Chronically Epileptic Human and Rat Neocortex Display a Similar Resistance Against Spreading Depolarization In Vitro

Anna Maslarova, MD; Mesbah Alam, PhD; Clemens Reiffurth, MD; Ezequiel Lapilover, MD; Ali Gorji, MD; Jens P. Dreier, MD

Background and Purpose—Experimental and clinical evidence suggests that prolonged spreading depolarizations (SDs) are a promising target for therapeutic intervention in stroke because they recruit tissue at risk into necrosis by protracted intracellular calcium surge and massive glutamate release. Unfortunately, unlike SDs in healthy tissue, they are resistant to drugs such as N-methyl-D-aspartate-receptor antagonists. This drug resistance of SD in low perfusion areas may be due to the gradual rise of extracellular potassium before SD onset. Brain slices from patients undergoing surgery for intractable epilepsy allow for screening of drugs, targeting pharmacoresistant SDs under elevated potassium in human tissue. However, network changes associated with epilepsy may interfere with tissue susceptibility to SD. This could distort the results of pharmacological tests.

Methods—We investigated the threshold for SD, induced by a gradual rise of potassium, in neocortex slices of patients with intractable epilepsy and of chronically epileptic rats as well as age-matched and younger control rats using combined extracellular potassium/field recordings and intrinsic optical imaging.

Results—Both age and epilepsy significantly increased the potassium threshold, which was similarly high in epileptic rat and human slices (23.6±2.4 mmol/L versus 22.3±2.8 mmol/L).

Conclusions—Our results suggest that chronic epilepsy confers resistance against SD. This should be considered when human tissue is used for screening of neuroprotective drugs. The finding of similar potassium thresholds for SD in epileptic human and rat neocortex challenges previous speculations that the resistance of the human brain against SD is markedly higher than that of the rodent brain. (Stroke. 2011;42:00-00.)

Key Words: epilepsy ■ human brain slices ■ neuroprotection ■ spreading depression

Several hundred Phase II and III clinical trials on presumed neuroprotective agents for stroke and brain trauma have failed in the last decades (see Washington University Internet Stroke Center, www.strokecenter.org). Obviously, novel approaches to the translation from “bench” to “bedside” have to be adopted. A new roadmap for neuroprotection was suggested by Donnan in 2007, the first 4 steps being: (1) better proof of efficacy in animal models; (2) in vitro efficacy in human tissue; (3) in vivo studies of the distribution of neuroprotectants in the human brain; and (4) efficacy in novel human models of acute neuronal injury to prove/disprove the concept. Only after Step 4, novel neuroprotective strategies should enter a Phase II clinical trial. We focused on the second step: “in vitro efficacy in human tissue.” Brain slices from patients with pharmacoresistant epilepsy provide the most physiological preparation of human tissue currently available for studying neuroprotectants in vitro. Unlike human cell cultures, they allow investigating neuroprotective effects on highly complex networks such as neocortex.

In neocortex, the term spreading depolarization (SD) describes abrupt near-complete breakdown of the electrochemical gradients across the neuronal membranes, which leads biochemically and morphologically to the cytotoxic edema. SD is observed extracellularly as a large negative slow field potential shift. Experimental evidence suggests that prolonged SDs recruit tissue at risk into necrosis by massive glutamate release and protracted rise of intracellular calcium. In the clinic, clusters of SDs were found in patients with delayed ischemic stroke after subarachnoid hemorrhage and in the course of malignant hemispheric stroke. Preliminary clinical findings suggest that very prolonged SDs are associated with lesion progression in a similar fashion to that in animals.
The cooperation of several ion channels mediates sodium and calcium inward fluxes that lead up to SD.\textsuperscript{6,7} Whereas N-methyl-D-aspartate-receptor antagonists can prevent SD in healthy brain tissue,\textsuperscript{8} their potency to suppress SD is greatly reduced under hypoxia, sodium pump inhibition, or artificial increase of extracellular potassium ([K\textsuperscript{o}]).\textsuperscript{9,10} Preliminary evidence suggests that such differences in susceptibility to N-methyl-D-aspartate-receptor antagonists also apply to SDs in the human brain.\textsuperscript{11,12}

