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Migraine prophylaxis, ischemic depolarizations and stroke outcomes in mice

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Abstract

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

Methods—We measured the cortical susceptibility to spreading depression and ischemic depolarizations, and determined tissue and neurological outcome 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 reduces the susceptibility to KCl- or electrical stimulation-induced spreading depressions as well as ischemic depolarizations in both wild-type and familial hemiplegic migraine type 1 mutant mice. Consequently, both tissue and neurological outcomes are improved. Notably, treatment with a single dose of either drug is ineffective.

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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.

Keywords

migraine prophylaxis; topiramate; lamotrigine; peri-infarct depolarization; anoxic depolarization; middle cerebral artery occlusion

INTRODUCTION

Migraine is the most common neurological condition, affecting 10-20% 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 more than 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 depolarizations (PIDs) worsen the metabolic mismatch in ischemic tissue and promote infarct growth during hyperacute stroke in both experimental animals[3-5] 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 $Ca_V2.1$ 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 upon chronic treatment in rats[14]. We tested lamotrigine because it also inhibits SD upon 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 post-ischemic dosing[18-22]. Therefore, neither drug has been tested as a prophylactic intervention in stroke.

We therefore tested these drugs in commonly employed experimental models of focal cerebral ischemia, and did this not only in wild-type (WT) but also in FHM1 mutant mice to

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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 outcome 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 (NIH Publication No. 85-23, 1996), and were approved by the institutional review board (MGH Subcommittee on Research Animal Care, 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 more than 10 generations. We studied mice between 2-6 months of age (23-30g), 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 due to their higher SD susceptibility compared to 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/d) or lamotrigine (30 mg/kg/d), 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 carried out with the investigators blinded, and confirmatory genotyping was done in mutant cohorts.

Study design

Study endpoints were defined *a priori*. Experiments were performed in three stages. First, efficacy of topiramate and lamotrigine was tested on SD susceptibility endpoints in WT and FHM1 mutant mice. Second, efficacy of both drugs on PID frequency and ischemic outcome was tested in two separate cohorts of WT mice. Lastly, 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 carried out each experimental stage. Experiments were carried out 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

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equipment and experimental setups, we could not perform comparisons of ischemic tissue and neurological outcome endpoints between WT and FHM1 mutant strains in this study.

Systemic physiological monitoring

Arterial pH, pO_2, pCO_2, and blood pressure were measured via a femoral artery catheter under isoflurane anesthesia (2.5% induction, 1.5% maintenance, in 70% N_2O and 30% O_2; Table 1) and maintained by endotracheal intubation and mechanical ventilation during electrophysiological recordings (i.e., 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 electrical 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. Electrical SD threshold was determined by escalating intensity cathodal square pulses (10-8000 μC) via a bipolar electrode placed on the occipital cortex, and then a 1-mm cotton ball soaked in 300 mM 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 two hemispheres to yield a single data point per animal. SD frequency and threshold were taken as primary endpoints. The amplitude, propagation speed (distance/latency between the two recording electrodes), and duration at half-amplitude of the first SD in each hemisphere were also measured as secondary endpoints. There was no technical failure leading to exclusion in this cohort.

Transient filament occlusion of the middle cerebral artery (fMCAO)

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% N_2O and 30% O_2) and laser Doppler monitoring (Perimed, Järfälla, Sweden), as described previously[13].

