M, Kang EJ, Pinczolits A, Grozea C, Hartings JA, Woitzik J, Dreier JP and others. Correlates of spreading depolarization in human scalp electroencephalography. Brain 2012;135(Pt 3):853-68.
3. Hofmeijer J, van Kaam CR, van de Werff B, Vermeer SE, Tjepkema-Cloostermans MC, van Putten MJAM. Detecting Cortical Spreading Depolarization with Full Band Scalp Electroencephalography: An Illusion? Front Neurol 2018;9:17.

Feasibility of a wireless, endovascular stent-electrode in the superior sagittal sinus for monitoring SD

Michael Ayad

New York Presbyterian Hospital, Brooklyn, USA

Introduction : Recording of spreading depolarization is difficult from scalp EEG leads, and generally requires placement of subdural strip or depth electrodes for continuous ECoG/ DC potential monitoring with high fidelity. However, implantation of these electrodes is not always ideal for certain patients and the availability of other techniques would be desirable.
Background : Currently, a stent incorporating electrodes equipped for wireless transmission of locally recorded potentials is available ; this was designed for endovascular placement in coronary arteries to monitor electrical signals from the heart. Similarly, a stent-electrode has been developed for use as a neural interface in paralyzed stroke patients, but requires transjugular placement with externalization of the leads.

Proposed is the development of a wireless stent-electrode placed percutaneously via transfemoral venous approach and deployed in the superior sagittal sinus of patients for monitoring of SD. Several considerations warrant discussion. Some patients exhibiting SD in the acute setting may have contraindications to antiplatelet therapy, which might be needed to prevent venous thrombosis. Antithrombotic stent coatings (e.g. Polyzene-F®) are currently being investigated to mitigate the need for antiplatelet therapy. While the medial location of such electrodes would be expected to capture the majority of SDs, it is unclear to what extent lateralized SD events might be missed. Other issues to be discussed include endothelialization, MRI-compatibility and potential device complications.

Therapies that influence spreading depolarizations

Edgar Santos[1], Arturo Olivares-Rivera[1], Sebastian Major[2,3,4], Renán Sánchez-Porras[1], Lorenz Uhlmann[5], Kevin Kunzmann[5], Roland Zerelles[1], Modar Kentar[1], Vasilis Kola[2], Adrian Hernandez Aguilera[1], Mildred Gutierrez Herrera[1], Johannes Woitzik[6], Jed A. Hartings[7,8], Oliver W. Sakowitz[1,9], Andreas W. Unterberg[1], Jens P. Dreier[2,3,4,10,11]

[1]Department of Neurosurgery, University Hospital Heidelberg, Ruprecht-Karls-University Heidelberg, Germany
[2]Center for Stroke Research Berlin, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
[3]Department of Neurology, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
[4]Department of Experimental Neurology, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
[5]Institute of Medical Biometry and Informatics, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany
[6]UC Gardner Neuroscience Institute, University of Cincinnati (UC) College of Medicine, Cincinnati, OH, USA
[7]Department of Neurosurgery, University of Cincinnati (UC) College of Medicine, Cincinnati, OH, USA
[8]Department of Neurosurgery, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
[9]Neurosurgery Center Ludwigsburg-Heilbronn, RKH Klinikum Ludwigsburg, Ludwigsburg, Germany
[10]Bernstein Center for Computational Neuroscience Berlin, Berlin, Germany
[11]Einstein Center for Neurosciences Berlin, Berlin, Germany

Introduction : Decreasing frequency and changing electrical and hemodynamic characteristics of Spreading Depolarizations (SDs) produce neuroprotection in animals with lissencephalic brain. The major objective of this project is to test different therapies to reduce the incidence and modify the hemodynamic characteristics of the SDs.
Methods : We studied the effect of different therapeutic approaches on SD incidence and characteristics in two swine animal models and the effect of long term s-ketamine in a collective of 66-SAH patients. SDs were monitored using electrocorticography (ECoG) in both animal and patients and intrinsic optical signal (IOS) imaging at the rage of 564nm±10nm FWHM in animals. This range correlates to cerebral blood volume changes. In animal model 1, SDs were induced with drops of 1M KCl in both hemispheres at 1h-intervals. In a model 2, the left middle cerebral artery group was occluded (MCAo) and SDs occurred spontaneously. Conditions tested in model 1 were hypothermia (33°, n=10 ; 33° with rewarming, n=5, 33° with cathecolamines to avoid hypotension, n=5), i.v. magnesium (10mg i.v., n=5), local magnesium application (1%, n=5), memantine (10 mg/h i.v. n=5 and 60mg i.v. bolus, n=5) and s-ketamine (2mg/kg/h and 4mg/kg/h). In model 2 conditions tested were : hypothermia and s-ketamine (5mg/kg/h).
Results : Hypothermia showed no influence on SD incidence in the model 1, but a significant reduction of SD incidence in model 2. Magnesium showed no effect when given i.v., but a reduction of SD incidence when applied locally in model 1. Memantine showed a moderate effect on decreasing both, near DC amplitude and IOS amplitude, but not decreasing SD incidence in model 1. S-ketamine shows significant results, showing a reduction on SD incidence and reduction of near-DC-shift, as well as reduction of the IOS signal in both models in all given doses. In patients,s-ketamine application started 4.2±3.5 days after aSAH. The mean dose was 2.8±1.4mg/kg BW/h and thus higher than the dose recommended for sedation. We found a significant decrease in SD incidence when s-ketamine was given (Poisson model, $p<0.001$). Thereafter, data was further divided into low-dose (0.1-2.0mg/kg BW/h) and high-dose (2.1-7.0mg/kg/h) segments. High-dose s-ketamine resulted in further significant decrease in SD incidence (Poisson model, $p<0.001$). There was little evidence of SD tolerance to long-term s-ketamine sedation through 5 days.
Conclusions : s-ketamine and hypothermia are the therapies that showed a significant reduction on SD incidence.

Ketamine reduces excitotoxic consequences of spreading depolarization after stroke

K. M. Reinhart[1], A.P. Carlson[2], and C.W. Shuttleworth[1]

[1]Department of Neurosciences, University of New Mexico School of Medicine, Albuquerque, NM, USA
[2]Department of Neurosurgery, University of New Mexico, Albuquerque, NM, USA