Ischemia causes a gradual rise in [K\textsuperscript{o}], before SD onset, presumably due to decline of sodium pump activity and activation of adenosine 5'-triphosphate- and calcium-sensitive potassium channels.\textsuperscript{13} This gradual rise of baseline [K\textsuperscript{+}], could be responsible, at least partially, for drug resistance of SDs under energy depletion.\textsuperscript{9} Hence, it has been suggested that the high potassium model in human brain slices from patients with intractable epilepsy allows for drug screening targeting drug-resistant SDs in human tissue. However, network changes associated with epilepsy may influence SD susceptibility. Therefore, we investigated effects of age and chronic epilepsy on the potassium threshold for SD in rat neocortical slices and compared the thresholds between chronically epileptic human and rat neocortex. We also investigated whether the effect of epilepsy could be due to changes of GABAergic mechanisms in epileptic tissue.

Materials and Methods
The study was approved by the local ethics committee. Written informed consent was obtained from each patient. For detailed description of the methods, see the online Supplemental Methods section (http://stroke.ahajournals.org).

Drugs
Bicuculline methiodide was purchased from Sigma.

Chronic Epilepsy
Chronic epilepsy was triggered by status epilepticus at 12 to 14 weeks due to intraperitoneal injection of pilocarpine as described previously (see online supplemental section).\textsuperscript{14}

Slice Preparation
Neocortical slices (500 \textmu m) from human temporal or frontal lobe resectates (Supplemental Table I) and horizontal temporohippocampal slices (400 \textmu m thick) from male Wistar rats (Charles River Laboratories, Sulzfeld, Germany; 10-month-old chronically epileptic rats, age-matched controls, and 8-week-old young controls) were prepared as described previously.\textsuperscript{9} The slices were stored in a humidified, carbogenated interface-type chamber perfused with pre-warmed (36°C) artificial cerebrospinal fluid (aCSF) containing (in mM) 129 NaCl, 3 KCl, 1.8 MgSO\textsubscript{4}, 1.6 CaCl\textsubscript{2}, 1.25 NaH2PO\textsubscript{4}, 21 NaHCO\textsubscript{3}, 10 glucose, pH 7.4, osmolality 303 mOsm/kg.

Extracellular Recordings and Stimulation
Combined field potential/potassium-sensitive microelectrodes were connected to a custom-made differential amplifier and recorded the direct current (DC) shift and [K\textsuperscript{o}] in layer III of human neocortex or temporal and ectorhinal rat neocortex. Signals were filtered at 1 kHz (field potential) and 3 Hz ([K\textsuperscript{o}]), then sampled at a rate of 5 kHz and 10 Hz, respectively, by a CED 1401 (Cambridge Electronic Design Limited, Cambridge, UK) and stored on a PC. Slice viability was tested by recording responses to orthodromic bipolar stimulation in layer V. Single or paired stimuli (0.1 ms, 1 to 10 V, 50-ms interval) were delivered using a stimulus isolator in constant voltage

### Table: Comparison of SD Parameters

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Young Control Rats</th>
<th>Group 2 Old Control Rats</th>
<th>Group 3 Chronically Epileptic Rats</th>
<th>Group 4 Chronically Epileptic Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>[K\textsuperscript{o}] for SD, mM</td>
<td>18.1 ± 0.9 (P&lt;0.001 vs Group 2)</td>
<td>20.8 ± 2.6 (P=0.027 vs Group 3)</td>
<td>23.6 ± 2.4 (P&lt;0.001 vs Group 4)</td>
<td>22.3 ± 2.8 (P&lt;0.001 vs Group 4)</td>
</tr>
<tr>
<td>Latency until first SD, min</td>
<td>20.9 ± 13.2 (P=0.001 vs Group 2)</td>
<td>54.4 ± 28.9 (P=0.001 vs Group 3)</td>
<td>82.8 ± 28.4 (P&lt;0.001 vs Group 4)</td>
<td>69.6 ± 31.3 (P&lt;0.001 vs Group 4)</td>
</tr>
<tr>
<td>Tissue [K\textsuperscript{o}] before first SD, mM</td>
<td>13.3 ± 2.9 (P&lt;0.001 vs Group 2)</td>
<td>16.0 ± 6.8 (P&lt;0.001 vs Group 3)</td>
<td>20.0 ± 5.2 (P&lt;0.001 vs Group 4)</td>
<td>18.0 ± 4.2 (P&lt;0.001 vs Group 4)</td>
</tr>
<tr>
<td>Tissue [K\textsuperscript{o}] peak during SD, mM</td>
<td>-14.7 ± 4.7 (P&lt;0.001 vs Group 2)</td>
<td>-13.7 ± 3.8 (P&lt;0.001 vs Group 3)</td>
<td>-9.6 ± 1.5 (P&lt;0.001 vs Group 4)</td>
<td>-18.0 ± 2.6 (P&lt;0.001 vs Group 4)</td>
</tr>
<tr>
<td>DC amplitude, mV</td>
<td>60 ± 21 (P&lt;0.001 vs Group 2)</td>
<td>100 ± 48 (P&lt;0.001 vs Group 3)</td>
<td>54 ± 29 (P&lt;0.001 vs Group 4)</td>
<td>81 ± 49 (P&lt;0.001 vs Group 4)</td>
</tr>
<tr>
<td>SD duration at half-maximal DC shift, sec</td>
<td>6.5 ± 1.5 (P&lt;0.001 vs Group 2)</td>
<td>4.0 ± 0.5 (P&lt;0.001 vs Group 3)</td>
<td>3.8 ± 1.1 (P&lt;0.001 vs Group 4)</td>
<td>4.9 ± 1.3 (P&lt;0.001 vs Group 4)</td>
</tr>
</tbody>
</table>