PID occurrence

To record PIDs after fMCAO, mice were transferred on 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 μm, and extracellular slow potential changes were recorded for approximately two hours starting approximately 20 minutes after the onset of fMCAO. PID frequency was taken as a primary endpoint. 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

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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 endpoint 24 hours after reperfusion, using a five-point scale: 0, normal; 1, forepaw monoparesis; 2, circling to left; 3, falling to left; 4, no spontaneous walking and depressed consciousness; 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 due to postmortem confounders. Infarct volume was calculated by integrating the infarct area in ten 1-mm-thick 2,3,5-triphenyltetrazolium chloride (TTC)-stained coronal sections. Infarct volume was calculated as a primary endpoint by subtracting the volume of ipsilateral non-infarcted tissue from contralateral hemisphere. Ischemic swelling volume was also calculated as a secondary endpoint 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 endpoint 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 due to technical reasons was taken as an *a priori* exclusion criterion for this dataset. Although this occurred more commonly, it resulted in the exclusion of only 16 out of 112 animals in which this secondary endpoint 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, 25-75% range; line, median; cross, mean) in the figures and mean ± standard deviation in the table. Statistical tests used to analyze each dataset, group sizes (n) and details of statistical outcomes are provided in the figure legends. *P* values are two-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 injections 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

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(Figure 1A). Both drugs also reduced the SD propagation speed by approximately 30%, albeit only in the FHM1 mutant. In addition, lamotrigine decreased SD duration, and tended to be more efficacious on all SD endpoints 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 also found that chronic treatment with valproate (200 mg/kg, i.p. for 6 weeks) also reduced the number of PIDs (3.1±0.6 PIDs/h) compared with vehicle (5.7±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.

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 approximately 30%, and improved neurological outcomes (Figure 3A). Smaller infarcts predominantly reflected less severe cortical involvement (71±10, 50±11 and 48±9 mm^3 in vehicle, topiramate and lamotrigine groups, respectively; $p<0.05$). Ischemic brain swelling, calculated by subtracting the contralateral from ipsilateral hemispheric volume, was also reduced by chronic topiramate or lamotrigine treatment compared with vehicle (8±2, 8±2 and 16±2 mm^3, respectively; $p<0.05$), possibly linked to less frequent PIDs. Neurological outcomes assessed using a combined death and disability score as a clinically relevant endpoint[13] 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 endpoints compared with vehicle after transient fMCAO (Figure 3B).

Delayed anoxic depolarization onset

Anoxic depolarization (AD) represents loss of membrane ionic gradients upon 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 administrating 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 approximately 25% (Figure 4A). The magnitude of CBF reduction in the ischemic core did not differ among groups (residual CBF 12±5%, 13±5% and 12±3% of baseline for vehicle, topiramate and lamotrigine, respectively), eliminating the possibility that slower AD onset was due to milder ischemia. A single dose of either drug did not affect the latency to AD (Figure 4B).

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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 30-35% (Figure 5); however, improved neurological function and the delay in the onset of AD reached statistical significance only in the lamotrigine group.

DISCUSSION

Migraine is an established risk factor for ischemic stroke. We have recently shown that genetically enhanced SD susceptibility worsens the impact of cerebral ischemia on the brain by facilitating ischemic depolarization events[13], as one mechanism to explain the increased risk of stroke in migraineurs, Conversely, we here show that pharmacological suppression of SD susceptibility by migraine prophylactic drugs inhibit AD and PIDs and improve 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 upon experimental stroke[28]. These data strongly support intrinsic SD susceptibility of brain tissue (i.e., *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 (e.g., 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 due to the cognitive and sedative side effects of such potent drugs[41-43]. In this respect, migraine prophylaxis may provide a

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better-tolerated anti-excitatory treatment alternative targeting to suppress 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.

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 non-migraineurs 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 due to 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.

