Spreading depolarizations : the missing link between mirgraine and stroke

Ayata, C.

CHAPTER 1

GENERAL INTRODUCTION

1. MIGRAINE

Migraine is a common and disabling episodic neurovascular disease[1] that affects ~15% of the world population; it is three times more common among women of reproductive age than men.[2, 3] Recurrent migraine attacks are characterized by severe headache accompanied to various degrees by nausea, vomiting, and increased sensitivity to light, sound, and smell, lasting anywhere between an hour to days (migraine *without* aura).[4] Attack frequency differs widely among patients, from one per year to several per week. Every day, millions of people are plagued by blistering migraines, which ranks among the most disabling medical conditions worldwide.[2, 5]

Migraine aura. One-third of migraine patients also experience transient focal neurological symptoms termed *aura* that usually precede or sometimes accompany the headache (migraine *with* aura).[4,6] Aura symptoms can be visual, sensory or motor, or involve language or brain stem function. Aura often shows a characteristic spread, such as a scintillating scotoma gradually expanding from a point of origin in one hemi visual field (Figure 1), or paresthesias marching up from the fingers and hand to sequentially involve the arm and then lower face. This form of spread pattern suggests an intracortical event propagating in a retinotopic or somatotopic fashion. It is widely accepted[7,8] that the symptomatology of migraine aura is caused by the electrophysiological event called *spreading depression (SD)*, an intense neuronal and glial spreading depolarization wave slowly propagating (~3 mm/min) in cerebral cortical or subcortical grey matter.[9-11] Mechanisms of migraine headache are still debated, although there is general agreement that activation and sensitization of the trigeminovascular nociceptive pathways is a critical step.[12,13] A large body of experimental evidence strongly suggests that SD is capable of activating the trigeminovascular system, and thus directly relevant for headache mechanisms.[11] The

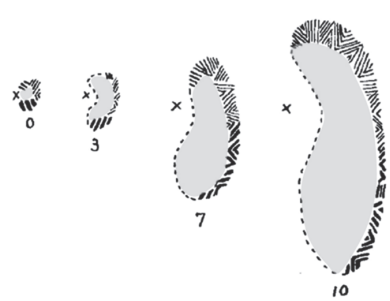

Figure 1. Scintillating scotoma of visual migraine aura. Fovea is indicated by x, and the time between each successive drawing by the numbers in minutes. The zig-zag pattern at the propagating wavefront represents scintillations (i.e., positive symptom at the wavefront) while the gray shaded area represents visual loss (i.e., scotoma, negative symptom).[15] Visual aura is caused by SD in occipital cortex.

trigeminovascular system consists of nociceptive trigeminal afferents surrounding the intracranial vessels. These perivascular trigeminal afferents project through the trigeminal ganglion to neurons in the trigeminocervical complex, and then relayed to the thalamus where all nociceptive inputs are integrated.[14]

2. SPREADING DEPRESSION

Definition. Spreading depression (SD) is an intense neuronal and glial depolarization wave that slowly propagates in brain tissue (~3 mm/min) by way of gray matter contiguity irrespective of functional divisions or vascular territories.[16] The near-complete loss of membrane potential is a result of massive transmembrane ionic and water shifts, including K^+ and glutamate efflux, and Na^+, Ca^{2+} and water influx, which last up to a minute and does not lead to injury in otherwise normal brain tissue. All these ionic and water shifts create a signature extracellular negative slow potential shift (aka DC shift) accompanied by suppression of action potentials and synaptic activity (Figure 2). As a result, electrocorticogram (ECoG) is depressed, hence the historical term ''spreading depression'' originally coined by Leao.[17] Similar spreading depolarization events also occur in ischemic brain, and are termed peri-infarct depolarizations (PIDs) or injury depolarizations (see below). Therefore, SD is a form of spreading depolarization event that occurs in otherwise normal brain.