Spreading depolarizations (SD) can contribute to the progression of stroke and traumatic brain injuries. We are interested in cellular determinants that confer SD vulnerability in order to develop interventions that can limit glutamate-mediated excitotoxicity during SD. We used here an *in vivo* model to extend prior slice work evaluating glutamate and Ca^{2+} during SD in relation to regional metabolic status. Laser speckle contrast imaging (LSCI) or wide-field fluorescence imaging was used to examine cerebral perfusion, neuronal intracellular Ca^{2+} (GCaMP5G), or extracellular glutamate (iGluSnFR) during SD in mice. After (60 min) permanent middle cerebral artery occlusion or sham surgery, SDs were initiated with focal KCl microinjection from a distant burr hole and confirmed electrophysiologically. Regions of interest (ROIs) in areas further from the occlusion ("remote") were compared to ROIs in closer proximity ("proximal/penumbra"). After stroke, cerebral perfusion was decreased in both ROIs prior to SD stimulation compared to sham animals ($P<0.0001$, $n=7$). The decrease was more prominent in proximal versus remote ROIs ($P=0.002$), indicating augmented metabolic compromise in areas closer to the stroke. During SD, perfusion decreases were exacerbated, and hyperemic responses attenuated, in proximal regions compared to remote areas within the same animal. DC shift durations in proximal regions were prolonged in stroke animals, but not in sham controls (proximal vs. remote : $P=0.0001$ and $P>0.99$ for stroke and sham, respectively). Results from iGluSnFR experiments suggest heterogeneity during SD with prolonged glutamate accumulation in proximal ROIs. These data are supported by GCaMP experiments where the initial time course of neuronal Ca^{2+} recovery during SD was delayed in proximal vs. remote ROIs in stroke animals ($P=0.0005$, $n=6$). In sham animals, there was not a significant effect of ROI location on Ca^{2+} recovery during SD ($P>0.99$, $n=3$). The NMDAR antagonist ketamine, at a dose that did not block SD (5mg kg^{-1} i.p.), accelerated intracellular Ca^{2+} recovery in proximal stroke ROIs compared to vehicle ($P=0.022$, $n=6$). DC shift durations in proximal recording sites were shorter in ketamine vs. vehicle controls ($P=0.037$). Furthermore, remote and proximal DC shift durations were no longer significantly different in the presence of ketamine ($P=0.410$, $n=6$). These results extend conclusions from *in vitro* studies indicating that ketamine can reduce the excitotoxic consequences of SD in stroke brain. These results suggest that it may be useful to examine DC shift duration and ECoG suppression in ICU patients treated with low to moderate doses of ketamine.

A Single Spreading Depolarization can Induce Synaptic Strengthening and BDNF Upregulation

J. E. Weisend[1], K.M. Reinhart[1], A.P. Carlson[2], C.W. Shuttleworth[1]

[1]Department of Neurosciences, University of New Mexico School of Medicine, Albuquerque, NM, USA
[2]Department of Neurosurgery, University of New Mexico, Albuquerque, NM, USA

Introduction : Previous work has described synaptic potentiation following spreading depolarization (SD), although the underlying mechanisms, duration and significance for pathophysiological states remain largely unknown. Increases in brain derived neurotrophic factor (BDNF) have been reported after series of repetitive SD, but the time course after a single SD has not been described. A mechanism coupling BDNF to synaptic strengthening is concomitant adenosine A2 receptor activation, which is of interest here because of large adenosine accumulations following a single SD. We therefore tested effects of a single SD on BDNF expression, synaptic potentiation and protection against subsequent SD injury.
Methods : KCl was used to induce SD in the hippocampal CA1 subregion of murine brain slices. Field excitatory postsynaptic potentials (fEPSPs) and DC shifts were recorded ; q-PCR was used to measure BDNF mRNA expression. Theta-burst stimulation (TBS) was used to test long-term potentiation. In some experiments, slices were preconditioned with SD and later challenged with a second SD in metabolically compromised conditions.
Results : In healthy brain slices, fEPSP slope was persistently increased by ~20%, 20 min after a single SD ($P<0.02$ vs. baseline, n=11). Both total and activity-dependent (L) BDNF increased by ~2-fold reaching peak levels at 45 minutes after SD ($P<0.01$ and $P=0.003$ for total and L, respectively ; n=5-7). Potentiation after SD was unaffected by the A2A receptor antagonist, ZM241385. When tested at the peak of BDNF increases (i.e. 45 min after SD), TBS-induced potentiation was not enhanced, in comparison with time-matched control slides that were not subjected to SD. We next evaluated if preconditioning improved recovery from SD in conditions of brain slice metabolic compromise. SD preconditioning (single SD in healthy conditions, 1 hour prior to SD challenge in metabolically-compromised conditions) improved fEPSP recovery to ~40% of baseline compared to only ~20% recovery in control slices ($P=0.03$, n=6-7). A combination of ketamine (30uM) pre-exposure with SD preconditioning further increased recovery to ~85% after SD (n=6).
Conclusion : These data suggest significant BDNF upregulation after a single SD, although mechanisms other than adenosine A2 receptor activation likely couple any effect of BDNF to synaptic strengthening at these time points. Preconditioning with a single SD was also shown to partially protect slices from SD-mediated injury during subsequent metabolic challenge. Additional studies will be required to elucidate mechanisms underlying SD-mediated plasticity, and potential relevance in injured brain.

Trigeminal Nerve Stimulation To Modulate Cortical Spreading Depolarizations After Brain Injury

Chunyan Li[1,2], Kevin Shah[1], Raj K. Narayan[1,2]

[1]Department of Neurosurgery, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY, USA
[2]Center for Bioelectronic Medicine, Feinstein Institute for Medical Research, Northwell Health, Manhasset, NY, USA

Introduction : Pharmacological interventions to block cortical spreading depolarization (CSD) are systemic and typically have significant side effects. Therefore, new strategies are needed to selectively reduce deleterious consequences of CSDs in the injured brain. Whether or not injury occurs after CSDs depends greatly on the capacity of tissues to re-establish ionic gradients in the aftermath of CSDs. The trigeminal nerve is the largest cranial nerve, forming an extensive network of connections throughout the CNS. It is unique because of its intimate connection with cerebral and meningeal blood vessels, referred to as the trigemino-cerebrovascular system. It is also capable of activating the so called 'diving reflex', whose primary role is to conserve oxygen for sensitive brain and heart tissue. In this study, we aim to investigate the effect of trigeminal nerve stimulation (TNS) to minimize CSD induced brain injuries.

Methods : Studies were performed on 32 male Sprague-Dawley rats. Animals were randomized to four study groups in injured brain with middle cerebral artery occlusion (MCAO) : (1) control animals with permanent MCAO; (2) MCAO animals with Pre-TNS (intermittent TNS for 60 min) ; (3) MCAO animals with 3-hour post-TNS (open-loop) ; and (4) MCAO animals with targeted TNS (closed-loop). TNS was performed by introducing two Teflon-coated bipolar wires bilaterally. Rectangular biphasic pulses (25 Hz, 0.5 ms) with amplitude of 1.5 V were delivered by an electrical stimulator. The number of CSDs, CBF and oxygen tension were recorded and analyzed. Brain tissues were collected at 24 h after MCAO to measure the lesion volume.

Results : MCAO resulted in a sequence of changes in CBF and DC potentials. Upon occlusion, CBF immediately fell by 68±11%. Spontaneous waves of depolarization appeared in the ischemic penumbra zone, averaging about eight events (8.1±2.1 ; n=8) over the 3 h after occlusion. The first CSD episode appeared at 7.1±3.6 min after occlusion. In the MCAO rats, TNS pre-treatment didn't alter the amplitude or duration of each CSD. However, TNS significantly lengthened the latency until the appearance of the first CSD almost 7-fold, and decreased their number by 53% (3.8±0.8 vs. 8.2±2.1 ; n=8). TNS pretreatment just before MCAO also significantly reduced infarction volumes by 34% (from 218.5±42.6 to 143.1±24.7 mm^3 ; n=8). Both open-loop and closed-loop post-TNS also significantly reduced infarction volumes by 47% and 39%, respectively.

Discussion : The results of our study demonstrate that TNS can selectively reduce the deleterious consequences of CSDs in the injured brain, by initiating cerebral vasodilation and increasing energy substrate levels.