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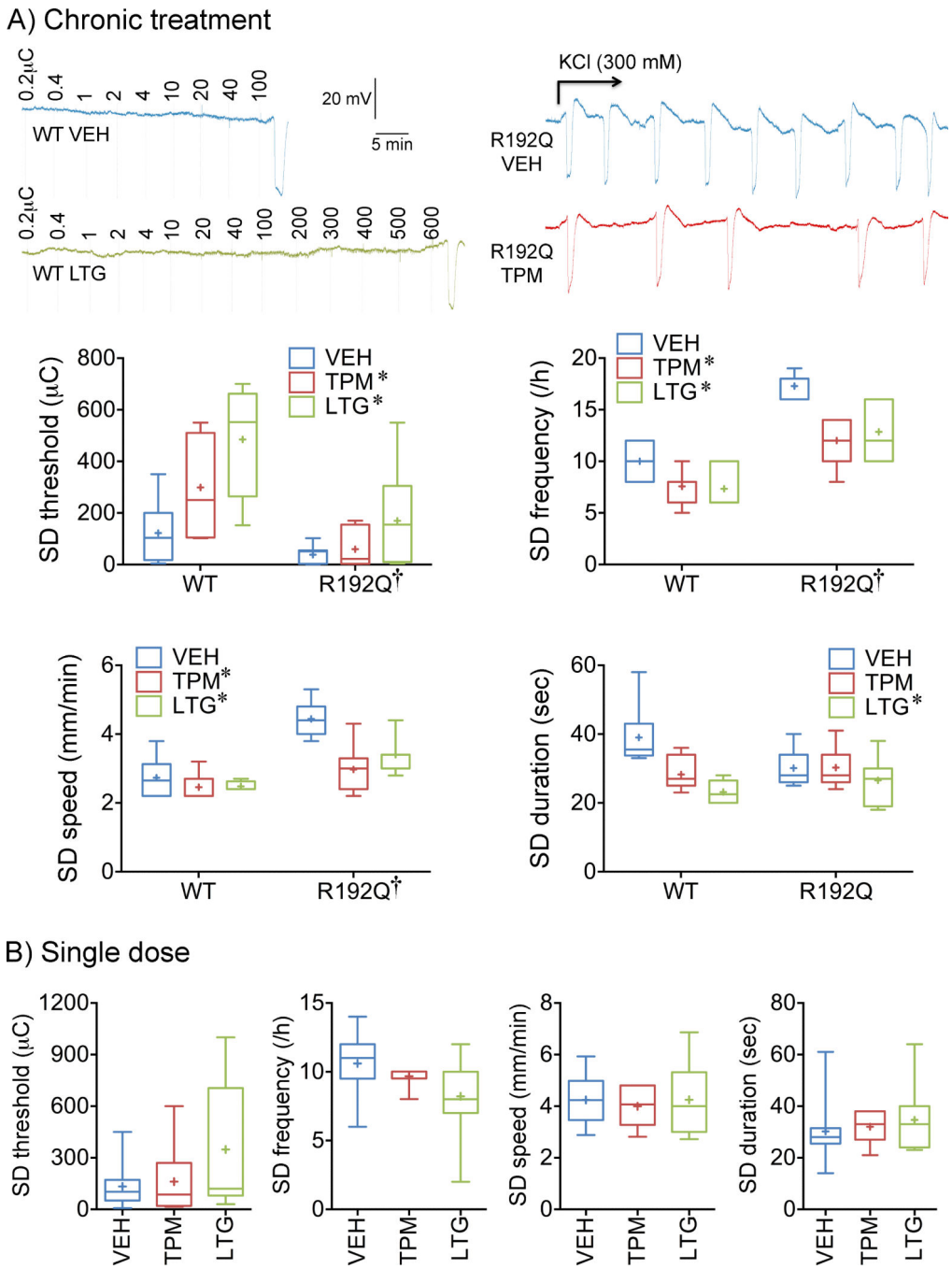

Figure 1. Chronic topiramate and lamotrigine treatment suppresses SD susceptibility

A) Representative electrophysiological tracings show SD triggered upon 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 1h onto the cortex to determine SD frequency (right), in wild-type (WT) or FHM1 (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,

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topiramate and lamotrigine groups, respectively; n=7 R192Q mice in vehicle, topiramate, and lamotrigine groups each. Twoway ANOVA followed by Sidak's and Tukey's 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's multiple comparisons test. Treatment effects were not statistically significant.