Basic electrophysiology. SD is triggered when a sufficiently strong stimulus (e.g., topical application of concentrated KCl solution, direct cathodal electrical stimulation, seizure activity or tissue ischemia) simultaneously depolarizes a minimum critical volume of brain tissue estimated to be ~1 mm^3 in rodent cortex, *in vivo*.[18] The depolarizing stimulus overloads the extracellular K^+ ($[K^+]_e$) clearance mechanisms causing $[K^+]_e$ to exceed a critical threshold concentration of ~12 mM.[18-24] These thresholds can vary in different species and brain regions depending on neuronal and excitatory synaptic density among other factors.[25,26] The inciting event causes a sudden drop in membrane resistance via opening of non-selective large conductance cation channels, the presence and identity of which are yet incompletely understood.[27] As a result, both intracellular and extracellular ions move along their transmembrane concentration gradients. Massive K^+ efflux raises $[K^+]_e$ from ~3 mM at resting state[28-30] to about ~30-50 mM, and sometimes as high as 80 mM, in an all-or-none fashion, in most species, tissues and model systems.[19,22,29,31-34] This large K^+ efflux is reciprocated by Na^+ and Cl^- influx that pulls water, causing cell swelling.[22,35-40] Extracellular space shrinks by as much as 50%.[41, 42] Depolarization also triggers Ca^{2+} influx and a more than 10-fold drop in $[Ca^{2+}]_e$,[22,43,44] which, along with Na^+ and water influx, leads to release of many if not all neurotransmitters and neuromodulators within the depolarized tissue. Extracellular glutamate, aspartate, glycine, GABA and taurine concentrations increase during SD,[32,45,46] and similar increases have been shown for catecholamine and ascorbate levels.[47-49]

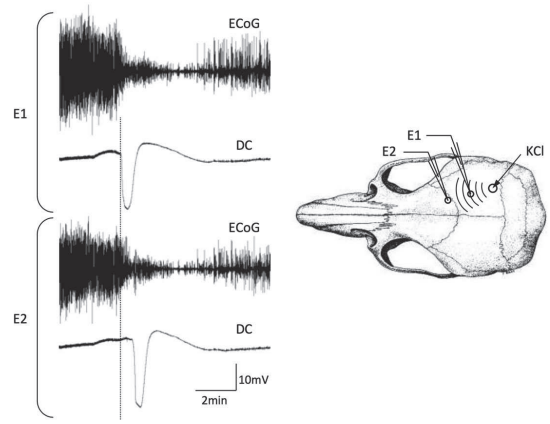

Figure 2. Representative tracings of electrocorticogram (ECoG) and extracellular DC potential during a cortical SD wave in rat brain, triggered by 1M KCl briefly applied on to the occipital cortex, and recorded by two intracortical glass microelectrodes placed in series with the KCl application site. SD is characterized by transient depression of cortical electrical activity and a slow DC shift 20-30 mV in amplitude lasting less than 1 min. When triggered by an intense stimulus that simultaneously depolarizes a minimum critical volume of brain tissue, SD spreads centrifugally to be detected first at the proximal (E1), and after a latency, at the distal (E2) microelectrode. In otherwise normal tissue, ECoG activity starts to recover a few minutes after the onset of SD, but may take up to 10 min to return to normal. The speed of propagation is calculated from the distance and the latency between the electrodes. Modified from [10].

Propagation of SD. It is believed that the rise in $[K^+]_e$ and glutamate act as chemical signals diffusing to and depolarizing adjacent cells, and in this way the depolarization slowly spreads. The massive rise in $[K^+]_e$ to levels sufficient to depolarize neighboring cells is the critical factor mediating the contiguous spread of the wave.[20,22,50] Elevated extracellular levels of the strongly depolarizing excitatory amino acids (glutamate and aspartate) further fuel SD and facilitate its propagation by activating NMDA receptors.[51,52] For the released K^+ and glutamate to reach the critical depolarization threshold of adjacent cells, high neuronal and synaptic density and low extracellular space volume are required; therefore, white matter is characteristically resistant to SD. However, SD can be triggered in subcortical grey matter structures such as striatum, thalamus and hippocampus, with the exception of brain stem, which is resistant to SD unless tested in immature animals or after pharmacological preconditioning (e.g., K^+-channel blockade).[53-57] Lastly, cortical SD can propagate into subcortical structures that have direct gray matter contiguity with the cortex.[58]