The beneficial effect of FP receptor antagonism on spreading depolarization in cerebral ischemia

Dániel P. Varga, Írisz Szabó, Viktória É. Varga, Armand R. Bálint, Ákos Menyhárt, Ferenc Bari, Eszter Farkas

Department of Medical Physics and Informatics, University of Szeged, Szeged, Hungary

Introduction: The prostaglandin F2a - FP receptor signaling pathway leads to Ca^{2+} mobilization in neurons and vascular smooth muscle cells. The inhibition of the FP receptor has recently been shown to limit neurodegeneration in brain ischemia. Spontaneous, recurrent spreading depolarizations (SD) are increasingly more appreciated as the pathomechanism behind ischemic brain injuries. Therefore, we set out to test the hypothesis that FP receptor blockade may achieve neuroprotection by the inhibition of SD.

Methods: Global forebrain ischemia/reperfusion was induced in isoflurane-anesthetized, young, adult, male Sprague-Dawley rats (n=16) by the bilateral occlusion and later release of the common carotid arteries. Two open craniotomies on the right parietal bone served the continuous elicitation of SD for one hour with 1M KCl (caudal), and the acquisition of local field potential (rostral). The entire dorsal cranium was thinned to track regional cerebral blood flow (CBF) variations by laser speckle contrast imaging. The femoral artery was prepared for the monitoring of mean arterial pressure (MAP) and for blood sampling for arterial blood gas analysis. The femoral vein was used for the infusion of an FP receptor antagonist (AL-8810; 1mg/bwkg) or its vehicle (0.1% dimethyl sulfoxide, DMSO). Apoptosis was evaluated five hours following ischemia induction by cleaved-caspase 3 immunohistochemistry.

Results: Physiological parameters were similar in the two groups (e.g. MAP: 82.7±8 vs. 84.5±9.1 mmHg; AL-8810 vs. vehicle). However, AL-8810 markedly reduced the duration of individual SDs (30±10 vs. 56±14 s; AL8810 vs. vehicle), as well as the cumulative depolarization time (2711 vs. 4511 s, AL-8810 vs. vehicle). The CBF response to SD involved a more restricted cortical surface in the AL-8810-treated animals suggesting the involvement of a smaller tissue volume in SD. Both the incidence (8 vs.12, AL-8810 vs. vehicle) and the amplitude of SD-related hypoperfusion as a result of inverse neurovascular coupling (-6.8±3 vs. -13.4±3.2 pp; AL-8810 vs. vehicle) were reduced in the AL-8810 group. Further, the amplitude of reactive hyperemia after reperfusion initiation was substantially greater (94.9±20 vs. 79.7±16%; AL-8810 vs. vehicle). The qualitative evaluation of the cleaved-caspase 3 immunolabeled brain sections revealed a decreased rate of Ca^{+}-dependent neurodegeneration after AL-8810 treatment in the parietal cortex bearing SD.

Discussion: In summary, the antagonism of FP receptors (located in the vascular wall or neurons) emerges as a promising approach to inhibit the evolution of injurious SDs in cerebral ischemia.

Funding:
GINOP-2.3.2-15-2016-00006; Laserlab-Europe IV. (654148); NRDIO (NKFIH_K120358 and K111923); NTP-NFTÖ-18-B-0173; SzSA (No. 34232-3/2016/INTFIN).

Surviving neurons in the ischemic core following focal stroke in mice

Rasha H. Mehder and R. David Andrew

Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Canada

While many studies have examined the properties of the compromised neocortex in the first several days following ischemia, there is less information regarding the initial 12 hours post-stroke. Here we examine live mouse neocortical slices harvested immediately and 12 hours after a 30-minute middle cerebral artery occlusion (MCAo). We compared non-ischemic and ischemic hemispheres with regard to the propensity for tissue swelling and for generating spreading depolarization (SD) as well as single pyramidal neuron morphological and electrophysiological properties.

Methods : Male C57/BL6 mice (20-25g) were anaesthetized using isoflurane in O_2 and air (80% : 20%). Focal cerebral ischemia was induced by intraluminal occlusion of the left MCAo for 30 minutes. Brains were harvested 30 min post-MCAo or after 12 hours of in vivo reperfusion. Following decapitation, the brain was immersed in artificial CSF. Then the brain was either processed for Golgi-Cox microscopy or live brain slices were prepared. During oxygen/glucose deprivation (OGD), a slice was imaged using changes in light transmittance to detect SD. Neurons were recorded using whole-cell patch clamp.

Results : Spontaneous SD was imaged in 7% of slices on the non-stroked side and 25% in the stroked side following the 30 min MCAo. Spontaneous SD was rare in 12 hr recovery slices. The region of the ischemic core and surround in slices was not susceptible to SD induced by OGD. At the neuronal level, neocortical gray matter was surprisingly unaltered in brain slices harvested immediately post-stroke. However by 12 hours, the fields of pyramidal and striatal neurons that comprise the infarcted core were electrophysiologically silent because the majority are morphologically devastated. Yet there remained a subset of diffusely distributed "healthy" pyramidal neurons in the neocortical core at 12 hours post-MCAo. Their intact intrinsic electrophysiological properties and normal dendritic morphology (including abundant spines) indicated a surprisingly selective resilience to stroke at the neuronal level.

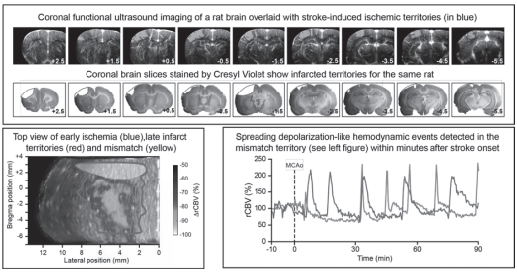
Conclusions : It is generally accepted that the injured core region of a focal stroke lack functional neurons and that they have each undergone terminal SD. Our study shows that some neocortical neurons, although surrounded by devastated neighbors, can maintain their structure and electrical properties. Our results are supported by a 2017 histochemical study showing scattered neuronal survivors extending out to at least 72 hr post-stroke (*Brain Pathol* 27:480–498). Identifying how these pyramidal neurons are protected may point to new molecular strategies for reducing stroke injury.

Whole brain functional ultrasound imaging (fUSi) for detection and tracking of spreading depolarization during stroke in anesthetized rats

C. BRUNNER[1], N. LAGUMERSINDEZ[2], M. GRILLET[1], G. MONTALDO[1], M. ENDRES[2], Alan URBAN[1]

[1]NeuroElectronics Research Flanders, IMEC, VIB, KU Leuven, Leuven, Belgium
[2]Dept. of Neurology, Exptl. Neurol., Charité-medical Univ. of Berlin, Berlin, Germany

