



Universiteit
Leiden
The Netherlands

Spreading depolarizations : the missing link between migraine and stroke

Ayata, C.

Citation

Ayata, C. (2016, November 24). *Spreading depolarizations : the missing link between migraine and stroke*. Retrieved from <https://hdl.handle.net/1887/44385>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/44385>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/44385> holds various files of this Leiden University dissertation.

Author: Ayata, C.

Title: Spreading depolarizations : the missing link between mirgraine and stroke

Issue Date: 2016-11-24

CHAPTER 3B

MIGRAINE PROPHYLAXIS, ISCHEMIC DEPOLARIZATIONS, AND STROKE OUTCOMES IN MICE

Katharina Eikermann-Haerter,^{1,*} Jeong Hyun Lee,^{1,*} Nilufer Yalcin,¹ Esther S. Yu,¹
Ali Daneshmand,¹ Ying Wei,¹ Yi Zheng,¹ Anil Can,¹ Buse Sengul,¹ Michel D. Ferrari,²
Arn M. J. M. van den Maagdenberg,^{2,3} Cenk Ayata^{1,4}

¹Neurovascular Research Laboratory, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown. ²Department of Neurology, University of Duisburg-Essen, Essen, Germany. ³Department of Neurology, and ⁴Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands. ⁴Stroke Service and Neuroscience Intensive Care Unit, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston.

*Equal contribution

Stroke 2015; 46: 229-236.

ABSTRACT

Background and Purpose: Migraine with aura is an established stroke risk factor, and excitatory mechanisms such as spreading depression (SD) are implicated in the pathogenesis of both migraine and stroke. Spontaneous SD waves originate within the peri-infarct tissue and exacerbate the metabolic mismatch during focal cerebral ischemia. Genetically enhanced SD susceptibility facilitates anoxic depolarizations and peri-infarct SDs and accelerates infarct growth, suggesting that susceptibility to SD is a critical determinant of vulnerability to ischemic injury. Because chronic treatment with migraine prophylactic drugs suppresses SD susceptibility, we tested whether migraine prophylaxis can also suppress ischemic depolarizations and improve stroke outcome.

Methods: We measured the cortical susceptibility to SD and ischemic depolarizations, and determined tissue and neurological outcomes after middle cerebral artery occlusion in wild-type and familial hemiplegic migraine type 1 knock-in mice treated with vehicle, topiramate or lamotrigine daily for 7 weeks or as a single dose shortly before testing.

Results: Chronic treatment with topiramate or lamotrigine reduced the susceptibility to KCl-induced or electric stimulation-induced SDs as well as ischemic depolarizations in both wild-type and familial hemiplegic migraine type 1 mutant mice. Consequently, both tissue and neurological outcomes were improved. Notably, treatment with a single dose of either drug was ineffective.

Conclusions: These data underscore the importance of hyperexcitability as a mechanism for increased stroke risk in migraineurs, and suggest that migraine prophylaxis may not only prevent migraine attacks but also protect migraineurs against ischemic injury.

INTRODUCTION

Migraine is the most common neurological condition, affecting 10% to 20% of the population.¹ Stroke is a major cause of death and disability worldwide. An intriguing association between migraine and stroke is well established. Epidemiological studies identified migraine with aura as an independent factor increasing stroke risk by >2-fold.² The relative risk is particularly high in otherwise healthy young adults without cardiovascular risk factors. The prevalence of migraine is on par with that of other known stroke risk factors.

Spreading depression (SD), an intense depolarization that underlies migraine aura, also occurs in peri-infarct tissue as an overlapping mechanism between migraine and stroke. Although SD does not cause injury in the healthy brain, recurrent peri-infarct SDs and peri-infarct depolarizations (PIDs) worsen the metabolic mismatch in ischemic tissue and promote infarct growth during hyperacute stroke both in experimental animals³⁻⁵ and in humans.^{6,7}

Indirect evidence implicates enhanced cerebral excitability in common migraine,^{8,9} as well as in familial hemiplegic migraine (FHM). FHM1 mutations enhance $\text{Ca}_v2.1$ channel open probability, presynaptic calcium influx and cortical glutamate release, and render the brain hyperexcitable.¹⁰ As a result, FHM1 mutations markedly enhance SD susceptibility.^{11,12} Underscoring the importance of SD in migraine and stroke, transgenic mice expressing FHM1 mutations exhibit faster onset of anoxic depolarization (AD) and rapid infarct growth linked to higher frequency of PIDs during experimentally induced focal cerebral ischemia.¹³

Chronic treatment with widely prescribed migraine prophylactic drugs of various pharmacological classes dose-dependently suppresses SD susceptibility in rats as a possible mechanism of action.¹⁴ The majority of these drugs, however, are ineffective after a single dose, reminiscent of the delayed onset of action requiring chronic treatment in migraine prophylaxis. We, therefore, examined the efficacy of migraine prophylactic drugs on stroke outcome and its mechanisms in relation to ischemic depolarizations. We chose topiramate as a prophylactic drug because it is efficacious in migraine prophylaxis,¹⁵ and inhibits experimental SD on chronic treatment in rats.¹⁴ We tested lamotrigine because it also inhibits SD on chronic treatment in rats,¹⁶ although its efficacy in migraine is not proven.¹⁷ Both drugs have been studied previously in experimental focal ischemia models without consistent efficacy, albeit as a single dose or as short-term postischemic dosing.¹⁸⁻²² Therefore, neither drug has been tested as a prophylactic intervention in stroke.

We, therefore, tested these drugs in commonly used experimental models of focal cerebral ischemia, and did this not only in wild-type (WT) but also in FHM1 mutant mice to test drug efficacy on a background of cerebral hyperexcitability modeling migraine. Here, we show that chronic daily treatment for 7 weeks with the migraine

prophylactic drugs topiramate or lamotrigine delays AD, inhibits PID occurrence and improves tissue and neurological outcomes after filament occlusion of the middle cerebral artery in both WT and FHM1 mutant mice. In contrast, single doses of each drug are ineffective, suggesting that the efficacy of migraine prophylactic drugs in stroke corresponds to their efficacy on SD, and that SD susceptibility is a critical but modifiable determinant of vulnerability to ischemic injury.