Recovery of SD. The massive redistribution of ions, water and neurotransmitters is self-limited. A number of mechanisms, including the Na^+/K^+-ATPase, intracellular buffering of $[Ca^{++}]_i$, reuptake and metabolism or spatial buffering by the astrocytic network, and quite likely vascular clearance, all help restore the homeostasis usually

within a minute. The process is in part energy-dependent and strongly stimulates O_2 and glucose consumption. Therefore, in severely hypoperfused (i.e., ischemic) tissue, restoration of homeostasis is delayed, with deleterious consequences on tissue viability.

SD and migraine. Since the discovery of SD decades ago, similarities between the electrophysiological properties of SD and the neurological signs and symptoms during migraine aura suggested a causative link between the two.[59-61] Experimental evidence also suggests that SD can trigger headache by activating the trigeminovascular system.[11,62-67] Although whether an asymptomatic SD triggers migraine headache without a perceived aura is still debated, suppression of SD susceptibility by migraine prophylactic drugs as a class effect supported this notion since these drugs have been equally efficacious in migraine with or without a perceived aura. Therefore, SD is now considered a potential therapeutic target in migraine, and experimental models of SD susceptibility are increasingly being used in migraine drug screening.

SD susceptibility. The ease with which SD can be initiated and sustained is often termed *SD susceptibility*, and can be used as an experimental surrogate for migraine (with aura) susceptibility. Indeed, modulation of SD susceptibility by drugs appears to be the basis for migraine prophylaxis.[68] There are several experimental models that can be used to measure SD susceptibility.[69] The most common attributes used to define SD susceptibility are: i) the threshold stimulus intensity that triggers an SD (i.e., electrical charge measured in Coulombs, or KCl concentration threshold) determined by sequential stimuli of stepwise escalating intensity until an SD is triggered, ii) the frequency of SDs triggered during continuous constant suprathreshold stimulus for up to an hour (i.e., topical high concentration of KCl), and iii) the propagation speed of SD.

3. ISCHEMIC STROKE

Definition of the problem. Ischemic stroke is an acute cerebrovascular catastrophe and a leading cause of death and disability worldwide.[70] It is caused by occlusion of an artery supplying the brain or spinal cord. Despite intense research into the pathophysiology and treatment of ischemic stroke, the only proven and widely adopted therapeutic modality is thrombolysis to achieve reperfusion in a timely manner. Brain tissue is highly sensitive to ischemia. Infarction ensues unless reperfusion is achieved within a few hours after cerebral arterial occlusion. Therefore, the therapeutic efficacy and safety window of thrombolysis is limited to only 4.5 hours after stroke onset.[71]

Ischemic core. Cerebral arterial occlusion typically creates a focal perfusion defect within its territory (Figure 3). Some degree of collateral blood flow reaches the focal ischemic tissue via surrounding patent arteries. However, efficacy of collateral flow drops as a function of distance, creating an inverted bell-shaped gradient of blood flow from the relatively well-perfused periphery towards the severely ischemic center. Irreversible ischemic injury (i.e., infarct) starts in the center and gradually expands over minutes to days into the periphery. Energy shortage in the severely ischemic center results in failure of Na^+/K^+ ATPase, gradual increase in extracellular potassium concentrations ($[K^+]_e$). Within a few minutes after the arterial occlusion, when $[K^+]_e$ reaches a critical threshold of ~12 mM (from a normal resting level of ~3-4 mM), neurons develop a sudden onset catastrophic loss of resting membrane potential (V_m), termed *anoxic depolarization (AD)*, which marks the *ischemic core*. The process is analogous to the triggering of SD as described above, with the critical distinction being tissue inability to restore the transmembrane ionic gradients because of ongoing ischemia. In that sense, AD is a persistent form of SD that can become permanent. It is generally believed that AD that continues longer than ~10-15 minutes results in irreversible cell injury and eventual death by necrosis and/or apoptosis.

