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Spreading depolarizations : the missing link between migraine and stroke

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SUMMARY

The research in this thesis was aimed at investigating the central hypothesis that susceptibility to SD determines both the susceptibility to migraine with aura and the susceptibility to hypoxic/ischemic injury in the same direction. We envisage that factors that enhance the susceptibility to SD increase the likelihood of migraine with aura as well as ischemic stroke. To this end we assess to what extent genetic, hormonal and pharmacological modulators of SD susceptibility will influence the susceptible to ischemic injury. Thus we will unravel underlying mechanisms of SD susceptibility and susceptibility to ischemic injury. Central to this research is the use of two transgenic mouse models of migraine that carry migraine-relevant FHM1 gene mutations in voltage-gated $\text{Ca}_v2.1 \text{ Ca}^{2+}$ channels.

Chapter 1 provides an overview of the pathophysiology of migraine and aura, acute stroke and its progression, and the clinical association between migraine and stroke. The concepts of spreading depression, spreading depolarization, peri-infarct and injury depolarizations are introduced to put the data presented in **Chapters 2-4** in proper context.

In **Chapter 2A**, we show that two FHM1 missense mutations in the *Cacna1a* gene (R192Q and S218L) enhance CSD susceptibility, and that the S218L mutation, which is associated with a more severe clinical phenotype than the R192Q mutation, enhances CSD susceptibility more than the R192Q mutation. We also show an allele-dosage effect where heterozygous mutants have an intermediate phenotype between homozygous mutants and wild-type controls. We demonstrate that the neurological signs (hemiparesis and circling behavior) precipitated by CSD induced within the cortex in R192Q and S218L knock-in mice closely mimic the clinical signs in FHM patients expressing the respective mutations. Furthermore, we dissect the modulatory role of female gonadal hormones by showing that female FHM1 mutants have higher CSD susceptibility than males, that ovariectomy diminishes and estrogen replacement restores the sex difference in CSD phenotype. These data are congruent with higher migraine susceptibility in women of reproductive age, and suggest an interaction between estrogen and the genetic determinants of migraine susceptibility.

An alternative explanation for the female susceptibility to migraine is a protective effect by androgens. Therefore, in **Chapter 2B**, we investigate male gonadal hormone modulation of SD susceptibility. Using well-controlled electrophysiological methods, *in vivo*, we show that orchiectomy enhances and chronic testosterone replacement, but not a single dose, restores SD susceptibility, in an androgen receptor-dependent manner in FHM1 mutants. These data suggest that male and female gonadal hormones have opposite effects on SD susceptibility. As with the female mice, male gonadal hormones did not modulate SD susceptibility in wild-type animals, once again suggesting an interaction between hormonal status and genetic susceptibility factors.

In **Chapter 2C** we turn our attention to the severe and prolonged neurological deficits (e.g., coma and hemiparesis) that FHM1 mutant mice develop after a cortical SD (shown in **Chapter 2A**). Because cortical somatosensory evoked potentials recovered at about the same rate in wild-type and FHM1 mutant mice, delayed functional recovery of cortex did not appear to be the culprit in creating the severe and prolonged neurological deficits. Therefore, in **Chapter 2C** we investigate the alternative hypothesis that subcortical rather than cortical dysfunction is responsible for the severe and prolonged neurological deficits in FHM1. We employ multifocal intracerebral electrophysiological recordings in FHM1 mutants to show that SD susceptibility is significantly enhanced in striatum, thalamus and hippocampus, and that SD triggered in cortex is capable of propagating into all of these structures via direct gray matter connections, with the same genetic and sex-related modulation patterns described in **Chapters 2A and 2B**. Moreover, a subcortical SD can propagate back into the cortex, creating re-entrant and reverberating cortico-subcortical SDs in the highly susceptible FHM1 mutants, to explain the neurological deficits mimicking those observed in FHM patients.

In **Chapter 3A**, we test the central hypothesis that genetic and hormonal determinants of SD susceptibility sensitize the brain to ischemia, using *in vivo* electrophysiology, MRI, optical imaging of cerebral blood flow, combined with standard models of focal cerebral ischemia, all of which demonstrate that FHM1 mutations worsen the tissue and neurological outcome after focal cerebral ischemia. We show sex differences in ischemic outcome (female mice fare worse than males), which are consistent with the gonadal hormone influences on SD susceptibility (shown in **Chapters 2A and B**), but in the opposite direction compared with wild-type animals. We also demonstrate an allele-dosage effect where the homozygous FHM1 mutants fare worse than heterozygotes. Moreover, we identify enhanced AD and PIDs as the mechanism accelerating infarct growth in FHM1 mutants, akin to enhanced SD susceptibility explaining migraine aura. Therefore, we show data supporting the hypothesis that enhanced SD susceptibility worsens ischemic outcome, and form a direct mechanistic link between migraine with aura and ischemic stroke.

It is known that migraine prophylactic drugs suppress SD susceptibility as one mechanism of action in migraine. Therefore, migraine prophylaxis presents an opportunity to modulate SD susceptibility in the opposite direct compared with migraine mutations. We, therefore, show in **Chapter 3B** that, conversely, suppression of SD susceptibility by chronic (>4 weeks) treatment with migraine prophylactic drugs topiramate and lamotrigine renders the brain resistant to ischemic injury. We show that the effect is present not only in wild-type mice but also in FHM1 mutants. In this chapter, we also confirm the efficacy of migraine prophylactic drugs on SD in mice, which has only been shown in rats in previous studies. Moreover, we show that

treatment with a single dose of these drugs shortly before testing is ineffective, once again demonstrating the need for chronic treatment with these drugs. Lastly, we show that smaller ischemic infarcts are also associated with more favorable functional neurological outcomes, thus underscoring the clinical implications of our findings.

Chapter 4 discusses current knowledge on shared mechanisms of migraine and stroke.

Finally in **Chapter 5**, we put all our experimental findings, and others from the literature, together with the clinical observations on the association between migraine and stroke, in a comprehensive review focusing on potential mechanisms to explain the association, including the ones investigated in this thesis. We hope that the manuscript will spur thought and discussion and lead to new investigations on the association between migraine and cerebrovascular disease, discovery of new mechanisms or confirmation of the proposed ones, and increase awareness among the practicing physicians.