SD indicates spreading depolarization; [K\textsuperscript{o}] extracellular potassium; aCSF, artificial cerebrospinal fluid; DC, direct current.
mode (ISO Flex; AMPI Instruments, Jerusalem, Israel) controlled by a Master-8 (AMPI Instruments). Only slices showing maximal amplitudes of the population spike $\pm 1$ mV were used.

Intrinsic Optical Imaging
Intrinsic optical signals were obtained with a CCD camera at the same time as transilluminating slices with white light to record SD propagation.9

Data Analysis
Data analysis was performed with Spike2 (Version 6; Cambridge Electronic Design Limited, Cambridge, UK) and MATLAB. Data are given as mean value $\pm$ SD. The groups were compared using 1-way analysis of variance with Fisher least significant difference post hoc test unless otherwise stated. $P \leq 0.05$ was considered statistically significant.

Results
Potassium Threshold for SD
The SD threshold in response to stepwise rises of the potassium concentration in the aCSF ($[K^+]_{acSF}$) was compared among brain slices of chronically epileptic human neocortex (EpiHum, n=10), chronically epileptic rats (EpiRats, n=7), age-matched (old) control rats (OCoRats, n=9), and young control rats (YCoRats, n=8). $[K^+]_{acSF}$ was increased initially to 17.5 mmol/L and increased further by 2.5 mmol/L every 30 minutes until the first SD occurred. The $[K^+]_{acSF}$ threshold for SD was significantly lower in the YCoRats compared with those of the other groups (Table). Moreover, OCoRats showed a significantly lower threshold than EpiRats and the thresholds were similar in neocortex of EpiRat and EpiHum tissue. Linear regression of the pooled data of all groups demonstrated that the time until the first SD correlated significantly with $[K^+]_{o}$ as measured in the tissue immediately before the first SD ($R=0.508, P=0.004$). This indicates a difference in tissue tolerance toward higher levels of $[K^+]_{o}$ without SD generation rather than different rates of potassium buffering between the groups. Peak-to-peak amplitude of the DC shift was significantly larger in the EpiHum group, whereas it was significantly smaller in EpiRat neocortex (Table). SD propagation velocity in YCoRats was significantly higher than that in the other groups (Table; Figures 1 and 2). No significant differences in peak concentrations of $[K^+]_{o}$ and SD duration (at 50% of the maximal DC amplitude) were observed.