Ischemic penumbra. Surrounding the core, there is sufficient collateral flow to maintain the resting V_m, but neural activity and synaptic transmission are suppressed via intrinsic protective mechanisms creating a ring of electrical silence (Figure 3). This 'still-viable tissue at risk for infarction' is called *ischemic penumbra*. The spatiotemporal evolution of injury in penumbra is complex. Depending on residual tissue perfusion and the intrinsic tissue sensitivity to ischemia, portions of penumbra succumb to AD, and get incorporated into the ischemic core over time. Therefore, ischemic core gradually expands into the penumbra (Figure 4). A critical factor that facilitates ischemic injury, expands the infarct and worsens the neurological outcome is the occurrence of spontaneous spreading depression waves within the peri-infarct tissue, called peri-infarct depolarizations.

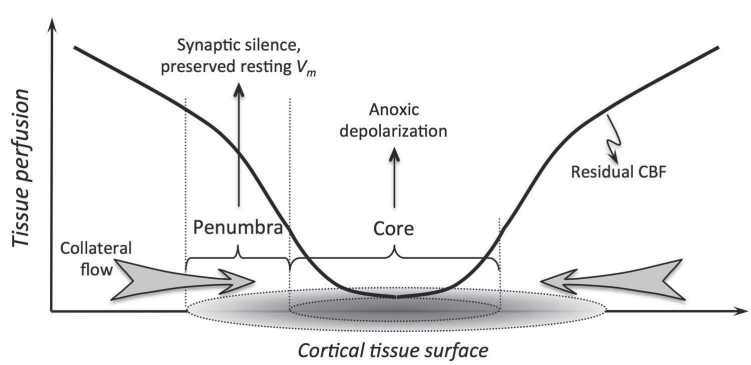

Figure 3. The hemodynamic status and membrane potential of acute focal ischemic brain tissue showing the definitions of ischemic penumbra and core. V_m, membrane potential.

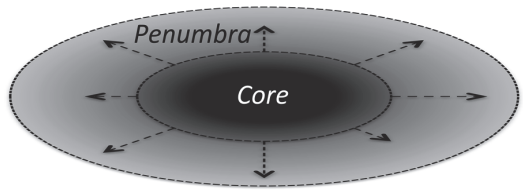

Figure 4. Ischemic core gradually expands into the penumbra because of persistent ischemia and the occurrence of peri-infarct depolarizations.

Peri-infarct depolarizations. In addition to AD, ischemic brain also develops spontaneous recurrent *peri-infarct spreading depolarization waves* (PIDs) that originate at the boundary and propagate throughout the ischemic and non-ischemic tissue. When PIDs enter the non-ischemic tissue, they are electrophysiologically indistinguishable from SD, the substrate of migraine aura, suggesting that PIDs are analogous to SDs (Figure 5). The local rise in $[K^+]_e$ and the hypoxic suppression of Na^+/K^+-ATPase in ischemic brain serve as the strongly depolarizing stimuli that trigger PIDs, analogous to the topical KCl or electrical stimulation to trigger SDs. Importantly, occurrence of PIDs have been shown in human brain as well.[72] Therefore, SDs and PIDs represent a mechanistic overlap between migraine and stroke.