METHODS

Experimental Animals. All experimental procedures were carried out in accordance with the Guide for Care and Use of Laboratory Animals (National Health Institutes Publication No. 85-23, 1996) and were approved by the institutional review board (Massachusetts General Hospital Subcommittee on Research Animal Care [MGH SRAC]). In addition to C57BL/6J WT mice, transgenic knock-in *Cacna1a* migraine mouse models homozygous for the R192Q FHM1 mutation were used, generated by a gene targeting approach,^{11,23} and backcrossed on C57BL/6J background for >10 generations. We studied mice between 2 and 6 months of age (23–30 g) because stroke risk is highest in young adult migraineurs. We studied male mice in stroke experiments to avoid the confounding effects of female hormones on outcome,^{24,25} and female mice in SD experiments because of their higher SD susceptibility compared with males,¹² and because migraine is more prevalent in women.

Treatment Paradigm. In the chronic treatment group, we treated mice for 7 weeks with once a day orogastric gavage doses of migraine prophylactic drugs topiramate (80 mg kg⁻¹ d⁻¹) or lamotrigine (30 mg kg⁻¹ d⁻¹), and compared these with vehicle (Ora plus/Ora sweet); the last daily dose was administered 2 hours before the experiment. In a separate cohort, we tested the efficacy of a single dose of these drugs administered 2 hours before the experiment. We selected the doses based on previously reported efficacy in other experimental models in mice.^{26,27} All experiments were performed with the investigators blinded, and confirmatory genotyping was done in mutant cohorts.

Study Design. Study end points were defined a priori. Experiments were performed in 3 stages. First, efficacy of topiramate and lamotrigine was tested on SD susceptibility end points in WT and FHM1 mutant mice. Second, efficacy of both drugs on PID frequency and ischemic outcome was tested in 2 separate cohorts of WT mice. Finally, efficacy of both drugs on ischemic outcome was tested in FHM1 mutant mice. Animals were randomly assigned to the treatment groups for each cohort. A different experimenter blinded to the treatment performed each experimental stage. Experiments were performed according to the intention-to-treat principle; therefore, data points were excluded only if technical failures prevented reliable data collection. Because focal cerebral ischemia experiments in WT and FHM1 mutant mouse cohorts were

separated in time, and performed by different operators using different equipment and experimental setups, we could not perform comparisons of ischemic tissue and neurological outcome end points between WT and FHM1 mutant strains in this study.

Systemic Physiological Monitoring. Arterial pH, pO₂, pCO₂, and blood pressure were measured via a femoral artery catheter under isoflurane anesthesia (2.5% induction, 1.5% maintenance, in 70% N₂O and 30% O₂; Table) and maintained by endotracheal intubation and mechanical ventilation during electrophysiological recordings (ie, SD susceptibility, PID frequency). In 24-hour survival experiments, these interventions were not performed to minimize morbidity and improve survival rates. Rectal temperature was controlled at 37°C.

SD Susceptibility. As described previously,¹² 3 burr holes were drilled under saline cooling at the following coordinates (mm from bregma): 3.5 posterior, 2 lateral (2 mm diameter for electric stimulation and KCl application onto occipital cortex); 1.5 posterior, 2 lateral (1 mm diameter, recording site 1); 0.5 anterior, 2 lateral (1 mm diameter, recording site 2). The dura was kept intact to minimize trauma. Two glass capillary microelectrodes were placed to record extracellular steady (DC) potential and electrocorticogram. Electric SD threshold was determined by escalating intensity cathodal square pulses (10-8000 μ C) via a bipolar electrode placed on the occipital cortex, and then a 1-mm cotton ball soaked in 300 mmol/L KCl was topically applied for 1 hour to record the frequency of evoked SDs. The protocol was then repeated on the opposite hemisphere. Data were averaged between the 2 hemispheres to yield a

Experiment	Treatment Duration	Genotype	Drug	Blood Pressure	pH	pCO ₂	pO ₂
SD	Chronic	WT	Control	92±7	7.41±0.04	28±4	132±18
		WT	Topiramate	98±9	7.34±0.03	30±4	140±12
		WT	Lamotrigine	89±7	7.37±0.04	30±2	113±15
		R192Q	Control	96±5	7.38±0.04	28±4	132±16
		R192Q	Topiramate	88±7	7.33±0.04	27±3	142±11
		R192Q	Lamotrigine	95±8	7.36±0.03	29±2	129±13
	Acute	WT	Control	88±6	7.31±0.04	32±4	134±11
		WT	Topiramate	81±7	7.25±0.02	34±5	153±5
		WT	Lamotrigine	82±4	7.33±0.04	34±5	137±16
PID	Chronic	WT	Control	95±16	7.38±0.03	38±5	122±30
		WT	Topiramate	88±11	7.37±0.05	37±6	131±23
		WT	Lamotrigine	94±10	7.40±0.05	33±6	136±25
	Acute	WT	Control	92±13	7.40±0.04	36±3	116±13
		WT	Topiramate	82±11	7.35±0.07	37±6	129±12
		WT	Lamotrigine	85±10	7.40±0.04	34±6	123±25

Table. Physiological Parameters. Data are displayed as mean±SD. PID indicates peri-infarct depolarization; SD, spreading depression; and WT, wild-type.

single data point per animal. SD frequency and threshold were taken as primary end points. The amplitude, propagation speed (distance/latency between the 2 recording electrodes), and duration at half-amplitude of the first SD in each hemisphere were also measured as secondary end points. There was no technical failure leading to exclusion in this cohort.

