GRAY MATTERS

WORKSHOP REPORT

Michael Foundation Forum 2012, Berlin, Germany

From October 4th to 6th, 2012, Berlin hosted the 11th edition of the Michael Forum. The special occasion of the 50th anniversary jubilee of the Michael Foundation brought together 24 Michael laureates to discuss the latest advancement in epilepsy research. The prestigious biannual Michael Prize, established in 1963 "to support scientific research on most effective treatment methods as well as the causes of illnesses associated with seizures, and to fight against individual and social consequences of epileptic diseases," awards young investigators for their innovative contributions to clinical and experimental epilepsy research. Michael Forum 2012 was sponsored by UCB.

The first session of the Forum focused on *epilepsy research in the developing, immature brain*. Epileptogenesis and seizure recurrence in the immature brain is associated with significant comorbidity and mortality. *Eli Mizrahi* noted that current animal models of epilepsy may not reflect conditions in the human neonate in terms of patterns, generators, and burden of seizures. Although potential new therapies and understanding of epilepsy dynamics have been generated in the laboratory, the new clinical strategies they suggest may not directly translate to the human neonate. *Solomon Moshé* presented a rat model of symptomatic infantile spasms (IS) induced by intracerebral administration of doxorubicin and lipopolysaccharide at postnatal (PN) day 3. Spasms appeared at PN4–13, followed at PN9 by other seizure types associated with neurodevelopmental and learning deficits. Spasms are adrenocorticotropic hormone (ACTH)-refractory, transiently sensitive to vigabatrin, and resistant to phenytoin. This model has been used to identify antiepileptic drugs (AEDs) that suppress human spasms acutely and modify the disease course (carisbamate, rapamycin), and it is well qualified for preclinical screening of disease-modifying and antiepileptogenic therapies and associated neurodevelopmental comorbidities. Progressive cognitive impairment due to underlying etiology, epileptiform discharges, and AEDs can be a devastating comorbidity of early life epilepsy. *Gregory Holmes* discussed how remedial training helps restoring memory function after early life seizures. Seizure-exposed rats showed initial difficulties in learning memory task, but performed similar to control rats when training was extended. During memory tasks, *theta* oscillatory activity increased in prefrontal cortex during early memory training and plateaued during extra training. CA1-prefrontal *theta* coherence was enhanced in correct (delayed) compared to incorrect (early) trials. The data support the idea that adapted involvement of hippocampal–prefrontal circuits following early life seizures may underpin cognitive rehabilitation following neurologic insults. Cardiac arrhythmia and respiratory deficiency have long been suspected to contribute to sudden unexpected death in epilepsy (SUDEP) following seizures both in pediatric and adult patients. *Jeffrey Noebels* reported that human mutations of *KCNQ1*, a gene that encodes for a potassium channel expressed in both heart (responsible for a lethal cardiac arrhythmia) and brain, produce a combined phenotype of epilepsy, cardiac long QT-interval syndrome (LQTS), and sudden death. LQT genes alter cardiorespiratory control centers that may impact SUDEP risk. Clinical and scientific research programs aimed at integrating a worldwide collection of SUDEP and near-SUDEP cases and their families have been planned to discover clinically useful genes. *George Kostopoulos* discussed the implication of thalamocortical circuits in altered cortical excitability in generalized spike and wave discharges (GSWDs), typical of childhood absence epilepsy and juvenile myoclonic epilepsy (JME). Bistability is evident in thalamus (tonic–phasic activity) and cortex (*down–up* states) in sleep–wake cycles/stages and during non–rapid eye movement (REM) cyclic alternating pattern (CAP). In patients, GSWDs appear mainly in cortical *up*-state of CAPs and show different topographic distribution compared to interictal and pregeneralization focal SWDs. During sleep transitions, corticothalamic responses become strong enough to generate autonomous GSWDs emerging from a preset system of bilaterally distributed cortical and subcortical networks.

JME is a form of epilepsy easy to control with AEDs, but likely to relapses after stress and sleep deprivation. *Dieter Janz* reviewed the evidence that from four longitudinal studies, demonstrating a relatively low frequency of relapses after AED withdrawal and a corresponding high rate of cured patients, when long observation periods (20–30 years) are evaluated, suggesting that long-term prognosis of JME without AED treatment is better than previously assumed. Combination with absences and polytherapy were identified as significant predictors for a poor seizure outcome.