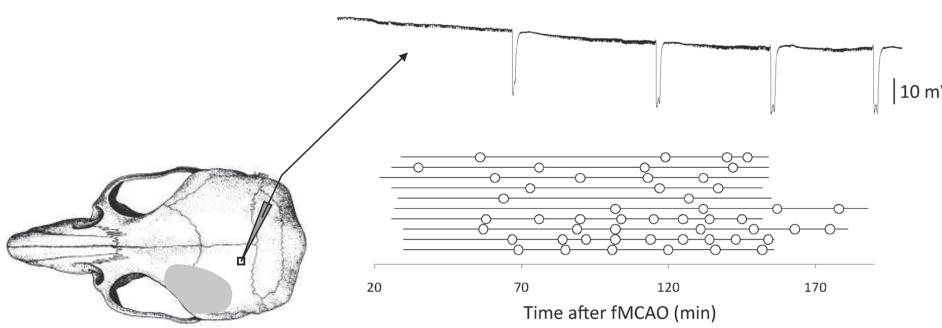

Figure 5. Recurrent peri-infarct injury depolarizations (PIDs; four negative deflections on the tracing) recorded during filament occlusion of the middle cerebral artery by an intracortical glass micropipette placed outside the ischemic tissue in a representative mouse. These PIDs are indistinguishable from SDs triggered by intense depolarization in otherwise normal cortex. Lower right panel is the summary of 10 experiments, where each horizontal line shows the beginning and end of electrophysiological recordings after the onset of ischemia, and each circle represents a PID. Grey shaded area shows the approximate location of the perfusion defect upon arterial occlusion. Recurrent PIDs appear spontaneously throughout the recording period in all mice.

Acute infarct progression on MRI. Ionic changes and cell swelling in tissue that has undergone AD cause a characteristic decrease in apparent diffusion coefficient (ADC) of water on diffusion-weighted MRI (DWI) as the *MRI signature of core* infarction (Figure 6). Perfusion-weighted MRI (PWI) outlines the total tissue at risk for infarction. Therefore, the mismatch between DWI and PWI lesion volumes is a measure of viable tissue at risk for infarction (i.e., penumbra). Ischemic penumbra is the primary target of all acute stroke rescue interventions. The volume of penumbra present at any given time is a good measure of how much tissue is available that can be rescued from ischemic infarction.

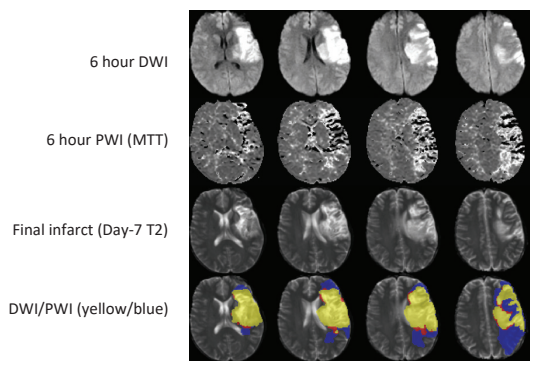

Figure 6. Acute MRI of a typical middle cerebral artery stroke. Diffusion-weighted imaging (DWI) shows the infarcted tissue (yellow), whereas perfusion-weighted imaging (PWI; measured by mean transit time, MTT, blue) delineates the hypoperfused tissue. The DWI/PWI mismatch (blue tissue outside the yellow) is a surrogate MRI measure of ischemic penumbra, defined as 'still-viable tissue at risk for infarction'. Day 7 T2 MRI shows the final infarct volume in this patient. Courtesy of Dr. Hakan Ay.

4. CLINICAL ASSOCIATION BETWEEN MIGRAINE AND STROKE

Migraine has traditionally been viewed as a benign chronic episodic condition. However, accumulating evidence suggests that migraine with aura, can be associated with increased risk for stroke and white matter lesions.[73-79]

Observational data. Abundant data from retrospective and population- or hospital-based case-control studies, as well as small and large population-based prospective studies including tens of thousands of subjects, have firmly established a link between migraine and ischemic stroke.[77,80,81] The odds ratio (OR) for ischemic stroke is ~2 among migraineurs (95% confidence interval [CI] 1.72-2.43). Important insights are:

I. The association is explained by migraine with aura alone (OR 2.5, 95% CI 1.5-4.1), and does not reach statistical significance for migraine without aura (OR 1.3, 95% CI 0.8-2.1),