Transient Filament Occlusion of the Middle Cerebral Artery. A nylon monofilament was inserted into the internal via the external carotid artery followed by reperfusion after 60 minutes, under isoflurane anesthesia (2.5% induction, 1.5% maintenance, in 70% N₂O and 30% O₂) and laser Doppler monitoring (Perimed, Järfälla, Sweden), as described previously.¹³

PID Occurrence. To record PIDs after transient filament occlusion of the middle cerebral artery (fMCAO), mice were transferred to a stereotaxic frame and two 0.5-mm diameter burr holes were carefully drilled under saline irrigation at the following coordinates (mm from bregma): 1.5 anterior, 0.5 lateral; 3.5 posterior, 0.5 lateral. These coordinates were chosen to be reliably outside the focal ischemic cortex to allow detection of PIDs. Two intracortical glass micropipettes were inserted at a depth of 250 μ m, and extracellular slow potential changes were recorded for $\approx$ 2 hours starting $\approx$ 20 minutes after the onset of fMCAO. PID frequency was taken as a primary end point. Technical failures occurred in WT cohorts only, and led to the exclusion of 1 chronic and 1 single dose vehicle, 1 chronic and 1 single dose topiramate, and 3 single dose lamotrigine-treated mice for PID assessments. Extensive surgery, intubation, mechanical ventilation, and arterial cannulation for PID monitoring precluded 24-hour survival. Therefore, infarct volumes were determined in a separate cohort.

Assessment of Tissue and Neurological Outcome After fMCAO. After reperfusion, mice were transferred to a temperature-controlled incubator with access to food and water ad libitum. Neurological outcomes were scored as a primary end point 24 hours after reperfusion, using a 5-point scale: 0, normal; 1, forepaw monoparesis; 2, circling to left; 3, falling to left; 4, no spontaneous walking and depressed consciousness; and 5, death. Premature death after ischemia was incorporated in the neurological outcome scale because of the intention-to-treat design; however, infarct volume data from these mice were not measured because of postmortem confounders. Infarct volume was calculated by integrating the infarct area in ten 1-mm-thick 2,3,5-triphenyltetrazolium chloride-stained coronal sections. Infarct volume was calculated as a primary end point by subtracting the volume of ipsilateral noninfarcted tissue from contralateral hemisphere. Ischemic swelling volume was also calculated as a secondary end point by subtracting the volume of contralateral hemisphere from the volume of ipsilateral hemisphere. Technical failures occurred in FHM1 cohorts only, and led to the exclusion of 2 chronic vehicle and 1 chronic topiramate-treated mice for tissue and neurological outcome assessments.

Measurement of AD Latency. The latency between fMCAO and AD onset was measured as a secondary end point using the characteristic secondary hypoperfusion caused by AD on laser Doppler tracings, as described in detail previously.¹³ We measured this parameter in all WT mice undergoing fMCAO either for PID frequency determination or infarct and neurological outcome assessment. Absence of a detectable secondary hypoperfusion because of technical reasons was taken as an a priori exclusion criterion for this data set. Although this occurred more commonly, it resulted in the exclusion of only 16 of 112 animals in which this secondary end point was studied, distributed relatively evenly among experimental groups.

Statistical Analysis. Data were analyzed using SPSS (v11.0) and GraphPad Prism 6, and presented as whisker-box plot (whiskers, full range; box, 25% to 75% range; line, median; cross, mean) in the figures and mean $\pm$ SD in the table. Statistical tests used to analyze each data set, group sizes (n) and details of statistical outcomes are provided in the figure legends. *P* values are 2-tailed, and *P*<0.05 was considered statistically significant.

RESULTS

Suppression of KCl-Induced or Electrically Triggered Cortical SD. We have previously shown in rats that migraine prophylactic drugs suppress SD susceptibility.¹⁴ To first test whether migraine prophylactic drugs are also efficacious in mice, we treated WT and FHM1 knock-in mice with chronic daily doses of topiramate or lamotrigine for 7 weeks. Chronic treatment with topiramate or lamotrigine elevated the electrical threshold for SD induction and reduced the frequency of KCl-induced SDs (Figure 1A). Both drugs also reduced the SD propagation speed by $\approx$ 30%, albeit only in the FHM1 mutant. In addition, lamotrigine decreased SD duration, and tended to be more efficacious on all SD end points compared with topiramate. A single dose of either drug administered 2 hours before SD, tested in WT mice only, did not affect any of the SD attributes although a trend for lamotrigine to elevate the electrical threshold and reduce KCl-induced SD frequency was noted (Figure 1B).

Suppression of Cortical PIDs During Middle Cerebral Artery Occlusion. We next tested whether migraine prophylactic drugs also suppress PIDs, akin to SD. Intracortical microelectrode recordings during fMCAO showed that chronic treatment with topiramate or lamotrigine reduced PID occurrence by 50% and 80%, respectively (Figure 2A). A single dose of topiramate 2 hours before ischemia onset was ineffective, whereas lamotrigine showed a strong trend (Figure 2B). In a separate cohort of mice, we found that chronic treatment with valproate (200 mg/kg, IP for 6 weeks) also reduced the number of PIDs (3.1 $\pm$ 0.6 PIDs/h) compared with vehicle (5.7 $\pm$ 0.5 PIDs/h; *P*<0.001, n=5 each), consistent with its inhibitory effect on KCl or electrically induced SDs previously shown in rats,¹⁴ and suggesting a class effect for migraine prophylactic drugs on PIDs.