Introduction : Spreading depolarization (SD) is a dramatic failure of brain ion homeostasis that propagates across the cerebral gray matter. SD are mainly characterized by a large negative shift of the extracellular direct current potential on electrocorticogram (ECoG). In healthy brain tissue, SD is associated with an increase in cerebral blood flow (CBF) required for reestablishment of ionic gradients and repolarization. Conversely, when neurovascular coupling is compromised like in ischemic condition, the SD-related hemodynamic response shift to hypoperfusion. There is strong experimental and clinical evidence suggesting presence and potential detrimental effects of SD in patients with acute cortical ischemic stroke, severe traumatic brain injury, and subarachnoid hemorrhage but appropriate animal models are still needed to provide a more comprehensive picture of how SD evolves in the ischemic brain and not only the cortex.
Materials & Methods : Here, we used functional ultrasound imaging (fUSi) to assess the characteristic features of ischemia-induced SDs at high spatiotemporal resolution (100um^3 voxel size, 2Hz imaging frequency) in two models of stroke accounting for either mild (permanent MCA occlusion) or severe ischemia (pCCA + pMCA occlusion). fUSi data were analyzed at several time points before, immediately after occlusion and continuously during 90mins. We established the first 3D stroke map including over 800 individual brain regions including cortical and subcortical area. These data were further compared to postmortem quantification of the infarct volume measured 24 hours after stroke.
Results : We demonstrated that only 5 mins after stroke, the volume of ischemic tissue and its level of hypoperfusion was much pronounced in the severe ischemia as compared to the mild model. We did not observe significant change over time of these parameters within the 90mins of the fUSi recording. Additionally, we observed quickly after the stroke onset transient SD-related hemodynamic waves originating from the frontal cortex and spreading all around the ischemic core. Importantly, we observed a significant mismatch between the location and the volume of the ischemic core as measured in vivo with fUSi and the infarcted brain tissue. We hypothesize that SD-related hemodynamic waves may trigger late tissue infarction which spatially correlates with the site of elicitation (focus) of these events.
Discussion : The fUSi technology is suitable for efficient prognosis of the infarct territory by allowing a precise measurement of the ischemic territory in the entire brain and by precisely mapping SD-related hemodynamic waves. We demonstrated that these 2 parameters are sufficient to predict tissue infarction within 5 min after stroke onset. Our results confirmed that SDs begin preferentially in sensory cortex in anesthetized rats and suggest a vulnerability of sensory cortex to injury-associated depolarizations. Here we showed that fUSi is a promising technology for high precision stroke mapping in preclinical studies that may help for understanding the effect of neuroprotective therapies considering the prevention of SD being a possible treatment.



Multispectral photoacoustic imaging of hemodynamics during spreading depolarization

Adrian H. Aguilera[+1], Thomas Kirchner[+2,3], Mildred A. Herrera[1], Tim Adler[2,4], Janek Gröhl[2,5], Lena Maier-Hein[*2,5], Edgar Santos[*1]

[1]Department of Neurosurgery, University Hospital Heidelberg, Heidelberg, Germany
[2]Division of Computer Assisted Medical Interventions, German Cancer Research Center (DKFZ), Heidelberg, Germany
[3]Faculty of Physics and Astronomy, Heidelberg University, Heidelberg, Germany
[4]Faculty of Mathematics and Computer Science , Heidelberg University, Heidelberg, Germany
[5]Medical Faculty, Heidelberg University, Heidelberg, Germany
+These authors contributed equally.
*These authors share senior authorship.

Introduction : Multispectral photoacoustic tomography (PAT) is a novel imaging technique which is ideally suited to image hemodynamics by estimating changes in total hemoglobin (THb) and blood oxygenation (sO_2). PAT can achieve centimeters imaging depth with submillimeter resolution by measuring the acoustic responses produced by the local absorption of short laser pulses. So far, photoacoustic imaging of the brain has mainly been performed in small animal models. The purpose of this work was to investigate if multispectral PAT can assess the hemodynamics of a gyrencephalic brain during spreading depolarization (SD).

Methods : As SD causes tissue hypoxia, we aim to visualize SD by continuously estimating sO_2 in parasagittal sections of in vivo porcine brains using our custom-built intraoperative hybrid photoacoustic and ultrasonic imaging system. For this, our custom PAT probe is fixed to a stereotaxic frame in acoustic contact with the cortex, following an extensive craniotomy to view the cortex bilaterally. With this setup, SD induction experiments by KCl stimulation are performed on five animals while recording ECoG for reference.

Results and Discussion : According to our experiments, PAT is able to image estimated sO_2 and THb changes in the entire depth of the perfused gray matter of the porcine cerebral cortex. Hemodynamic changes associated with SD have been observed with PAT consistent with ECoG reference measurements in all five animals of our preliminary study. Furthermore, the integration of PAT in a clinical ultrasound system allowed for real-time in vivo imaging of the gyrencephalic brain and allowed for PAT guided KCl stimulation. We conclude that multispectral PAT can be a potentially valuable tool for the study of hemodynamics in the cerebral cortex of a gyrencephalic brain in depth with high spatiotemporal resolution. PAT could yield new insights in SD, stroke, and brain injury research.

Near-infrared reflectance imaging for monitoring spreading depolarizations and accompanying lesion progression in a rat focal cerebral ischemia model

Satoko Kawauchi[1], Izumi Nishidate[2], Hiroshi Nawashiro[3], Shunichi Sato[1]

[1]Division of Bioinformation and Therapeutic Systems, National Defense Medical College Research Institute, Tokorozawa, Saitama, Japan
[2]Graduate School of Bio-Applications & Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan
[3]Division of Neurosurgery, Tokorozawa Central Hospital, Tokorozawa, Saitama, Japan

Introduction : To understand the pathophysiology and to examine neuroprotection method for ischemic stroke, in vivo imaging of spreading depolarization (SD) and evolution of tissue damage is desired. Since these events are accompanied by cellular morphological changes, light-scattering signals, which are sensitive to cellular and subcellular morphology, would be useful to monitor SD and tissue alteration. In this study, we performed transcranial imaging of near-infrared (NIR) diffuse reflectance at ~800 nm which is sensitive to light-scattering change and examined how NIR reflectance signals are correlated with cerebral blood flow (CBF) simultaneously measured for a rat middle cerebral artery occlusion (MCAO) model[1)].
Methods : Male Sprague-Dawley (SD) rats were anesthetized with pentobarbital sodium and placed in a stereotactic frame. A cranial window (~ 3 mm x ~4 mm) was made to occlude distal MCAs in the left hemisphere, and the entire left cortex was imaged though the skull with a laser speckle CBF imaging system that was combined with non-coherent NIR light illumination for diffuse reflectance imaging.
Results and Discussion : After MCAO, wave-like NIR reflectance changes indicating occurrence of SDs were generated and propagated around the ischemic core for ~90 min, during which time NIR reflectance signal intensity increased not only within the ischemic core but also in its peripheral region. The area with increased reflectance signal expanded with increase in the number of SD occurrences, the correlation coefficient being 0.7686 (n=5). The area with increased reflectance signal became infarcted at 24 h after MCAO. The infarct region was found to be associated with hypoperfusion or no-flow response to SD, but hyperemia or hypoperfusion followed by hyperemia response to SD was also observed in the region infarcted. Such a regional heterogeneity of blood flow response to SD seemed to be connected with the rat cerebrovasculature and hence existence/absence of collateral flow. The results suggested that NIR reflectance signals depicted early evolution of tissue damage and lesion progression, which were not seen by CBF imaging in the present stroke model.

Reference:

1) S. Kawauchi, I. Nishidate, H. Nawashiro, and S. Sato, "Near-infrared diffuse reflectance signals for monitoring spreading depolarizations and progression of the lesion in a male rat focal cerebral ischemia model," Journal of Neuroscience Research, 96 (5), 875-888 (2018).

Real time nitric oxide changes during spreading depolarization, ischemia and reperfusion.