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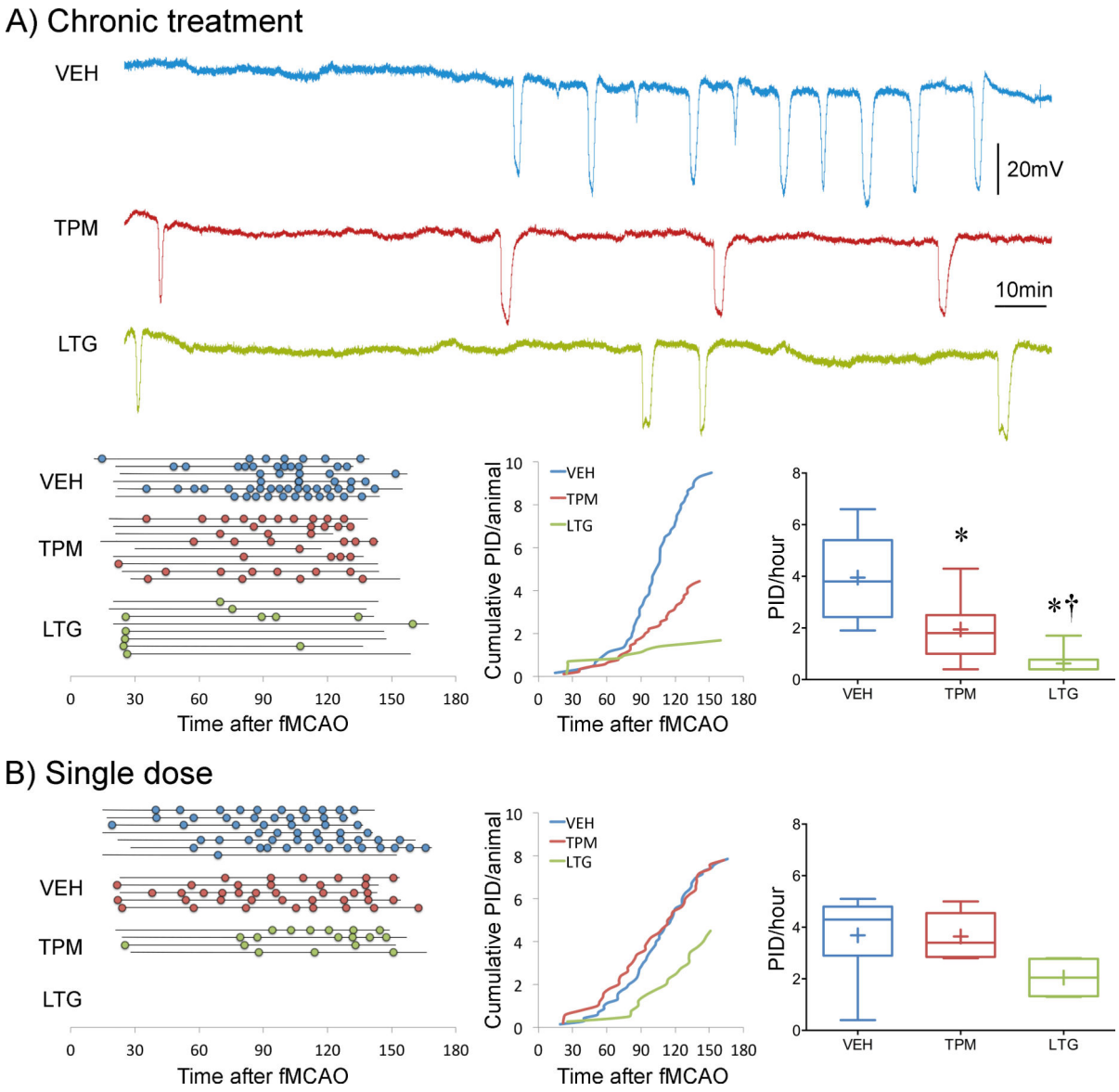

Figure 2. Chronic topiramate and lamotrigine treatment suppresses PIDs

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's 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.