SD Frequency Under Elevated Baseline $[K^+]_{acSF}$
Perfusion with 25 mmol/L $[K^+]_{acSF}$ for 45 minutes elicited multiple SDs in all groups. Average SD incidence was significantly higher in YCoRats than in the other groups. SD incidence in OCoRats was higher than in the epileptic groups: YCoRats: 14.4 $\pm$ 5.0 SDs (n=8); OCoRats: 8.3 $\pm$ 4.1 SDs (n=8); EpiRats: 4.0 $\pm$ 2.1 SDs (n=6); EpiHum: 2.0 $\pm$ 1.5 SDs (n=6); YCoRats versus EpiRats and EpiHum: $P<0.001$; YCoRats versus OCoRats: $P=0.003$; OCoRats versus EpiRats and EpiHum: $P=0.044$ and $P=0.005$, respectively. Incidence in the 2 epileptic groups was similar. SDs often merged in clusters of OCoRats and YCoRats as reported.
This phenomenon was not observed in the epileptic groups (Figure 3B). The DC shift was significantly smaller in EpiRats compared with the other groups: YCoRats: $9.0 \pm 3.2$ mV, $P < 0.05$; OCoRats: $-14.5 \pm 2.7$ mV, EpiHum: $-13.6 \pm 3.5$ mV. Differences in SD velocities and DC durations did not reach statistical significance between groups.

Bicuculline

aCSF containing bicuculline (50 $\mu$mol/L) induced spontaneous SDs in 4 of 7 YCoRats and 4 of 9 OCoRats. Moreover, interictal-like activity and seizure-like events were triggered in 5 and 1 YCoRats, respectively, and 5 and 2 OCoRats, respectively. Neither SDs nor epileptiform activities were induced by bicuculline in the epileptic tissues ($n=6$ EpiRats, $n=5$ EpiHum). Artificial rise of $[K^+]_{aCSF}$ always terminated epileptiform activity in the control groups. In presence of bicuculline, SD number per 45 minutes significantly increased in slices of OCoRats (8.3$\pm$4.1 versus 13.4$\pm$4.3 SDs $[n=8]$, $t$ test, $P=0.03$) as well as EpiRats (4.0$\pm$2.1 versus 7.7$\pm$2.0 SDs $[n=6]$, $P=0.011$) and EpiHum tissue (2.0$\pm$1.5 versus 4.4$\pm$0.9 SDs $[n=5]$, $P=0.014$). There was no significant effect in YCoRats (14.4$\pm$5.0 versus 15.7$\pm$4.9 SDs $[n=7]$). Bicuculline did not affect SD duration and DC amplitude. Only in YCoRats, SD propagation velocity was significantly faster in presence of bicuculline (11.8$\pm$4.6 mm/min versus 6.0$\pm$1.5, $P=0.015$).

In YCoRats, we possibly missed the enhancing effect of bicuculline on SD frequency because the SD frequency was already very high at 25 mmol/L $[K^+]_{aCSF}$, even in the absence of bicuculline. Therefore, we tested whether bicuculline would influence the potassium threshold for SDs in YCoRats starting with $[K^+]_{aCSF}$ at 12.5 mmol/L and increasing it by 2.5 mmol/L every 30 minutes. Bicuculline lowered significantly the threshold from 17.8$\pm$2.1 to 14.4$\pm$3.1 mmol/L ($n=8$, paired $t$ test, $P=0.01$). We then tested thresholds in presence of bicuculline in 8 patients to verify that bicuculline would also lower the potassium threshold in epileptic human neocortex. Consistently, we found a significant decrease from 22.5$\pm$3.0 to 19.7$\pm$1.6 mmol/L ($n=8$, paired $t$ test, $P=0.015$).

Discussion

In the current study, we investigated 2 different measures of tissue susceptibility to SD in vitro: (1) the potassium threshold for SD; and (2) the SD frequency under elevated $[K^+]_{aCSF}$. The latter approach was also used previously in vivo. Both age and epilepsy increased resistance against SD in rats; resistance was not different between chronically epileptic rat and human neocortex. Slices from young control rats showed faster SD propagation similar to a previous study in gerbils. Bicuculline increased SD susceptibility in all groups and propagation velocity in young control rats.

The terms epileptiform activity and SD describe the 2 fundamental spectra of pathological network disturbances in the cortex. Whereas epileptiform ictiform activity is character-
Thus, resistance against SD seems to increase in models for acute status epilepticus. Similar observations also exist in patients with acute neuronal injury in whom SDs were significantly more abundant than epileptic activities, but experimental evidence suggests that status epilepticus increases resistance against SD and whether this protects against ischemic damage. To our knowledge, this is unknown but experimental evidence suggests that status epilepticus early in life in fact modifies infarct volume later in life. However, this effect seems complex because it depended on the inducer of status epilepticus.