Baptiste Balança[1,2,3], Eun-Jeung Kang[4], Coline Lemale[4,5], Anne Meiller[1], Stéphane Marinesco[1], Jens P. Dreier[4,5,6,7,8]

[1]Lyon neuroscience Research Center, Lyon, France
[2]Lyon University, Lyon, France
[3]Hospices civils de Lyon, department of anesthesiology and intensive care medicine
[4]Center for Stroke Research Berlin
[5]Departments of Experimental Neurology, Charité – Universitätsmedizin Berlin
[6]corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
[7]Bernstein Center for Computational Neuroscience Berlin, Berlin, Germany
[8]Einstein Center for Neurosciences Berlin, Berlin, Germany

Introduction : After ischemic stroke, nitric oxide (NO) may act in two different ways : (1) as a free radical it may lead to molecular damage, (2) NO deprivation may lead to SD-induced perfusion deficit (i.e. spreading ischemia, SI). In this study we aimed at investigating cortical NO concentration in control Wistar Kyoto (WKY) and spontaneously hypertensive stroke prone (SHRsp) rats during SDs under normal or ischemic conditions as well as during reperfusion.
Methods : Experiments were done in adult male rats under thiopental anesthesia (SHRsp n=10 and WKY n=10 in each group). The cortex was perfused with either aCSF, aCSF+LNNA, or aCSF+LNNA+carboxy PTIO. We measure brain oxygen partial pressure ($PbtO_2$) and NO with microsensors, and regional cerebral blood flow (rCBF) using laser speckle contrast analysis imaging. A first SD was triggered by KCl application at a remote window. 30min later, the ipsilateral carotid artery was occluded using a silastic loop. If no SD occurred spontaneously, an SD was induced by KCl. 30min later, the contralateral carotid artery was occluded. 30min after the onset of a terminal SD, both carotids were released. Group comparisons were performed using Kruskal-Wallis tests.
Results : Compared to WKY, SHRsp had a higher systolic arterial pressure (167mmHg [159 ; 176] vs 115mmHg [109 ; 123], p<0.001), and a higher $PbtO_2$ (46.6mmHg [28.6 ; 53.5] vs 20.2mmHg [7.7 ; 27.6], p=0.011). The baseline signals of the NO electrodes were not different in WKY (38.8nM [32.2 ; 53.1]) and SHRsp (49.8nM [33.6 ; 52.9], p=0.9). The first SD triggered an rCBF increase while $PbtO_2$ decreased. The NO sensors recorded a sharp peak followed by a slower increase. The signal was not affected by LNNA or LNNA+CPTIO (Fig1).
When rCBF decreased after ipsilateral occlusion, 3/10 WKY showed SI while the others a hyperemic response. When rCBF was further decrease after bilateral occlusion 4 WKY and 1 SHRsp presented SI before the onset of a terminal depolarization. During those SD NO sensors showed an increase not affected by LNNA or LNNA+CPTIO (Fig1). 20 min after reperfusion, rCBF and the PbtO2 were close to baseline concentrations and NO concentration was not elevated.
Discussion : An interesting finding was that SHRsp showed a higher baseline level of $PbtO_2$, and that SI were only observed in WKY under reduced rCBF. The signal recorded by the NO electrode was not affected by LNNA or CPTIO, suggestion that the signal changes were not due to NO. Instead, this may have resulted from the oxidation of another compound at the sensor's surface, or a distorted DC potential artefact. Our data suggest that NO changes during SD, ischemia and reperfusion remain below the detection limit of the sensor (i.e. 10 nM).

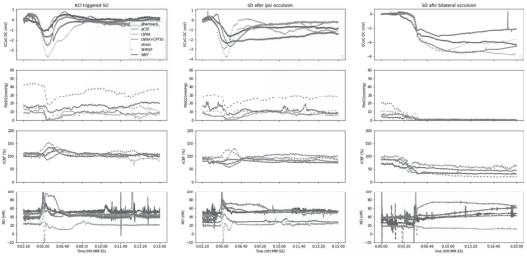


Fig1. SD induced rCBF, $PbtO_2$ and NO changes

Monitoring Rat Cortical Spreading Depolarizations Using Minimally-invasive microelectrode biosensors Based on Platinized Carbon Fibers

Stéphane Marinesco, Clelia Allioux, Baptiste Balança, Anne Meiller

Lyon Neuroscience Research Center, Team TIGER Faculty of Medicine, Lyon, France

Monitoring the chemical composition of the brain interstitial fluid is an important challenge both in basic neuroscience and in neuro-intensive care units. It can be achieved with microelectrode biosensors consisting in oxidase enzymes immobilized on a platinum microelectrode that oxidizes hydrogen peroxide (H2O2) produced by enzymatic activity. These devices can detect non-electroactive molecules like glucose and lactate, but their size is quite large and could potentially damage brain tissue, modifying the local composition of the interstitial fluid. Here, microelectrodes based on platinized carbon fibers were fabricated to obtain biosensors with less than 15 µm external diameter. Platinization was achieved by evaporation of a 10 nm Ti adhesion layer followed by 100 nm of Pt. Microelectrodes based on platinized carbon fibers could detect brain O2 in vivo, or be functionalized with oxidase enzymes to monitor glucose or lactate interstitial concentrations. After implantation in the rat parietal cortex, such biosensors detected smaller basal O2 and lactate concentrations and a slower diffusion of glucose through the blood brain barrier (BBB) compared to more conventional biosensors with 90-100 µm external diameter. This result, as well as histological images of BBB integrity using Evans blue, indicates that injury to blood vessels was reduced around small sensors. Interestingly, spreading depolarizations (SD) produced a smaller increase in lactate and a larger decrease in glucose at platinized carbon fiber microelectrode biosensors compared to larger sensors. Changes in tissue pO2 detected by small sensors were also wider and more rapid than with more conventional ones. These differences probably reflected the presence of a layer of compromised tissue around the probe, with limited O2 and glucose consumption, and acting as a low pass filter on the concentration changes occurring in the interstitial fluid. Therefore, these smaller, less invasive probes provided more physiological measurements from the brain interstitial fluid, allowing a more accurate characterization of the neurochemical signature of SDs in healthy or injured brain tissue.
Financial support from CNRS UMR 5292, Inserm U1028, Union des Blessés de la Face et de la Tête (Fondation Gueules Cassées, FGC 49-2016)

Spreading Depolarizations Are Associated with Worsening Pathologic Anatomy in Computed Tomographic Imaging after Severe Brain Trauma

Bryan Krueger[1], Laura B. Ngwenya[1–3], Jason M. Hinzman[1], Anthony J. Strong[4], David O. Okonkwo[5], and Jed A. Hartings[1–3]

[1]Department of Neurosurgery, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA
[2]Collaborative for Research on Acute Neurologic Injury (CRANI), University of Cincinnati (UC) College of Medicine, Cincinnati, Ohio, USA
[3]UC Gardner Neuroscience Institute, Cincinnati, Ohio, USA
[4]Department of Clinical Neuroscience, King's College London, London, UK
[5]Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Background: Computed tomographic (CT) imaging is the principal diagnostic method for management of traumatic brain injury (TBI). Serial CT studies are often obtained to assess evolution of cerebral edema, contusions, and hemorrhage as cause for protracted coma and indication for further intervention. Here we examined whether spreading depolarizations (SDs) are associated with worsening CT pathology.

Methods: 138 patients requiring surgical treatment of severe TBI were enrolled in a prospective COSBID study at five medical centers. A subdural electrode strip was placed during surgery and electrocorticography (ECoG) was recorded during neurointensive care. Patients were further screened for inclusion in this analysis based on availability of: (1) baseline post-surgical CT study (PS CT), (2) minimum 20 hr of valid ECoG following the PS CT, (3) a follow-up CT (FU CT) study after this minimum ECoG period and within 48 after ECoG end. FU and PS CTs were compared to determine the stability of anatomic pathology according to clinical judgment, with blinding to ECoG data. Binary logistic regression was used to assess the variables of age, sex, pupil reactivity, GCS motor score, Rotterdam CT score, INR, and SDs for prediction of CT worsening.