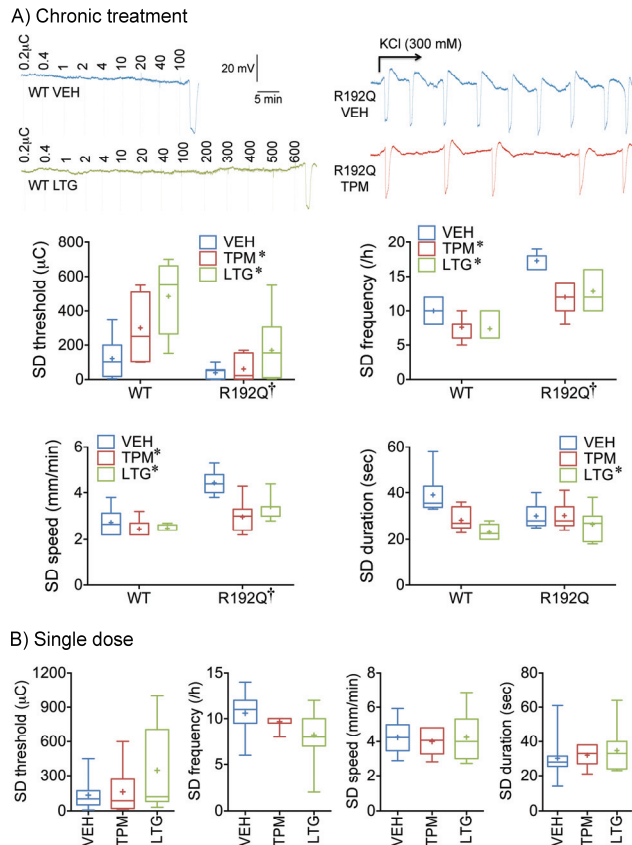
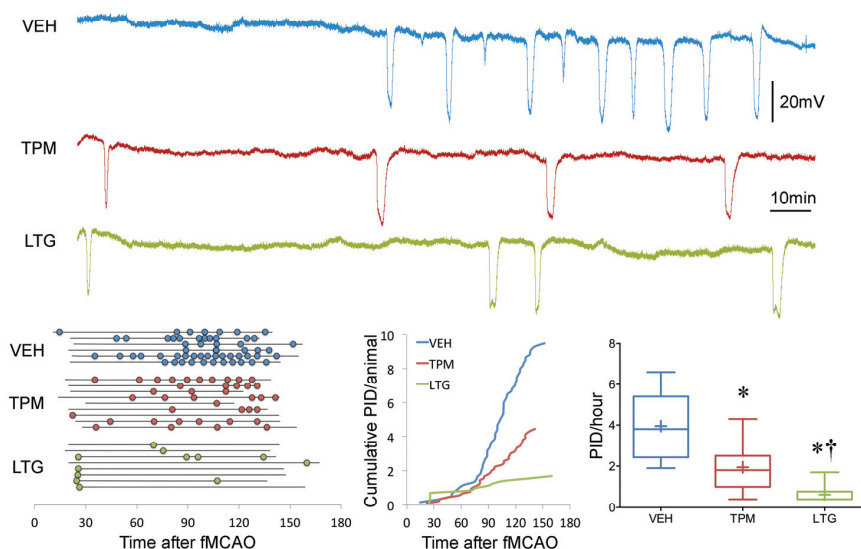


Figure 1. Chronic topiramate and lamotrigine treatment suppresses spreading depression (SD) susceptibility. (A) Representative electrophysiological tracings show SD triggered on stepwise escalating cortical cathodal stimulation at intensities indicated above each tracing to determine the SD threshold (left), and repetitive SDs triggered by continuous topical KCl application for 1 hour onto the cortex to determine SD frequency (right), in wild-type (WT) or familial hemiplegic migraine type 1 (R192Q) mutant mice after 7 weeks of daily treatment with vehicle (VEH, blue), topiramate (TPM, red), or lamotrigine (LTG, green). Whisker-box plots summarize the effects of chronic treatment on SD threshold, frequency, speed, and duration; n=6, 7, and 6 WT mice in vehicle, topiramate and lamotrigine groups, respectively; n=7 R192Q mice in vehicle, topiramate, and lamotrigine groups each. Two-way ANOVA followed by Sidak and Tukey multiple comparisons. SD threshold: genotype effect $F(1,34)=18.8$, $P=0.0001$; treatment effect $F(2,34)=8.4$, $P=0.0011$; interaction $F(2,34)=1.9$, $P=0.1674$. SD frequency: genotype effect $F(1,34)=83.8$, $P<0.0001$; treatment effect $F(2,34)=15.4$, $P<0.0001$; interaction $F(2,34)=1.8$, $P=0.1857$. SD speed: genotype effect $F(1,34)=42.8$, $P<0.0001$; treatment effect $F(2,34)=10.7$, $P=0.0002$; interaction $F(2,34)=4.8$, $P=0.0142$. SD duration: genotype effect $F(1,34)=0.3$, $P=0.5647$; treatment effect $F(2,34)=7.8$, $P=0.0016$; interaction $F(2,34)=3.8$, $P=0.0332$. Post hoc comparisons: * $P<0.05$ vs vehicle; * $P<0.05$ vs WT. **(B)** Whisker-box plots summarize the effect of a single dose of each drug on SD frequency, threshold, speed, and duration in WT mice. n=10, 6, and 9 mice in vehicle, topiramate, and lamotrigine groups, respectively. One-way ANOVA followed by Holm–Sidak multiple comparisons test. Treatment effects were not statistically significant.

A) Chronic treatment



B) Single dose

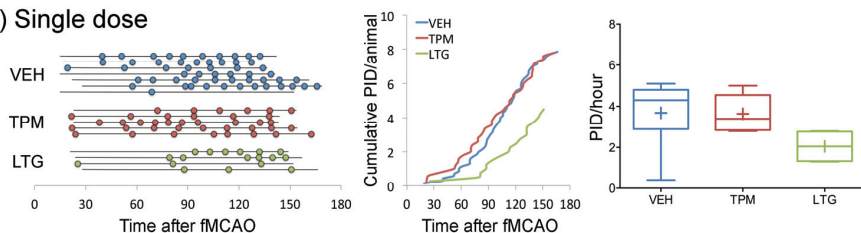


Figure 2. Chronic topiramate and lamotrigine treatment suppresses peri-infarct depolarizations (PIDs). (A) Upper panel shows representative electrophysiological tracings of repetitive PIDs that spontaneously arise around focal ischemic tissue during filament middle cerebral artery occlusion (fMCAO) after 7 weeks of daily treatment with vehicle (VEH, blue), topiramate (TPM, red), or lamotrigine (LTG, green) in WT mice. Lower left panel summarizes all experiments. Horizontal lines indicate the time of onset and end of electrophysiological recordings with respect to fMCAO onset in each mouse, and circles indicate PIDs. Line graph shows average cumulative PID occurrence per mouse as a function of time. When calculating the cumulative PID occurrence over time, differences in group sizes and recording durations were taken into account. Whisker-box plots show average overall PID frequency; $n=6$, 9 , and 8 mice in vehicle, topiramate, and lamotrigine groups, respectively. One-way ANOVA followed by Holm–Sidak multiple comparisons test. Treatment effect $F(2,23)=18.1$, $P<0.0001$. Post hoc comparisons: $*P<0.05$ vs VEH; $†P<0.05$ vs TPM. (B) Left panel summarizes all experiments where horizontal lines indicate the time of onset and end of electrophysiological recordings with respect to fMCAO onset in each mouse, and circles indicate PIDs. Line graph shows average cumulative PID occurrence per mouse as a function of time. When calculating the cumulative PID occurrence over time, differences in group sizes and recording durations were taken into account and corrected for. Whisker-box plots show average overall PID frequency; $n=7$, 5 , and 4 mice in vehicle, topiramate, and lamotrigine groups, respectively. One-way ANOVA followed by Holm–Sidak multiple comparisons test. Treatment effects were not statistically significant.