A role of GABAergic transmission for SD ignition was suggested previously. Those authors observed that subepileptic doses of bicuculline triggered SD in neocortical slices from healthy rats in contrast to slices from rats and patients with epilepsy in a similar fashion to the present study. Experimental evidence suggests that GABAergic transmission is disturbed in chronically epileptic human tissue. However, in the present study, bicuculline increased the susceptibility to SD under high [K+]o in a similar fashion in all groups. This suggests that differences in susceptibility to SD between groups were not mediated by altered GABAergic transmission or by differences in SK channel function. Further studies should determine why chronic epilepsy increases resistance against SD and whether this protects against ischemic damage. To our knowledge, this is unknown but experimental evidence suggests that status epilepticus early in life in fact modifies infarct volume later in life. However, this effect seems complex because it depended on the inducer of status epilepticus.

It has been proposed that SDs are a target for therapeutic intervention in the ischemic penumbra because their blockade may inhibit lesion progression. Previous studies reported that the resistance against SD is significantly higher in human epileptic tissue compared with that of healthy rats. We confirmed this here. Nevertheless, this was often related to species differences rather than structural and functional alterations of epileptic tissues because earlier reports had suggested that the gyrencephalic cortex of cats and monkeys is more resistant against SD than the lissencephalic cortex of rodents. However, we found that rat and human chronically epileptic tissues were similarly resistant against SD. Consistently, recent clinical trials suggested that patients with hemorrhagic and ischemic stroke or brain trauma display SDs in vivo in a similar fashion to rodents. Thus, species differences may be overestimated. The present data indicate that epileptic network changes could be related to alterations in potassium buffering: ictal activity is associated with moderate rise of [K+]o to the ceiling level of approximately 12 mmol/L. Repeated stimulus-induced rises of [K+]o to this level seem to boost up mechanisms for potassium reuptake, which should counteract SD ignition. However, our findings suggest that age and epilepsy increased the tissue’s ability to tolerate higher [K+]o rather than buffer it more efficiently.

By inhibiting GABAα receptors with bicuculline, we investigated whether altered GABAergic transmission is responsible for differences in SD threshold. GABAα receptors are ligand-gated ion channels concerned mainly with passing of chloride ions across the cell membrane. Moreover, bicuculline inhibits small-conductance calcium-activated potassium channels (SK channels), which mediate slow afterhyperpolarization after the action potential in many neurons. Above 5 μmol/L, bicuculline induces epileptiform activity in healthy neocortex. Such epileptiform activity was inhibited by the high potassium medium in the present study. Excess [K+]o forces chloride uptake and this may decrease the proepileptic effect of inhibiting GABAα receptors, which are dependent on the chloride equilibrium potential.

A role of GABAergic transmission for SD ignition was suggested previously. Those authors observed that subepileptic doses of bicuculline triggered SD in neocortical slices from healthy rats in contrast to slices from rats and patients with epilepsy in a similar fashion to the present study. Experimental evidence suggests that GABAergic transmission is disturbed in chronically epileptic human tissue. However, in the present study, bicuculline increased the susceptibility to SD under high [K+]o in a similar fashion in all groups. This suggests that differences in susceptibility to SD between groups were not mediated by altered GABAergic transmission or by differences in SK channel function. Further studies should determine why chronic epilepsy increases resistance against SD and whether this protects against ischemic damage. To our knowledge, this is unknown but experimental evidence suggests that status epilepticus early in life in fact modifies infarct volume later in life. However, this effect seems complex because it depended on the inducer of status epilepticus.

It has been proposed that SDs are a target for therapeutic intervention in the ischemic penumbra because their blockade may inhibit lesion progression. Previous studies reported that the resistance against SD is significantly higher in human epileptic tissue compared with that of healthy rats. We confirmed this here. Nevertheless, this was often related to species differences rather than structural and functional alterations of epileptic tissues because earlier reports had suggested that the gyrencephalic cortex of cats and monkeys is more resistant against SD than the lissencephalic cortex of rodents. However, we found that rat and human chronically epileptic tissues were similarly resistant against SD. Consistently, recent clinical trials suggested that patients with hemorrhagic and ischemic stroke or brain trauma display SDs in vivo in a similar fashion to rodents. Thus, species differences may be overestimated. The present data indicate that epileptic network changes could be more important than species differences for the SD threshold, and effects of neuroprotectants could be overestimated in epileptic tissue. It is unlikely that the results would have been different when the
patients had not been pretreated with antiepileptic drugs because experimental evidence suggests that antiepileptic drugs further increase the SD threshold.1,6