Results: 75 patients (age 46 ± 19; 80% male) met inclusion criteria. PS CTs were obtained 4 ± 8 hrs after ECoG start. FU CTs were obtained 19 ± 13 hrs after ECoG end in 34 cases and 48 ± 35 hrs before ECoG end in 41 cases. Total ECoG duration was 122 ± 42 hrs and the interval between PS and FU CTs was 101 ± 48 hrs. Thirty of 75 patients were judged to have stable CT pathology, while others showed worsening identified as significant swelling beyond craniectomy defect (n=19), contusion increase (46 ± 52 cc; n=37), midline shift increase >2 mm (4.4 ± 3.7 mm; n=14), and/or increased subdural (n=10) or intraventricular hemorrhage (n=6). CT worsening occurred in 33% (9/27) of patients with no SDs, 58% (11/19) of patients with isolated SDs only, and 86% (25/29) of those with SD clusters (≥3 SDs in 120 min)(X^2, $p<0.001$). Multivariate regression showed that Rotterdam CT score contributed to the regression model ($p = 0.085$), but SD scoring was the only statistically significant predictor of CT worsening ($p = 0.003$). Isolated SDs had an OR of 8.1 (CI 1.3 – 52.4, $p = 0.027$) and presence of SD clusters was the most significant variable (OR 26.8; CI 4.0 – 179.9, $p = 0.001$).

Conclusions: SDs are associated with worsening CT pathology. Real-time analysis of ECoG data should be considered to help guide clinical decision making in severe brain trauma.

The Impact of Physiological Variables on Spreading Depolarization and Neurovascular Coupling after Malignant Hemispheric Stroke

Leonie Schumm[1], Jens P. Dreier[2], M. Nieminen-Kelhä[1], C. Lemale[2], S. Major[2], A.Zdunczyk[1], P. Martus[3], N. Hecht[1], J.Woitzik[1,4]

[1]Department of Neurosurgery, Charité Universitätsmedizin Berlin, Germany
[2]Department of Neurology, Charité Universitätsmedizin Berlin, Germany
[3]Institute for Clinical Epidemiology and Applied Biometry, University of Tübingen, Germany
[4]Department of Neurosurgery, University of Oldenburg, Germany

Objectives : Decompressive hemicraniectomy is a surgical therapy for patients with malignant hemispheric stroke (MHS) to relieve intracranial pressure and to prevent or reverse cerebral herniation. The craniotomy allows the placement of a subdural electrode strip for the recording of spreading depolarizations (SD) which frequently occur in the early period after stroke. Physiological variables such as body temperature, arterial blood pressure (ABP) or intracranial pressure (ICP) are relevant in postoperative care and may influence the patients' outcome. However, little is known about the relationships between physiological variables and SD after MHS. The aim of the present study was (1) to experimentally investigate the impact of the physiological variables on SDs and neurovascular coupling after middle cerebral artery occlusion (MCAO), and (2) to study their effect on occurrence and features of SDs in MHS patients.

Methods : Experimental focal ischemia was induced by distal MCAO in adult, male C57BL/6J mice. SDs were recorded with an intracortical electrode and cerebral blood flow (CBF) with laser speckle contrast analysis imaging. Infarct progression was evaluated by T2-weighted magnetic resonance imaging (MRI). KCl- induced SDs under normothermia (37° C), hypothermia (34° C) or hyperthermia (39° C) (n=10 each). 60 patients with MHS requiring hemicraniectomy were included in the clinical study. Electrocorticography of the periinfarct zone was performed with a linear subdural electrode strip (6 Pt/Ir contacts spaced at 10mm). Body temperature, ABP and ICP were monitored continuously. Clinical outcome was assessed at 6 months according to the extended Glasgow Outcome Scale (eGOS).

Results : In the experimental setting, hypothermia was associated with longer hyperperfusion during SD (6.2±2.0min vs. 3.5±1.7min (hyperthermia, $p<.05$)) and increased perfusion thereafter (+1.8±3.8% vs. -2.2±2.3% (hyperthermia, $p<.05$)). No differences were found for the hypoperfusion phase but overall both magnitude (%CBF decrease, $R^2=0.35$, $p<.001$) and duration ($R^2=0.44$, $p<.05$) correlated with SD duration. Regarding lesion growth, hypothermic animals differed significantly from the other groups, showing no stroke progression at all (-10.4±6.8% vs. +1.7±6.6% (KCl^-, $p<.01$) and -1.9±3.9% (hyperthermia, $p<.05$). In the patients, 1690 SD occurred with a total depression time of 821h during a total recording time of 7480h. The statistical analysis of the clinical data is currently ongoing.

Conclusion: Neurovascular coupling to SD is influenced by body temperature in experimental stroke and could be a key mechanism for delayed stroke progression after SD.

Preliminary evidence that clinical manipulations are associated with increased cortical spreading depolarization in patients with acute neurologic injury

Andrew P. Carlson[1], Fares Qeadan[2], K. C. Brennan[3], C. William Shuttleworth[4]

[1]Department of Neurosurgery, University of New Mexico School of Medicine, Albuquerque, NM, USA
[2]Department of Internal Medicine/ Clinical Translational Science Center, Division of Biostatistics, University of New Mexico School of Medicine, Albuquerque, NM, USA
[3]Department of Neurology, University of Utah, Salt Lake City, UT, USA
[4]Department of Neuroscience, University of New Mexico School of Medicine, Albuquerque, NM, USA

Background : Secondary neurologic injury occurs in many conditions including aneurysmal subarachnoid hemorrhage (SAH), traumatic brain injury (TBI), and stroke. Massive waves of spreading depolarization (SD) have been strongly implicated as causal in secondary injury progression. Preclinical work has shown that tactile limb stimulation is sufficient to initiate damaging SD after stroke. If humans share this susceptibility, it would have significant implications for clinical care.
Objective: Determine whether clinical manipulations are temporally associated with increased occurrence of SD in patients with acute neurologic injury.
Methods : Prospective observational study of patients with acute neurological injury undergoing continuous video electrocorticography (ECoG) after surgical intervention. The exposure variable was frequency of clinical manipulations performed for routine care by staff or family. The primary outcome measure was Odds of SD with increasing frequency of clinical manipulations.
Results : 18 subjects were enrolled, 14 (78%) who had recorded SD. Overall, there was a significant increased odds of SD with increased frequency of manipulations (OR=1.494 for additional 10 manipulations/20 minutes, 95% CI= 1.021-2.185). In individual patient level analysis, we identified 8 subjects (57%) with individually significant, frequency dependent increase in occurrence of SD with manipulations, while the remaining 6 subjects demonstrated no measurable effect of manipulations. This effect was observed across all injury types.
Conclusions : These data provide the first evidence that clinical manipulations common in ICU care are associated with increased incidence of subsequent SD in patients with acute neurologic injury. The metabolic challenge of SD has been strongly linked to injury expansion. Whether or not a strategy of limiting very early stimulation in such compromised patients leads to improved outcomes should be studied in future clinical trials.

Effect of locally delivered nimodipine microparticles on spreading depolarization after aneurysmal subarachnoid hemorrhage.