Improved Stroke Outcomes After Chronic Treatment. We next tested whether suppression of PIDs translated into improved stroke outcomes in WT mice. Chronic treatment with either drug reduced the infarct size after transient fMCAO by $\approx 30\%$, and improved neurological outcomes (Figure 3A). Smaller infarcts predominantly reflected less severe cortical involvement (71 ± 10 , 50 ± 11 , and 48 ± 9 mm³ in vehicle, topiramate, and lamotrigine groups, respectively; $P < 0.05$). Ischemic brain swelling, calculated by subtracting the contralateral from ipsilateral hemispheric volume, was

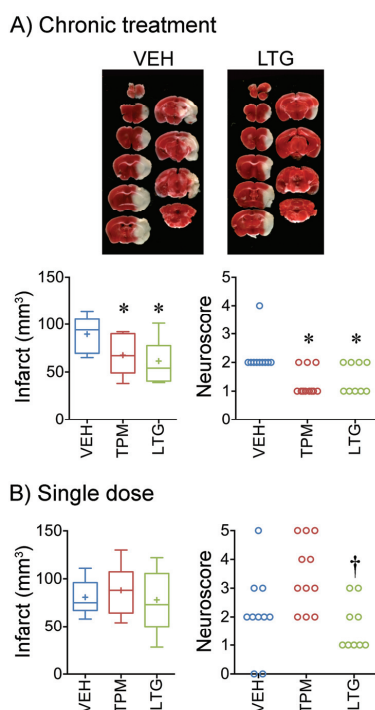


Figure 3. Chronic topiramate and lamotrigine treatment improves stroke outcomes. (A) Representative 2,3,5-triphenyltetrazolium chloride-stained 1-mm-thick coronal sections show the infarct 24 hours after 1-hour transient filament middle cerebral artery occlusion. Whisker-box plot summarizes the indirect infarct volumes after 7 weeks of daily treatment with vehicle (VEH, blue), topiramate (TPM, red) or lamotrigine (LTG, green) in wild-type mice. Neurological deficit scores are also shown in individual animals; $n=10$, 11 and 9 mice in vehicle, topiramate and lamotrigine groups, respectively. One-way ANOVA followed by Holm-Sidak multiple comparisons test for infarct volume, or Kruskal–Wallis followed by Dunn multiple comparisons test for neurological deficit score. Infarct volume: treatment effect $F(2,27)=5.5$, $P=0.01$. Neuroscore: treatment effect Kruskal–Wallis statistic 12.3, $P=0.0021$. Post hoc comparisons: $*P < 0.05$ vs vehicle. **(B)** Whisker-box plot summarizes the indirect infarct volumes after a single dose of vehicle, topiramate or lamotrigine; $n=10$, 11, and 9 mice in vehicle, topiramate, and lamotrigine groups, respectively. One-way ANOVA followed by Holm-Sidak multiple comparisons test. Neuroscore: treatment effect Kruskal–Wallis statistic 9.4, $P=0.009$. Post hoc comparisons: $*P < 0.05$ vs topiramate.

also reduced by chronic topiramate or lamotrigine treatment compared with vehicle (8 ± 2 , 8 ± 2 , and 16 ± 2 mm³, respectively; $P<0.05$), possibly linked to less frequent PIDs. Neurological outcomes assessed using a combined death and disability score as a clinically relevant end point¹³ were improved after chronic treatment with topiramate or lamotrigine compared with vehicle (Figure 3A). In contrast to chronic treatment, single doses of either drug did not affect any of the outcome end points compared with vehicle after transient fMCAO (Figure 3B).

Delayed AD Onset. AD represents loss of membrane ionic gradients on ischemic failure of Na⁺/K⁺-ATPase function. We have previously shown that migraine mutations hasten AD after focal ischemia and this correlated well with SD susceptibility and tissue outcome.¹³ Therefore, we assessed whether decreased SD susceptibility after administering migraine prophylactic drugs was associated with delayed AD onset in WT mice, detected by its cerebral vasoconstrictive effect as previously described.^{4,13} Chronic treatment with lamotrigine, but not topiramate, delayed the onset of AD by $\approx 25\%$ (Figure 4A). The magnitude of cerebral blood flow reduction in the ischemic core did not differ among groups (residual cerebral blood flow $12\pm 5\%$, $13\pm 5\%$, and $12\pm 3\%$ of baseline for vehicle, topiramate, and lamotrigine, respectively), eliminating the possibility that slower AD onset was because of milder ischemia. A single dose of either drug did not affect the latency to AD (Figure 4B).