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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's multiple comparisons test. Treatment effects were not statistically significant.

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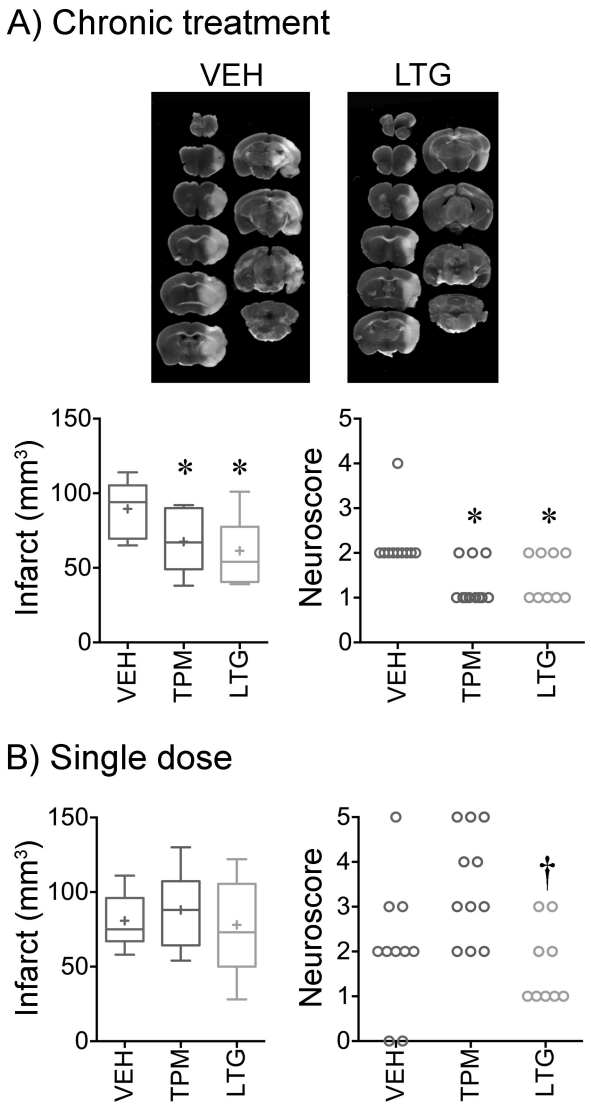

Figure 3. Chronic topiramate and lamotrigine treatment improves stroke outcomes

A) Representative TTC-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 WT 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's multiple comparisons test for infarct volume, or Kruskal-Wallis followed by Dunn's 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 in WT mice. n=10, 11 and 9 mice in vehicle, topiramate and lamotrigine groups, respectively. One-way ANOVA followed by Holm-Sidak's multiple

comparisons test. *Neuroscore*: treatment effect Kruskal-Wallis statistic 9.4, p=0.009. Post-hoc comparisons: †p<0.05 vs. topiramate

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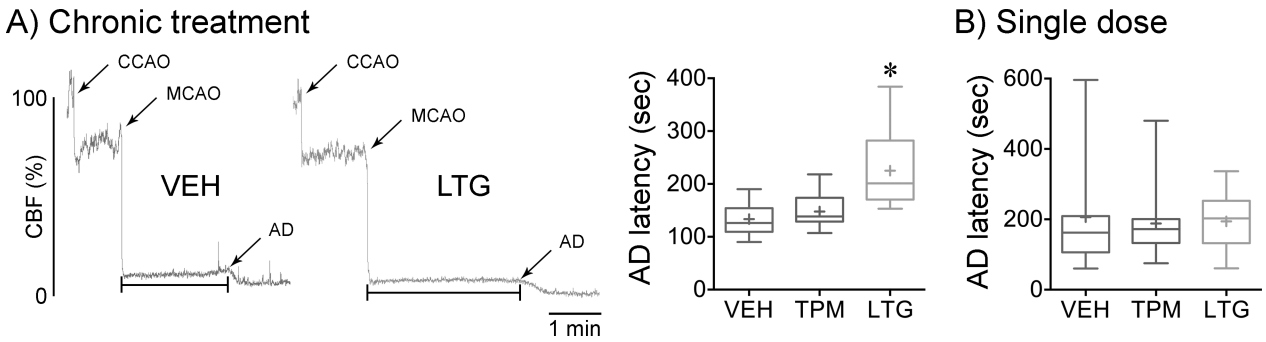