Andrew P. Carlson[1], R. Loch MacDonald[2], C. Bill Shuttleworth[3]

[1]Department of Neurosurgery, University of New Mexico School of Medicine, Albuquerque, NM, USA
[2]Department of Neurosurgery, University of Illinois at Chicago, Chicago, IL, USA
[3]Department of Neuroscience, University of New Mexico School of Medicine, Albuquerque, NM, USA

Background : Nimodipine is an efficacious therapy that improves outcomes after aneurysmal subarachnoid hemorrhage (SAH). Preclinical data support that at least some of this effect may be related to nimodipine's effects in suppression of spreading depolarization (SD) or improving the vascular response to SD. Thus, it would be helpful to determine the effect of different methods of nimodipine administration that are associated with different intracranial nimodipine concentrations.
Methods : We enrolled all eligible subjects undergoing clipping of a ruptured aneurysm into a prospective study of SD monitoring with ECog (electrocorticography). A subset of these subjects were then randomized to standard of care (SOC) oral nimodipine or an intracranially delivered sustained release formulation of nimodipine that leads to higher intracranial nimodipine concentrations than oral nimodipine (NEWTON 1 and 2 studies). Scoring of SD was blinded to study allocation. We then tested SD characteristics of subjects who received the investigational drug to all subjects with monitoring after SAH (both in NEWTON and not enrolled in NEWTON).
Results : There were 24 subjects with complete data sets available for review. 4 were in NEWTON 1, all of whom received the investigational drug. 5 were enrolled in NEWTON 2 : 2 were randomized to SOC oral nimodipine, and 3 to investigational drug. 17 standard of care subjects were available for comparison (including the 2 NEWTON 2 subjects). Monitoring times were similar. The overall rate of SD was the same in both groups (0.12 sd/hour/patient). The mean total daily depression duration appeared longer in the SOC group (13 minutes, 48 seconds) compared to the NEWTON group (2 minutes, 7 seconds, $p = 0.4$).
Conclusions : Locally delivered nimodipine may exert neuroprotective effects through SD related mechanisms, particularly in improved recovery from SD. Further studies in patients at high risk for delayed cerebral ischemia are warranted.

Characteristics of ictal DC shifts, another infraslow EEG, recorded by scalp EEG in epilepsy patients: Comparison between scalp- and subdural recording

Shunsuke Kajikawa[1], Tomohiko Murai[2], Masao Matsuhashi[3], Katsuya Kobayashi[4], Takehumi Hitomi[5], Masako Daifu[4], Yukihiro Yamao[6], Takayuki Kikuchi[6], Kazumichi Yoshida[6], Takeharu Kunieda[7], Takao Namiki[8], Ichiro Tsuda[9], Riki Matsumoto[10], Ryosuke Takahashi[1], Akio Ikeda[3]

[1]Department of Neurology, Kyoto University graduate school, Kyoto, Japan
[2]Department of Neurology, Kyoto city hospital, Kyoto, Japan
[3]Department of Epilepsy, Movement Disorders and Physiology, Kyoto University Graduate School of Medicine, Kyoto, Japan
[4]Epilepsy center, Cleveland Clinic
[5]Department of Clinical Laboratory, Kyoto University Graduate School of Medicine, Kyoto, Japan
[6]Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto, Japan
[7]Department of Neurosurgery, Ehime University Graduate School of Medicine, Ehime, Japan
[8]Department of Mathematics, Hokkaido University, Hokkaido, Japan
[9]Chubu University Academy of Emerging Sciences, Aichi, Japan
[10]Department of Neurology, Kobe University Graduate School of Medicine, Hyogo, Japan

Introduction : Epileptic ictal direct current shifts (ictal DC shifts) is another infraslow EEG activity similar to cortical spreading depolarizations (CSDs) from generator mechanism point of view. It was reported by subdural electrodes since 1996 in epilepsy surgery protocol (Ikeda et al.,1996) as well as by scalp EEG since 1996 (Ikeda et al, 1999). Currently ictal DC shifts becomes to be applied in epilepsy surgery as a surrogate marker of epileptogenicity.

Ictal DC shifts is usually recorded by time constant (TC) of 10 sec, but many institutes have the AC amplifier with TC 2 sec. Therefore, 1) we demonstrated that ictal DC shifts could be identified even by changing the display setting of TC from 10 sec to 2 sec for subdural electrodes in 21 patients with refractory focal epilepsy (Kajikawa et al., 2019). 2) By means of TC of 2 sec, we also defined 2 patterns of ictal DC shifts by means of cluster analysis : ① Rapid development pattern ; shorter peak latency (latency from the onset to peak of ictal DC shifts), ② Slow development pattern ; longer peak latency and higher amplitude attenuation (Kajikawa et al, 2019).

As proposed to these extensive analysis for subdural ictal DC shifts, the study to characterize scalp-recorded DC shifts was little done, and thus we attempted to classify the scalp-recorded ictal DC shifts with rapid- or slow development pattern.

Methods : We summarized 7 patients based on previous study in our institutes where scalp EEG were recorded by TC 10 sec . Ictal DC shifts of 3 out of 7 patients were reported previously (Ikeda et al., 1997, 1999), , and those 3 patients data were provided by publication materials.

We measured the peak latency of ictal DC shifts and classifed into rapid- and slow development patterns, i.e., as <9 sec from onset to peak latency in TC 10 sec, based on logistic analysis (Kajikawa et al, 2019)>.

Results : Five out of 7 patients were focal epilepsy and 2 were symptomatic generalized epilepsy (SGE). Occurrence rate of ictal DC shifts in focal epilepsy and SGE in seizure population were 26.1% and 69%, respectively. All patients tended to show rapid development pattern, <9 sec from onset to peak latency in TC 10 sec>.

Conclusion : Subdural DC shifts consisted of rapid- and slow development patterns whereas scalp-recorded DC shifts were all rapid development pattern. It may be because scalp-recorded slow development pattern is subject to blurring most likely by means of scalp bone.

Correlates of spreading depolarization, spreading depression and negative ultraslow potential in human epidural versus subdural electrocorticography

Jens P. Dreier[1,2,3,4,5], Sebastian Major[1,2,3], Coline L. Lemale[1,3,6], Vasilis Kola[1], Clemens Reiffurth[1,3], Karl Schoknecht[1,2,3], Nils Hecht[1,6], Jed A. Hartings[7,8], Johannes Woitzik[1,6]

[1]Center for Stroke Research Berlin
[2]Department of Neurology
[3]Department of Experimental Neurology
[6]Department of Neurosurgery, Charité University Medicine Berlin, Berlin, Germany
[4]Bernstein Center for Computational Neuroscience Berlin, Berlin, Germany
[5]Einstein Center for Neurosciences Berlin, Berlin, Germany
[7]UC Gardner Neuroscience Institute
[8]Department of Neurosurgery, University of Cincinnati (UC) College of Medicine, Cincinnati, OH, USA