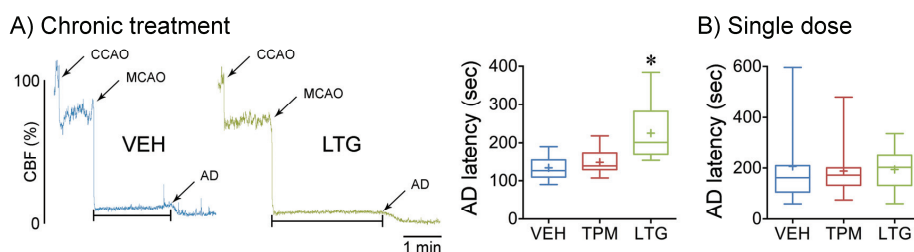


Figure 4. Chronic topiramate and lamotrigine treatment shortens anoxic depolarization (AD) latency after ischemia onset. (A) Left panel shows representative laser Doppler cerebral blood flow (CBF) reductions induced by occlusion of the common carotid artery and the middle cerebral artery, and the subsequent drop in CBF that marks the onset of AD. AD latency is measured as shown by the horizontal line. This secondary end point was measured in all transient filament occlusion of the middle cerebral artery experiments performed for peri-infarct depolarizations frequency and tissue and neurological outcome assessments. Whisker-box plot summarizes AD latency after 7 weeks of daily treatment with vehicle (VEH, blue), topiramate (TPM, red), or lamotrigine (LTG, green) in wild-type mice; $n=17$, 14 , and 13 mice in vehicle, topiramate, and lamotrigine groups, respectively. One-way ANOVA followed by Holm-Sidak multiple comparisons test. Treatment effect $F(2,41)=16.0$, $P<0.0001$. Post hoc comparisons: $*P<0.05$ vs vehicle and topiramate. (B) Whisker-box plot summarizes AD latency after a single dose of vehicle, topiramate or lamotrigine; $n=13$, 16 , and 13 mice in vehicle, topiramate, and lamotrigine groups, respectively. Oneway ANOVA followed by Holm-Sidak multiple comparisons test. Treatment effects were not statistically significant.

Improved Stroke Outcomes After Chronic Treatment in FHM1 Mice. After showing that migraine prophylaxis with topiramate and lamotrigine improves stroke outcomes in WT mice, we also tested whether efficacy is sustained in migraine-susceptible FHM1 brains. Chronic treatment with either topiramate or lamotrigine reduced infarct size after transient fMCAO in FHM1 mutants by 30% to 35% (Figure 5); however, improved neurological function and the delay in the onset of AD reached statistical significance only in the lamotrigine group.

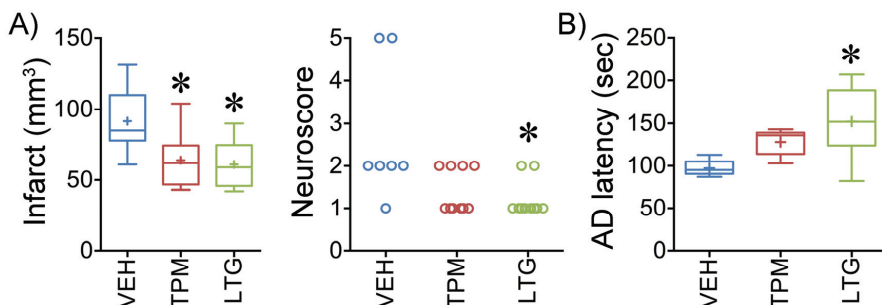


Figure 5. Chronic topiramate and lamotrigine treatment improves stroke outcomes in familial hemiplegic migraine type 1 mutant mice. (A) Whisker-box plot summarizes the indirect infarct volumes after 7 weeks of daily treatment with vehicle (VEH, blue), topiramate (TPM, red), or lamotrigine (LTG, green) in R192Q mutant mice. Neurological deficit scores are also shown in individual animals; n=7, 10, and 10 mice in vehicle, topiramate, and lamotrigine groups, respectively. * $P < 0.05$ vs vehicle. One-way ANOVA followed by Holm-Sidak multiple comparisons test for infarct volume, and Kruskal–Wallis followed by Dunn multiple comparisons test for neurological deficit score. Infarct volume: treatment effect $F(2,24)=6.0$, $P=0.0075$. Neuroscore: treatment effect Kruskal–Wallis statistic 8.6, $P=0.0136$. Post hoc comparisons: * $P < 0.05$ vs vehicle. (B) Whisker-box plot summarizes anoxic depolarization latency after a single dose of vehicle, topiramate, or lamotrigine (LTG, green) in R192Q mutant mice; n=5, 11, and 10 mice in vehicle, topiramate, and lamotrigine groups, respectively. One-way ANOVA followed by Holm-Sidak multiple comparisons test. Treatment effect $F(2,23)=6.8$, $P=0.0048$. Post hoc comparisons: * $P < 0.05$ vs vehicle and topiramate.

DISCUSSION

Migraine is an established risk factor for ischemic stroke. We have recently shown that genetically enhanced SD susceptibility worsens the effect of cerebral ischemia on the brain by facilitating ischemic depolarization events,¹³ as a mechanism to explain the increased risk of stroke in migraineurs. Conversely, we here show that pharmacological suppression of SD susceptibility by migraine prophylactic drugs inhibits AD and PIDs and improves stroke evolution in both WT and FHM1 mutant mice. The magnitude of SD suppression by each drug corresponded well with the magnitude of AD and PID suppression, and stroke outcome. Consistent with this, genetically reduced susceptibility to SD as observed in *rolling Nagoya* and *leaner* mice, which

have spontaneously arisen mutations in the *Cacna1a* gene leading to loss of Ca_v2.1 function, was associated with smaller infarcts, compared with WT on experimental stroke.²⁸ These data strongly support intrinsic SD susceptibility of brain tissue (ie, the tissue factor) as an important determinant of stroke outcome.

Although *in vitro* studies of topiramate and lamotrigine have suggested a neuroprotective effect,²⁹ *in vivo* studies were generally negative in various models of focal cerebral ischemia.^{18–22} All studies, however, have tested single doses or short-term treatment administered before or after ischemia onset. Our data suggest that chronic treatment is required for efficacy, as has been the case for SD suppression in rats^{14,16} and for the prophylactic effect on migraine in patients. Both topiramate and lamotrigine have been shown to acutely inhibit various voltage-gated ion channels as well as glutamatergic neurotransmission.^{30,31} However, whether chronic treatment simply enhances these effects by achieving higher tissue levels, or induces structural or gene expression changes, remains to be determined.