In conclusion, we recommend that results gained in human epileptic tissue are compared with those in both nonepileptic and epileptic tissues of old rodents. With this modification, preclinical studies according to step 2 of Donnan’s new roadmap for acute neuroprotection in stroke should reach higher validity.1

Sources of Funding
Supported by grants from the Deutsche Forschungsgemeinschaft (DFG SFB Tr3 D10), the Bundesministerium für Bildung und Forschung (Center for Stroke Research Berlin, 01 EO 0801), the Bernstein Center for Computational Neuroscience Berlin 01GQ1001C B2, and the Kompetenznetz Schlaganfall (J.P.D.).

Disclosures
None.

References
Chronically Epileptic Human and Rat Neocortex Display a Similar Resistance Against Spreading Depolarization In Vitro
Anna Maslarova, Mesbah Alam, Clemens Reiffurth, Ezequiel Lapilover, Ali Gorji and Jens P. Dreier

Stroke. published online August 11, 2011;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2011/08/11/STROKEAHA.111.621581

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2011/08/11/STROKEAHA.111.621581.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/
Supplemental Methods

Chronically Epileptic Rats

All animal experiments were performed in compliance with the Governmental Animal Care and Use Committee (Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit Berlin (LAGetSi)). Adult male Wistar rats (12-14 weeks old) were intraperitoneally injected with pilocarpine (350mg/kg body weight [BW]) 30min after pre-treatment with methylscopolamine (1mg/kg subcutaneously). All animals developed a generalized convulsive status epilepticus. In order to terminate the status, pentobarbital (50mg/kg BW) was injected 60min after onset. Behavioral signs of status epilepticus disappeared approximately 1h later. Animals were kept in separate cages under standard housing conditions and light-dark cycle. Video recordings were performed to monitor the development of spontaneous epileptic activity. A seizure-free interval of two to six weeks was followed by spontaneous recurrent seizures, characterized by headshaking, staring, oral automatism, rearing and loss of consciousness. The seizures lasted as long as the animals were alive.

Hippocampal Slice Preparation

Male wistar rats (8-weeks and 10-months old control and 10-months old chronically epileptic pilocarpine-treated rats) were anesthetized with ether and decapitated. The brains were rapidly removed in ice-cold, carbogenated artificial cerebrospinal fluid (aCSF) containing (in mM) 129 NaCl, 3 KCl, 1.8 MgSO4, 1.6 CaCl2, 1.25 NaH2PO4, 21 NaHCO3, 10 glucose, pH 7.4, osmolality 303 mOsm/kg. The two brain hemispheres were separated by a median sagittal cut and horizontal slices (400 μm thick) were obtained (including hippocampal formation, ento-and perirhinal cortex as well as ectorhinal and temporal neocortex) using a vibratome (Campden Instruments, Leicester, UK). The slices were immediately transferred to a humidified, carbogenated (5%CO2, 95% O2) interface-type recording chamber perfused at a rate of 1.7ml/min with prewarmed (36°C) aCSF. Slices were allowed to recover for at least 1h before recording.

Human Neocortical Slices

The study was approved by the local ethics committee and written informed consent was obtained from each patient. Experiments were performed on neocortical tissue of patients
suffering from drug-resistant temporal or frontal lobe epilepsy (see supplementary Table S1). In selected patients, subdural ECoG recordings were clinically indicated prior to surgery to locate the epileptic focus. Temporal or frontal lobe resectates from patients with intractable epilepsy were collected in the operating theater, and immediately immersed in ice-cold (4°C) carbogenated transport solution containing (in mM): 3 KCl, 1.25 NaH2PO4, 10 glucose, 2 MgSO4, 2 MgCl2, 1.6 CaCl2, 21 NaHCO3, 200 sucrose, 0.1 tocopherol, pH 7.4, osmolality 302 mOsm/kg. Neocortical slices (500μm) were obtained, transferred to a recording chamber and perfused with aCSF (see above). Recovery time of at least 5h was allowed before the recordings started.