Objectives: Spreading depolarizations (SD) are characterized by near-complete breakdown of the transmembrane ion gradients and neuronal edema. Terminal SD, the SD extreme in ischemic tissue, is characterized by prolonged depolarization in addition to the negative ultraslow potential (NUP). The NUP is the largest bioelectrical signal ever recorded from human brain and is thought to reflect progressive recruitment of neurons into cell death in the wake of SDs. However, it is unclear whether the NUP is a field potential or results from contaminating sensitivities of platinum/iridium (Pt/Ir) electrodes. In contrast to Ag/AgCl electrodes in animals, Pt/Ir electrodes are the gold standard for intracranial recordings in humans. Here, in an approach of reverse translation from bedside to bench, we studied the full continuum from short-lasting SDs under normoxia to long-lasting SDs under systemic hypoxia to terminal SD under severe global ischemia using Pt/Ir electrodes in rats. In an approach of classic translation from bench to bedside, we then studied the use of epidural titanium peg electrodes in patients.
Methods: A parietal craniotomy was performed in male Wistar rats (n=8). A K+-selective Ag/AgCl microelectrode was used to measure intracortical electrocorticogram and extracellular K+; two Pt/Ir plate electrodes were placed on the cortex. In 7 patients with either subarachnoid hemorrhage or malignant hemispheric stroke, two epidural peg electrodes were placed 10mm from a subdural strip.
Results: In the rats, sensitivities for detecting SDs, spreading depressions or NUP were 100% for both electrode types. However, the Pt/Ir-recorded NUP was ten times smaller in rats than humans. In the patients, 31/67 SDs on the subdural strip were also detected epidurally. SDs that had longer negative direct current shifts and spread more widely across the subdural strip were more likely to be observed in epidural recordings. One patient displayed an SD-initiated NUP while undergoing brain death despite continued circulatory function. The NUP's median amplitude was -150mV subdurally and -67mV epidurally. This suggests that the human NUP is a bioelectrical field potential rather than an artefact of platinum sensitivity to other factors, since the dura separates the epidural from the subdural compartment and the epidural microenvironment unlikely changed, given that ventilation, mean arterial pressure and peripheral oxygen saturation remained constant during the NUP.
Conclusions: Our data provide further evidence for the clinical value of invasive neuromonitoring, highlighting important possibilities as well as limitations of less invasive recording techniques.

販売名：SPプレート
一般的名称：体内固定用プレート
医療機器承認番号：22200BZX00866000

S&Brain
www.sbraincorp.jp

製造販売元
ケンテック株式会社
〒162-0043 東京都新宿区早稲田南町52-2
TEL.03-5155-2596 FAX.03-5155-2598

製造元
プロスパー株式会社
〒945-1355 新潟県柏崎市鵜井川2028番地6
TEL.0257-24-5277 FAX.0257-24-8620

販売元
エス・アンド・ブレイン株式会社
〒279-0021 千葉県浦安市富岡3-3 F415
TEL.047-316-0517 FAX.047-316-0518

INTEGRA®
LIMIT UNCERTAINTY

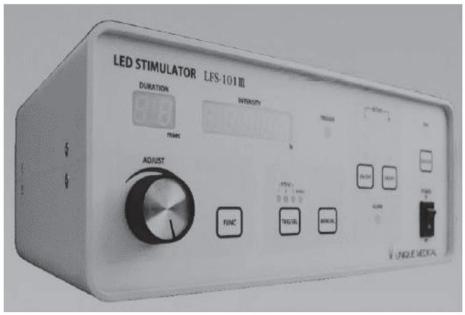
CODMAN NEURO

A NEW DAY
FOR NEUROSURGERY

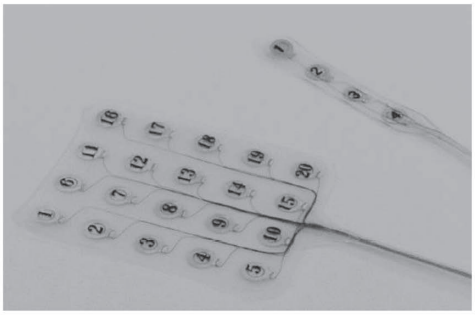
CODMAN® ICP Monitoring System

お問合せ先
Integra Japan 株式会社
〒107-0052 東京都港区赤坂 1−7−1 赤坂榎坂ビル9F
TEL：03-6809-0235　FAX：03-6809-0236
integralife.com

製造販売元：ジョンソン・エンド・ジョンソン株式会社
販売名：CODMAN MicroSensor ベーシック・キット　承認番号：21500BZY00397000
販売名：ICP エクスプレス　承認番号：20800BZY00959000



LED 光刺激装置　LFS-101Ⅲ
VEP による視覚路機能モニタリング



頭蓋内電極　UZN シリーズ
豊富な極数や形状の頭蓋内電極
術中脳波やてんかん焦点診断に

株式会社　ユニークメディカル
● 東京営業所 〒201-0003 東京都狛江市和泉本町2丁目7番12号
Tel(03)3480-7235 Fax(03)3480-6147 E-mail: tokyo@unique-medical.jp
● 大阪営業所 〒533-0031 大阪市東淀川区西淡路1丁目3番26号(弥生新大阪第1ビル5F)
Tel(06)6323-3706 Fax(06)6325-9126 Email: osaka@unique-medical.jp
● 九州営業所 〒814-0164 福岡市早良区賀茂3丁目7番24号(夢ビル2F)
Tel(092)864-3390 Fax(092)284-8724 Email: kyusyu@unique-medical.jp
URL http://www.unique-medical.jp/

抗てんかん剤　薬価基準収載

VIMPAT® ビムパット®錠 50mg 100mg

劇薬、処方箋医薬品：注意一医師等の処方箋により使用すること
一般名／ラコサミド(Lacosamide)

●「効能・効果」、「用法・用量」、「禁忌を含む使用上の注意」、「用法・用量に関連する使用上の注意」等については添付文書をご参照ください。

販売元（資料請求先）
Daiichi-Sankyo 第一三共株式会社
東京都中央区日本橋本町3-5-1

製造販売元
ucb ユーシービージャパン株式会社
東京都新宿区西新宿8丁目17番1号

2018年6月作成

抗てんかん剤
処方箋医薬品（注意一医師等の処方箋により使用すること）　薬価基準収載

イーケプラ® 錠250mg 錠500mg ドライシロップ50%

Ekeppra レベチラセタム製剤

抗てんかん剤
処方箋医薬品（注意一医師等の処方箋により使用すること）　薬価基準収載

イーケプラ® 点滴静注500mg

Ekeppra レベチラセタム注射液

●効能・効果、用法・用量、禁忌を含む使用上の注意等については添付文書をご参照ください。

販売
Otsuka 大塚製薬株式会社
東京都千代田区神田司町2-9

資料請求先
大塚製薬株式会社 医薬情報センター
〒108-8242 東京都港区港南2-16-4　品川グランドセントラルタワー

製造販売元
ucb ユーシービージャパン株式会社
東京都新宿区西新宿8丁目17番1号

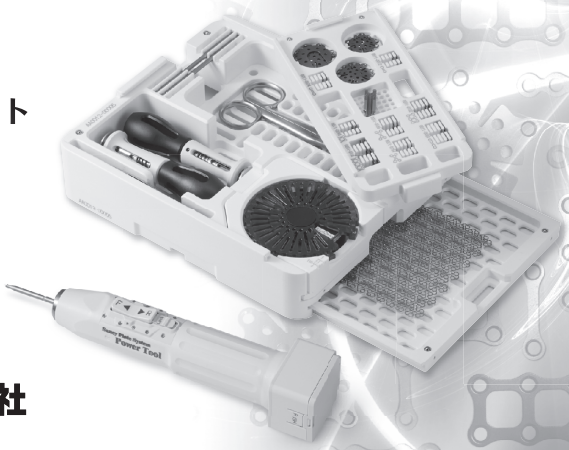
（'18.9 改訂）

未滅菌　頭蓋骨固定用プレートシステム

サンエープレートシステムⅡ

Saney Plate System Ⅱ

0.4mmの
ロープロファイルプレート



製造販売元
サンエー精工株式会社
埼玉県朝霞市泉水2-10-14
TEL.048-466-9551　FAX.048-463-9419
販売名：サンエープレートシステムⅡ　　医療機器製造販売承認番号：21900BZX00931000
販売名：サンエープレートシステムパワーツール　　一般医療機器届出番号：11B1X10002000012

iCSD 2019

International Conference on Spreading Depolarizations