Although PIDs are generally thought to enlarge infarcts by worsening the supply-demand mismatch, an alternative and possibly complementary mechanism is a further increase in cerebral excitability by SD shown in neocortical slices^{32,33}; PID inhibition by migraine prophylaxis may prevent this delayed hyperexcitability and improve outcome. Of course, glial cells critically modulate SD susceptibility, and glial protective effects of topiramate and lamotrigine^{34–36} may also contribute to PID suppression and infarct reduction.

It is well established that PIDs worsen stroke outcomes,^{6,37} and that drugs acutely inhibiting PIDs after a single dose (eg, NMDA receptor antagonists) are protective in focal cerebral ischemia both in experimental animals and in stroke patients.^{4,38–40} However, clinical translation of this neuroprotective target has been difficult because of the cognitive and sedative side effects of such potent drugs.^{41–43} In this respect, migraine prophylaxis may provide a better-tolerated antiexcitatory treatment alternative targeting SD and PIDs in stroke prophylaxis. Consistent with this notion, chronic treatment with lamotrigine was reported to diminish stroke-like episodes in a migraineur with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes,⁴⁴ suggesting that the approach may be even more efficacious in hyperexcitable subsets of patients.

Summary and Conclusions. In summary, our data suggest that pharmacological suppression of SD susceptibility may protect against ischemic injury in patients at high risk for stroke, migraineurs, and nonmigraineurs alike. Whether migraine prophylaxis clinically improves stroke outcomes or reduces the stroke risk remains to be tested in large population-based studies. Although chronic treatment purely as a form of stroke prophylaxis may not be justified at this time because of potential side effects, migraine patients who are already on a migraine prophylactic regimen may indeed see a reduction in their stroke risk as an additional benefit.

ACKNOWLEDGMENTS

This work was supported by the American Heart Association (10SDG2610275), the Claflin Distinguished Award from the Massachusetts General Hospital, National Institutes of Health (NS061505, NS055104, and NS35611); Fondation Leducq; The Heitman Foundation; The Ellison Foundation; Netherlands Organization for Scientific Research (903-52-291 and Vici 918.56.602; Spinoza 2009); EU BRAINPATH (612360); and the Center for Medical Systems Biology in the framework of the Netherlands Genomics Initiative.

REFERENCES

1. IHS. The international classification of headache disorders: 2nd edition. *Cephalalgia*. 2004;24(suppl 1):9–160.
2. Kurth T, Schürks M, Logroscino G, Gaziano JM, Buring JE. Migraine, vascular risk, and cardiovascular events in women: prospective cohort study. *BMJ*. 2008;337:a636.
3. Hopwood SE, Parkin MC, Bezzina EL, Boutelle MG, Strong AJ. Transient changes in cortical glucose and lactate levels associated with peri-infarct depolarisations, studied with rapid-sampling microdialysis. *J Cereb Blood Flow Metab*. 2005;25:391–401.
4. Shin HK, Dunn AK, Jones PB, Boas DA, Moskowitz MA, Ayata C. Vasoconstrictive neurovascular coupling during focal ischemic depolarizations. *J Cereb Blood Flow Metab*. 2006;26:1018–1030.
5. Strong AJ, Anderson PJ, Watts HR, Virley DJ, Lloyd A, Irving EA, et al. Peri-infarct depolarizations lead to loss of perfusion in ischaemic gyrencephalic cerebral cortex. *Brain*. 2007;130:995–1008.
6. Dreier JP, Woitzik J, Fabricius M, Bhatia R, Major S, Drenckhahn C, et al. Delayed ischaemic neurological deficits after subarachnoid haemorrhage are associated with clusters of spreading depolarizations. *Brain*. 2006;129(pt 12):3224–3237.
7. Dohmen C, Sakowitz OW, Fabricius M, Bosche B, Reithmeier T, Ernestus RI, et al. Spreading depolarizations occur in human ischemic stroke with high incidence. *Ann Neurol*. 2008;63:720–728.
8. Anttila V, Winsvold BS, Gormley P, Kurth T, Bettella F, McMahon G, et al. Genome-wide meta-analysis identifies new susceptibility loci for migraine. *Nat Genet*. 2013;45:912–917.
9. Anttila V, Stefansson H, Kallela M, Todt U, Terwindt GM, Calafato MS, et al. Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1. *Nat Genet*. 2010;42:869–873.
10. Tottene A, Conti R, Fabbro A, Vecchia D, Shapovalova M, Santello M, et al. Enhanced excitatory transmission at cortical synapses as the basis for facilitated spreading depression in Ca(v)2.1 knockin migraine mice. *Neuron*. 2009;61:762–773.
11. van den Maagdenberg AM, Pietrobon D, Pizzorusso T, Kaja S, Broos LA, Cesetti T, et al. A Cacna1a knockin migraine mouse model with increased susceptibility to cortical spreading depression. *Neuron*. 2004;41:701–710.
12. Eikermann-Haerter K, Dilekoz E, Kudo C, Savitz SI, Waeber C, Baum MJ, et al. Genetic and hormonal factors modulate spreading depression and transient hemiparesis in mouse models of familial hemiplegic migraine type 1. *J Clin Invest*. 2009;119:99–109.
13. Eikermann-Haerter K, Lee JH, Yuzawa I, Liu CH, Zhou Z, Shin HK, et al. Migraine mutations increase stroke vulnerability by facilitating ischemic depolarizations. *Circulation*. 2012;125:335–345.
14. Ayata C, Jin H, Kudo C, Dalkara T, Moskowitz MA. Suppression of cortical spreading depression in migraine prophylaxis. *Ann Neurol*. 2006;59:652–661.