During the second session of the Forum, the *use of human biologic data/specimens to study epilepsy* was discussed. The temporal cortex of patients with drug-resistant epilepsy undergoing epilepsy surgery treatment can be

used to study pharmacoresistance and to explore new AEDs. *Uwe Heinemann* examined drug resistance in postsurgery neocortical human in vitro slices by studying seizure-like events induced by pharmacologic manipulations. After AED administration, seizure-like events persisted in most slices, even with coadministration of drug-transport inhibitors and AEDs. These studies suggested that inhibition of multidrug-resistance proteins may modify responses to AEDs, but does not reverse drug resistance. Moreover, adenosine and sK channel agonists inhibited seizure events in human tissue. Evidence to support the hypothesis of multidrug-transporter overexpression as a mechanism of pharmacoresistance was presented by *Matthias Koepp*. Using [11C]verapamil positron-emission tomography (PET) in human temporal lobe epilepsy (TLE), he showed that more efficient Pgp function was found in drug-refractory in comparison to drug-sensitive patients, resulting in lower brain AEDs concentrations. Tariquidar administration demonstrated overexpression of Pgp in epileptogenic hippocampus of pharmacoresistant patients. The availability of imaging biomarkers, such as verapamil-PET, supports new treatment strategies targeted at multidrug transporter to reverse pharmacoresistance. Mechanisms of AED transporter regulation in epilepsy were further discussed by *Heidrun Potschka*. Overexpression of blood–brain barrier efflux transporters, putatively contributing to drug resistance, has been repeatedly described in rodent epilepsy models and in the human epileptic brain. An endothelial *N*-methyl-D-aspartate (NMDA) receptor/cyclooxygenase-2/EP1 receptor pathway that induces enhanced functional expression of Pgp was identified as the cause of seizure-associated induction of this efflux transporter in rodent epilepsy models. The same signaling pathway seems to be active in human capillaries prepared from tissue dissected during epilepsy surgery. The data might render a translational basis for targeting approaches to control Pgp. *Eleonora Aronica* discussed the role of astrocytes in the regulation of the immune/inflammatory response in epileptic human brain tissue. Accumulating experimental evidence indicates that proinflammatory molecules can alter glioneuronal communication and contribute to the generation of seizures and seizure-related neuronal damage. In this context, understanding of the astroglial inflammatory response in the epileptic brain and the mechanisms underlying its regulation may provide new strategies to target astrocyte-mediated epileptogenesis. The lateral nucleus of the amygdala (LA) gives rise to most of the projections from the amygdala to the hippocampal area. *Erwin Josef Speckmann* updated knowledge on functional mesiotemporal amygdala connections in postsurgical human specimens. Combined neurophysiologic and imaging experiments on in vitro amygdala slices obtained from mesial temporal lobectomies revealed peculiar patterns of activity propagation that sustain anatomofunctional longitudinal interactions within the lateral amygdala.

The third session was dedicated to the *studies on epileptogenesis* and to the development of antiepileptogenic strategies. *Alon Friedman* reviewed in vivo and in vitro experiments in which rat brains were exposed to "serum-like" conditions. BBB protection from extravasation of serum proteins is crucial to control neuronal excitability, glia functions, brain immune response, and vascular response to neuronal activation. BBB function is a key to pharmacotherapeutics of peripheral-acting and central-acting drugs. BBB opening may allow the delivery of peripheral-acting drugs into the central nervous system, and may also alter the effect of brain-permeable drugs and contribute to pharmacoresistance. Matrix metalloproteinases (MMPs) are extracellular matrix proteases involved in tissue repair, cell death, and morphogenesis. Evidence has been generated that implicates involvement of MMPs in epilepsy and epileptogenesis. *Chrysanthy Ikonomidou* presented data on the involvement of MMP9 in trauma-related and seizure-related cell injury after pilocarpine-induced seizures in the developing brain. Different proepileptogenic brain insults downregulate the K^+-Cl^- cotransporter (KCC2) and upregulate Na^+-K^+-$2Cl^-$ cotransporter (NKCC1), leading to an increase in intracellular Cl^- that may contribute to the development of neuronal hyperexcitability. *Wolfgang Löscher* showed that the selective NKCC1 inhibitor, bumetanide, may counteract the upregulation of NKCC1 during epileptogenesis. This drug exerts disease-modifying effects when coadministered with phenobarbital after a pilocarpine-induced status epilepticus in rats. Derivatives of bumetanide with longer half-life and with masked acid group to enhance brain penetration to prolong maintenance in the brain were developed. The most promising prodrug (BUM5) exerted anticonvulsant and disease-modifying effects in mouse and rat models of epilepsy. *Istvan Mody* examined the hypothesis that dentate gyrus granule cells "caught" by the epileptogenic insult around 4 weeks of age are the key elements in temporal lobe epileptogenesis. At this postnatal age, these cells present signs of greatest neuronal plasticity (spine appearance, new synaptic contacts, shift of Ca^{2+} binding proteins, basal dendrite formation, and so on). Neurons born 4 weeks before pilocarpine- or kainic acid–induced status epilepticus tested during the period of epileptogenesis show doublet spiking that could provide a powerful "detonator" drive for the mossy fiber-to-CA3 pyramidal synapses. These findings suggest that 4-week-old granule cells could be the true "hub" cells of the epileptogenic hippocampal formation.