Figure 4. Chronic topiramate and lamotrigine treatment shortens AD latency after ischemia onset

A) Left panel shows representative laser Doppler cerebral blood flow (CBF) reductions induced by occlusion of the common carotid artery (CCAO) and the middle cerebral artery (MCAO), and the subsequent drop in CBF that marks the onset of AD. AD latency is measured as shown by the horizontal line. This secondary endpoint was measured in all fMCAO experiments performed for PID 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 WT mice. n=17, 14 and 13 mice in vehicle, topiramate and lamotrigine groups, respectively. One-way ANOVA followed by Holm-Sidak's 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 in WT mice. n=13, 16 and 13 mice in vehicle, topiramate and lamotrigine groups, respectively. One-way ANOVA followed by Holm-Sidak's multiple comparisons test. Treatment effects were not statistically significant.

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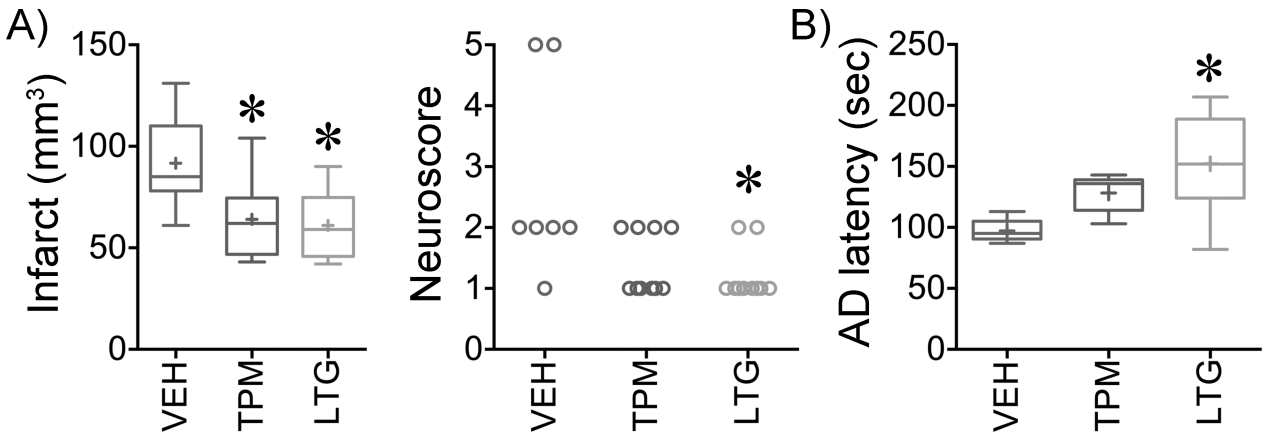

Figure 5. Chronic topiramate and lamotrigine treatment improves stroke outcomes in FHM1 mutant mice

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's multiple comparisons test for infarct volume, and Kruskal-Wallis followed by Dunn's 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 AD 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's multiple comparisons test. Treatment effect $F(2,23)=6.8$, $p=0.0048$. Post-hoc comparisons: *p<0.05 vs. vehicle and topiramate.

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Table 1

Physiological parameters.

Experiment	Treatment Duration	Genotype	Drug	BP	pH	pCO_2	O_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

Data are displayed as mean ± standard deviation.