15. Brandes JL, Saper JR, Diamond M, Couch JR, Lewis DW, Schmitt J, et al. Topiramate for migraine prevention: a randomized controlled trial. *JAMA*. 2004;291:965–973.
16. Bogdanov VB, Multon S, Chauvel V, Bogdanova OV, Prodanov D, Makarchuk MY, et al. Migraine preventive drugs differentially affect cortical spreading depression in rat. *Neurobiol Dis*. 2011;41:430–435.
17. Linde M, Mulleners WM, Chronicle EP, McCrory DC. Antiepileptics other than gabapentin, pregabalin, topiramate, and valproate for the prophylaxis of episodic migraine in adults. *Cochrane Database Syst Rev*. 2013;6:CD010608.
18. Madden K, Clark W, Lessov N. Failure of ischemic neuroprotection by potentiators of gamma-aminobutyric acid. *Clin Med Res*. 2003;1:119–124.
19. Yang Y, Shuaib A, Li Q, Siddiqui MM. Neuroprotection by delayed administration of topiramate in a rat model of middle cerebral artery embolization. *Brain Res*. 1998;804:169–176.
20. Ataus SA, Onal MZ, Ozdem SS, Locke KW, Balkan S. The effects of citicoline and lamotrigine alone and in combination following permanent middle cerebral artery occlusion in rats. *Int J Neurosci*. 2004;114:183–196.
21. Traystman RJ, Klaus JA, DeVries AC, Shaivitz AB, Hurn PD. Anticonvulsant lamotrigine administered on reperfusion fails to improve experimental stroke outcomes. *Stroke*. 2001;32:783–787.
22. Smith SE, Meldrum BS. Cerebroprotective effect of lamotrigine after focal ischemia in rats. *Stroke*. 1995;26:117–121.
23. van den Maagdenberg AM, Pizzorusso T, Kaja S, Terpolilli N, Shapovalova M, Hoebeek FE, et al. High cortical spreading depression susceptibility and migraine-associated symptoms in Ca(v)2.1 S218L mice. *Ann Neurol*. 2010;67:85–98.
24. Hurn PD, Macrae IM. Estrogen as a neuroprotectant in stroke. *J Cereb Blood Flow Metab*. 2000;20:631–652.
25. Krause DN, Duckles SP, Pelligrino DA. Influence of sex steroid hormones on cerebrovascular function. *J Appl Physiol*. 2006;101:1252–1261.
26. Kaminski RM, Banerjee M, Rogawski MA. Topiramate selectively protects against seizures induced by ATPA, a GluR5 kainate receptor agonist. *Neuropharmacology*. 2004;46:1097–1104.
27. Cuadrado A, Bravo J, Armijo JA. Synergistic interaction between felbamate and lamotrigine against seizures induced by 4-aminopyridine and pentylene-tetrazole in mice. *Eur J Pharmacol*. 2003;465:43–52.
28. Tian X, Zhou Y, Gao L, He G, Jiang W, Li W, et al. Analysis of ischemic neuronal injury in Cav2.1 channel $\alpha 1$ subunit mutant mice. *Biochem Biophys Res Commun*. 2013;434:60–64.
29. Costa C, Martella G, Picconi B, Prosperetti C, Pisani A, Di Filippo M, et al. Multiple mechanisms underlying the neuroprotective effects of antiepileptic drugs against in vitro ischemia. *Stroke*. 2006;37:1319–1326.
30. Martella G, Costa C, Pisani A, Cupini LM, Bernardi G, Calabresi P. Antiepileptic drugs on calcium currents recorded from cortical and PAG neurons: therapeutic implications for migraine. *Cephalalgia*. 2008;28:1315–1326.
31. Rogawski MA, Löscher W. The neurobiology of antiepileptic drugs. *Nat Rev Neurosci*. 2004;5:553–564.
32. Berger M, Speckmann EJ, Pape HC, Gorji A. Spreading depression enhances human neocortical excitability in vitro. *Cephalalgia*. 2008;28:558–562.
33. Ghadiri MK, Kozian M, Ghaffarian N, Stummer W, Kazemi H, Speckmann EJ, et al. Sequential changes in neuronal activity in single neocortical neurons after spreading depression. *Cephalalgia*. 2012;32:116–124.

34. Angehagen M, Rönnbäck L, Hansson E, Ben-Menachem E. Topiramate reduces AMPA-induced Ca^{2+} transients and inhibits GluR1 subunit phosphorylation in astrocytes from primary cultures. *J Neurochem*. 2005;94:1124–1130.
35. Angehagen M, Ben-Menachem E, Rönnbäck L, Hansson E. Topiramate protects against glutamate- and kainate-induced neurotoxicity in primary neuronal-astroglial cultures. *Epilepsy Res*. 2003;54:63–71.
36. Pavone A, Cardile V. An in vitro study of new antiepileptic drugs and astrocytes. *Epilepsia*. 2003;44(suppl 10):34–39.
37. Hartings JA, Watanabe T, Bullock MR, Okonkwo DO, Fabricius M, Woitzik J, et al. Spreading depolarizations have prolonged direct current shifts and are associated with poor outcome in brain trauma. *Brain*. 2011;134:1529–1540.
38. Sakowitz OW, Kiening KL, Krajewski KL, Sarrafzadeh AS, Fabricius M, Strong AJ, et al. Preliminary evidence that ketamine inhibits spreading depolarizations in acute human brain injury. *Stroke*. 2009;40:e519–e522.
39. Taghibiglou C, Martin HG, Lai TW, Cho T, Prasad S, Kojic L, et al. Role of NMDA receptor-dependent activation of SREBP1 in excitotoxic and ischemic neuronal injuries. *Nat Med*. 2009;15:1399–1406.
40. Tu W, Xu X, Peng L, Zhong X, Zhang W, Soundarapandian MM, et al. DAPK1 interaction with NMDA receptor NR2B subunits mediates brain damage in stroke. *Cell*. 2010;140:222–234.
41. Ikonomidou C, Turski L. Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? *Lancet Neurol*. 2002;1:383–386.
42. Lees KR, Asplund K, Carolei A, Davis SM, Diener HC, Kaste M, et al. Glycine antagonist (gavestinel) in neuroprotection (GAIN International) in patients with acute stroke: a randomised controlled trial. GAIN International Investigators. *Lancet*. 2000;355:1949–1954.
43. Dyker AG, Edwards KR, Fayad PB, Hormes JT, Lees KR. Safety and tolerability study of aptiganel hydrochloride in patients with an acute ischemic stroke. *Stroke*. 1999;30:2038–2042.
44. Finsterer J, Barton P. Regression of stroke-like lesions in MELAS syndrome after seizure control. *Epileptic Disord*. 2010;12:330–334.