The last session focused on understanding the nature and the *mechanisms of generation of seizures (ictogenesis)* and its control by *AEDs*. Any "normal" brain can be provoked into seizures, for example, following a convulsive

electroshock in human, or following the injection of various chemical compounds in experimental models of epilepsy. *Christophe Bernard* proposed that seizures belong to the dynamic repertoire of possible brain activities. Their relative stereotypy across both different brain regions and different neuroarchitectures suggests the existence of generic laws governing seizure dynamics. Seizures can be fully described by five state variables that contribute to predict seizure onset, development, and offset, which were verified experimentally. The concept that diverse seizure patterns in specific brain regions are possibly sustained by different network mechanisms was discussed by *Marco de Curtis.* Pharmacologic treatments of the in vitro isolated guinea pig brain with either γ-aminobutyric acid ($GABA_A$)-receptor antagonists or potassium channel blocker (4-aminopyridine, 4AP)–induced independent network–specific epileptiform patterns in olfactory areas and in the hippocampal–parahippocampal region. Interictal and ictal discharges were sustained by different sequences of events, confirmed to be region specific. In both systems, seizure onset was associated with reduction of principal neuron activity. Prolonged synaptic depression was observed in olfactory cortices but not in limbic areas. *Massimo Avoli* discussed the relationships between interictal-like and ictal-like discharges in limbic areas of in vitro brain slices during 4AP. Interictal events have a wide range of duration and interval of occurrence, and ictal discharges either are shortly preceded by an isolated "slow" interictal discharge or initiate suddenly from a pattern of polyspike interictal discharge. High-frequency oscillations (HFOs) were recorded during slow interictals and at ictal onset. Therefore, the HFOs during the interictal period may define ictal-onset characteristics that are reminiscent of those seen in vivo when seizure onset is characterized by low-voltage fast activity. It has recently become possible to control specific subsets of neurons utilizing light-sensitive channels or pumps (opsins), allowing for unprecedented specific and immediate control of cell populations of interest in the behaving animal. However, the widespread use of these novel optogenetic technologies has so far been difficult to apply to epilepsy, in part because of the unpredictable nature of the seizures. *Ivan Soltez* described the development and the use of a closed-loop system for detecting and using optogenetic methods to respond to spontaneous electrographic and behavioral seizures in mice. These results suggest that diverse on-demand optogenetic strategies can be effective at controlling seizure activity. *Rüdiger Köhling* explored the role of afterhyperpolarizing potentials (AHPs) in the control of neuronal epileptic excitability. The impact of $GABA_A$-receptor blocker (gabazine)–induced epileptiform activity on AHP was studied in hippocampal rat slices using intracellular recordings of CA1 neurons. Spontaneous epileptiform discharges induced a depolarizing shift of the resting membrane potential and AHP reduction. These changes were prevented by glutamate receptor antagonists and by protein kinase blockers H-9 and H-89, suggesting that the AHP is suppressed due to a phosphorylation process involving protein kinase A. The use of $GABA_A$-receptor modulating agents, including benzodiazepine (BZD), is limited by drug's psychopharmacological profile, tolerance, and additive potentials. *Chris Rundfeldt* examined seizure control by low affinity, partial agonists of BZD receptor (imepitoin and AWD 131–139), and showed potent anticonvulsant and anxiolytic activity without sedative, hypnotic, or muscle relaxant properties. In drug discrimination trials, monkeys trained to discriminate full BZD agonists did not recognize imepitoin as BZD-like drug and imepitoin did not substitute for cocaine in self-administration trials. Phase I clinical trials revealed excellent tolerability for this compound class. For AWD 131–139, dose-dependent CNS modulation was proven using pharmaco—electroencephalography (EEG). *Les Turski* reported history of development of the selective and noncompetitive glutamate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) antagonist, perampanel, first identified in 1998. Despite hesitation to develop glutamate antagonists raised after failure of NMDA antagonists in clinical trials, perampanel was shown in clinical trials to be safe, and well tolerated, and to decrease seizure frequency over at least 1 year of exposure in patients with refractory partial-onset seizures.

Acknowledgments

The attendees of the Forum are grateful to the Michael Foundation for the opportunity to meet every 2 years, providing a unique platform to discuss latest findings in epilepsy research. UCB supported the Forum with an unrestricted educational grant and did not influence the program of the meeting or the contents of this report.

Disclosure

The author has no conflict of interest to declare. I confirm that I have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Letter

Seizures and the neuro-cardio-endocrine axis

To the Editors:

We read with interest the article by Hocker et al. (2012) reporting on the spectrum of cardiac injury in