II. The association is stronger in women (OR 2.9, 95% CI 2.4-3.5),[77] and in subjects younger than 45 (OR 2.7, 95% CI 1.4-5.0),
III. The risk is higher in patients who experience active migraine attacks (OR 1.9, 95% CI 1.2-3.1), and not in those with just a history of migraine without recent attacks,[82]
IV. The risk is higher in those who experience >12 attacks per year (OR 1.7, 95% CI 1.1-2.8).[83]

Neuroimaging. A number of neuroimaging studies over the past decade revealed a higher prevalence of subclinical brain lesions in migraineurs, including infarcts and white matter hyperintensities, suggesting acute or chronic ischemic disease. CAMERA (Cerebral Abnormalities in Migraine and Epidemiological Risk Analysis) was a cross-sectional, population-based MRI lesion prevalence study in subjects between ages 30 and 60 (mean age 48; 161 migraine with aura, 134 migraine without aura and 140 matched controls) randomly selected from the Genetic Epidemiology of Migraine study. Data suggested an increased risk of subclinical posterior circulation infarct-like lesions, mostly located in the cerebellum, in migraineurs compared to controls (OR 7.1, 95% CI 0.9-55).[78,84] Important insights are:

I. The risk was substantially higher in migraineurs with aura (OR 13.7, 95% CI 1.7-112),
II. The risk was also higher with frequent migraine attacks (≥1 attack/month) (OR 15.8, 95% CI 1.8-140),
III. The risk was independent of triptan use or vascular risk factors,
IV. In the 9-year follow up of the same cohort, none of the lesions disappeared, and new posterior circulation lesions were found in 5% of migraineurs compared with none in control subjects.[85]

The conclusions of CAMERA study were later independently confirmed in the Age Gene/Environment Susceptibility Reykjavik study,[86] and in the population-based Epidemiology of Vascular Aging study (780 subjects, mean age of 69).[87] Clinical contrasts between migraine and stroke make this association highly intriguing:

1. Unlike the perceived benign nature of migraine (i.e., no imminent risk of injury), stroke is: (i) an acute and often catastrophic cerebrovascular event, (ii) the leading cause of acquired physical disability in adults in the US,[88] and (iii) the second leading cause of mortality worldwide.[70]
2. Stroke is predominantly a disease of the elderly, while migraine prevalence peaks around age 40.
3. Stroke risk is higher in males than females of reproductive age, whereas migraine is higher in females of reproductive age and increases stroke risk most prominently in this group.

Investigation of the mechanisms underlying the association between migraine and stroke can impact hundreds of millions of people worldwide.

5. TRANSGENIC ANIMAL MODELS OF HUMAN MIGRAINE SYNDROMES

Modeling susceptibility to migraine. In order to elucidate the mechanisms linking migraine with aura and stroke, animal models are needed. While animal models of stroke are plenty, animal models of migraine with aura are relatively scarce. Migraine is an inherited disease.[89] Genetic determinants of migraine susceptibility, occurrence and severity range from rare monogenic inherited conditions (e.g., a single mutation is sufficient to cause disease in a patient) with large effect sizes to common polygenic influences (e.g., multiple polymorphisms are needed to cause disease in a patient) with small effect sizes. While the latter (i.e., common variants with small effects) is difficult to model experimentally, transgenic mouse models expressing human migraine mutations have been generated that recapitulate the clinical features of monogenic inherited conditions characterized by migraine with aura as well as ischemic stroke (e.g., CADASIL[90, 91]). Among these are mutant mouse models of familial hemiplegic migraine (FHM).

Familial hemiplegic migraine. Hemiplegic migraine is a rare monogenic form of migraine with aura characterized by motor weakness during the attacks sometimes accompanied by sensory, aphasic, visual and basilar symptoms, that can be sporadic, or familial (autosomal dominant), linked to genes involved in ion regulation (Table 1). Auras are often severe and prolonged, and a third of patients can experience a decrease in level of consciousness and even coma, which may be prolonged.[92] The net result of all FHM mutations identified to date is dysregulation of membrane ionic equilibrium and hyperexcitability, predicting enhanced susceptibility to SD as the overarching theme and the overlapping feature between migraine and stroke. FHM has been used as a model for more common forms of migraine with aura because of shared clinical features and trigger factors, female preponderance, and because two thirds of FHM patients and their first-degree relatives also suffer from attacks of common migraine with and without aura.