**Recording and Stimulation Electrodes**
Double-barreled K⁺-selective microelectrodes (Fluka 60031 ionophore, 150 mM NaCl reference), were prepared and tested as described previously.⁹ Only electrodes showing slopes of 55-60mV for a 10-fold change in potassium concentration were used. Bipolar stimulation electrodes were made of platinum wires, 50μm diameter, and 200μm tip separation. Single or paired stimuli (0.1ms, 1-10V, 50ms interval) were delivered using a stimulus isolator in constant voltage mode (ISO Flex, AMPI Instruments, Jerusalem, Israel) controlled by a Master-8 (AMPI Instruments, Jerusalem, Israel).

**Intrinsic Optical Imaging**
The massive ion transfer during SD is accompanied by swelling of neurons, glial cells and cell organelles and can be optically detected as increase in the light scattering properties of the tissue.⁹ We monitored intrinsic optical signals with a CCD camera while transilluminating slices with white light. The signal was digitized with a frame grabber board. The first image in a series, captured before SD onset, served as control (T0) and was subtracted from each subsequent image, revealing changes in light transmittance (ΔT) over time. The image series was used to estimate the origin and spreading pattern of SD as well as to calculate its propagation velocity.
### Supplemental Table S1 Clinical Data

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Seizure Type</th>
<th>Seizure Frequency</th>
<th>Years of seizures</th>
<th>Tissue</th>
<th>AED</th>
<th>Histology</th>
<th>[K(^+)](_{\text{aCSF}}) threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>42</td>
<td>CPS GS</td>
<td></td>
<td>26</td>
<td>rFL</td>
<td>TPX PHB</td>
<td>Dysplasia</td>
<td>22.5</td>
</tr>
<tr>
<td>M</td>
<td>24</td>
<td>CPS GS</td>
<td>3-6 /d</td>
<td>10</td>
<td>rFL</td>
<td>CBZ</td>
<td>Gliosis</td>
<td>22.5</td>
</tr>
<tr>
<td>F</td>
<td>24</td>
<td>SPS CPS GS</td>
<td>2-3 /w</td>
<td>5</td>
<td>rTL</td>
<td>LEV OXC</td>
<td>WG2 Temporo-mesial dysplasia</td>
<td>27.5</td>
</tr>
<tr>
<td>F</td>
<td>32</td>
<td>SPS CPS</td>
<td>5-6 /w</td>
<td>30</td>
<td>rFL</td>
<td>OXC CZ</td>
<td>Polymicrogyria</td>
<td>25</td>
</tr>
<tr>
<td>M</td>
<td>38</td>
<td>CPS GS</td>
<td>5 /m</td>
<td>12</td>
<td>rTL</td>
<td>LTG OXC</td>
<td>Heterotopias, Gliosis</td>
<td>20</td>
</tr>
<tr>
<td>M</td>
<td>22</td>
<td>SPS SGS</td>
<td>3 /m</td>
<td>12</td>
<td>rTL</td>
<td>LEV LTG</td>
<td>CA1 WG2, cortical dysplasia</td>
<td>20</td>
</tr>
<tr>
<td>M</td>
<td>61</td>
<td>SPS SGS</td>
<td>2 /m</td>
<td>4</td>
<td>lTL</td>
<td>GPT</td>
<td>HS WG3</td>
<td>22.5</td>
</tr>
<tr>
<td>M</td>
<td>17</td>
<td>SPS CPS</td>
<td>5 /d</td>
<td>1</td>
<td>rTL</td>
<td>OXC</td>
<td>Oligodendroglia</td>
<td>22.5</td>
</tr>
<tr>
<td>F</td>
<td>47</td>
<td>SPS CPS GS</td>
<td>13 /m</td>
<td>46</td>
<td>lTL</td>
<td>LTG</td>
<td>HS WG4</td>
<td>22.5</td>
</tr>
<tr>
<td>M</td>
<td>27</td>
<td>SPS CPS GS</td>
<td>2-6 /m</td>
<td>11</td>
<td>rTL</td>
<td>LTG</td>
<td>WG1, polymicrogyria</td>
<td>17.5</td>
</tr>
</tbody>
</table>

### Table Legend

Human tissue resectates were used from 10 patients with chronic epilepsy. The last column displays the [K\(^+\)]\(_{\text{aCSF}}\) threshold as observed in the *in vitro* experiments.