Table 1. Familial hemiplegic migraine genes identified to date. Modified from Russell and Ducros, 2011.[92]

	FHM1	**FHM2**	**FHM3**
Chromosome	19p13	1q23	2q24
Gene	*CACNA1A*	*ATP1A2*	*SCN1A*
Protein	Pore-forming α1 subunit of neuronal $Ca_V2.1$ (P/Q-type) voltage-gated Ca^{2+} channels	Catalytic α2 subunit of a glial and neuronal Na^+/K^+ ATPase	Pore-forming α1 subunit of neuronal $Na_V1.1$ voltage-gated Na^+ channels

Familial hemiplegic migraine type 1. FHM1 is caused by mutations in the pore-forming α_{1A} subunit of neuronal $Ca_V2.1$ (P/Q-type) voltage-gated Ca^{2+} channels (Figure 7). Mutations shift the voltage-current relationship of the channel to the left so that channels open at more negative membrane potentials (i.e., upon smaller membrane depolarizations), and stay open longer, increasing the Ca^{2+} influx. $Ca_V2.1$ channels are major regulators of presynaptic glutamate release. The net result is increased presynaptic Ca^{2+} entry and glutamate release resulting in enhanced cerebral excitability[93] that may be shared with more common forms of migraine.[13] Among the FHM1 mutations identified to date, the S218L missense mutation confers stronger gain of $Ca_V2.1$ channel function and a more severe clinical phenotype with prolonged auras that can progress to coma.[94,95] In contrast, the R192Q mutation is associated with modest gain of $Ca_V2.1$ channel function and pure hemiplegic auras.[96]

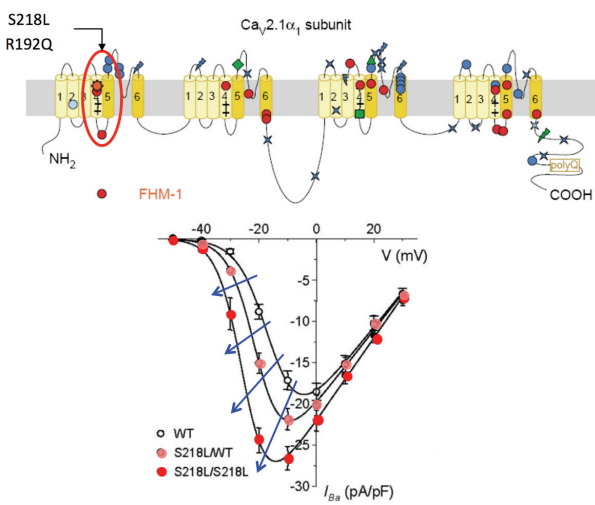

Figure 7. FHM1 mutations and the $Ca_V2.1$ gain-of-function. The two missense mutations studied as part of this thesis are also shown (S218L and R192Q). Blue arrows show the direction of change by the S218L mutation with an allele dosage effect. Modified from Pietrobon, 2005.[97]

6. OUTLINE OF THE THESIS

The overall hypothesis. The central hypothesis states that susceptibility to SD determines both the susceptibility to migraine with aura and the susceptibility to hypoxic/ischemic injury in the same direction. Therefore, factors that enhance the susceptibility to SD increase the likelihood of migraine with aura as well as ischemic stroke. Such factors that can modulate the susceptibility to SD may include genes, hormones and pharmacological agents.

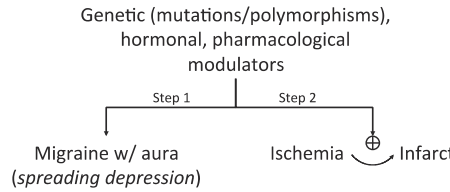

Figure 8. The central hypothesis.

The hypothesis predicts that genetic, hormonal and pharmacological modulators that enhance or suppress SD susceptibility will render the brain more or less susceptible to ischemic injury, respectively (Figure 8). As such, SD is hypothesized to be the missing link between migraine and ischemic stroke. In order to test the hypothesis in a logical sequential manner, it is first necessary to identify and characterize a set of factors that modulate the susceptibility to SD that mimic the clinical observations of susceptibility to migraine aura (Figure 8, step 1), and then study the impact of those factors on outcome of a cerebral ischemic event (Figure 8, step 2).

The aim of this thesis is to investigate how genetic, hormonal and pharmacological modulators of SD susceptibility will influence the susceptible to ischemic injury. To this end we will unravel underlying mechanisms of SD susceptibility and susceptibility to ischemic injury by making use of two transgenic mouse models of migraine that carry migraine-relevant gene mutations in voltage-gated $Ca_V2.1$ Ca^{2+} channels (**Chapters 2 and 3**) and will review all relevant clinical and experimental data from the literature (**Chapter 4**).

In **Chapter 2**, we describe the genetic and gonadal hormone modulation of cortical SD susceptibility using state-of-the-art *in vivo* electrophysiological studies under full systemic physiological monitoring and maintenance in mice. To characterize the genetic modulation, we utilize two transgenic (knock-in) mouse models of FHM1 expressing the *Cacna1a* R192Q or S218L missense mutation in $Ca_V2.1$ voltage-gated (P/Q-type) Ca^{2+} channels. As mentioned above, the magnitude of single channel gain-of-function is larger in S218L mutants compared with R192Q, and with homozygous mutants compared with heterozygotes. Therefore, the use of two mutations and three genotypes (wild-type controls, heterozygous and homozygous mutants) allows a graded modulation the SD susceptibility. **Chapter 2A** also dissects the female gonadal hormone modulation of CSD susceptibility by gonadectomy (i.e., ovariectomy) and hormone replacement, and draws parallels between the clinical symptomatology of FHM and the neurological signs displayed by FHM1 mutant mice after an SD. Because higher SD susceptibility in female mutants can in part be due to an inhibitory effect of male gonadal hormones on SD in male mice, in **Chapter 2B** we investigate the effect of

androgens on SD once again using gonadectomy (i.e., orchiectomy). Ischemic events are not limited to cortex; therefore, in **Chapter 2C** we study the SD susceptibility in subcortical structures, including striatum, thalamus and hippocampus, and its genetic and gonadal hormone modulation, with the same graded approach using different mutants, genotypes and sexes as described above. Altogether, studies in **Chapter 2** set the stage to investigate the same genetic and hormonal modulators on the outcome of cerebral ischemic events in **Chapter 3**.

In **Chapter 3**, we build upon the data obtained in **Chapter 2** and investigate how SD modulators impact the outcome of cerebral ischemic events. In **Chapter 3A**, we test the impact of genetic susceptibility to SD on susceptibility to ischemic injury by making use of FHM1 knock-in mouse models. By comparing the R192Q and S218L mutants, different genotypes (heterozygotes vs. homozygotes) and sexes, we get an opportunity to study the correspondence between SD susceptibility and ischemic sensitivity across a wide range of phenotypes. **Chapter 3B** tests the opposite effect, i.e., decreased SD susceptibility, using chronic treatment by migraine prophylactic drugs known to suppress SD. By studying two drugs and comparing the effects of chronic treatment (i.e., mimicking migraine prophylaxis) to acute effects of a single dose, we once again get an opportunity to study a wide range of effect size. And finally in **Chapter 3C**, we take the first step towards clinical translation in a retrospective case-control study, by testing whether a history of migraine, particularly migraine with aura, accelerates infarction of ischemic penumbra in acute human stroke as well.

Lastly, **Chapter 4** puts the data generated in this thesis and all relevant clinical and experimental data from the literature together into a comprehensive review to form a synthesis that can explain the clinical association between migraine and stroke. Novel hypotheses and potential experimental approaches to test them are proposed to elucidate potential mechanisms.

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