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SUMMARY

The research in this thesis was aimed at identifying and understanding mechanisms underlying modulating factors for and consequences of cortical spreading depression (CSD), the pathophysiological substrate for migraine aura that occurs in one-third of migraine patients. In this thesis, experimental studies on CSD were performed in wild-type (WT) and transgenic migraine mice, which express Ca\(_{\text{V}}\)2.1 Ca\(^{2+}\) channels with a mutated \(\alpha_1\) subunit that contains the R192Q missense mutation. The R192Q mutation was previously identified in patients with familial hemiplegic migraine type 1 (FHM1) and causes gain-of-function effects in terms of neuronal Ca\(^{2+}\) influx, neurotransmission, and susceptibility to experimentally induced CSD. Using various experimental strategies, in this thesis, the FHM1 R192Q mouse model was used to study pathophysiological mechanisms of the initiation and modulation of CSD as well as of neurobiological and molecular changes that accompany CSD events. In Chapter 2, we investigated in what way physiological factors that vary in animals during surgery affect readouts of CSD experiments, CSD frequency and threshold. We determined to what extent the composition of the gas mixture and the choice of the experimental paradigm, i.e., monitoring or controlling physiological parameters pO\(_2\), pCO\(_2\), pH and blood pressure, or no monitoring at all, affected the CSD readouts in FHM1 R192Q and WT animals. The physiological monitoring paradigm entails that physiological parameters are measured in femoral artery blood; the physiological control paradigm ensures that variations in physiological parameters are adjusted via subtle changes of the breathing condition of the animal by making adjustments in the mechanical ventilation using tracheotomy. We showed that physiological control unmasks a gender effect on visual cortex CSD susceptibility in R192Q mice that remained hidden when physiological parameters of the animal were not controlled. This finding indicates that the CSD readouts appear sensitive to changes in pH, pO\(_2\), pCO\(_2\) or blood pressure. We could also show that CSD readouts differed between visual versus motor cortex and showed a gender difference in R192Q mice, when the experiments were performed in the absence of physiological control. All in all, our study demonstrates that parameters of CSD susceptibility may be masked or unmasked depending on the experimental paradigm used. Although controlling the physiological status of an animal seems the preferred experimental paradigm, it has the risk that certain characteristics of CSD susceptibility in mutant mice are missed, when they depend on differences in physiological status between R192Q and WT mice.

A way to overcome the methodological issues related to CSD susceptibility measurements in anesthetized animals is to perform recordings of brain activity under freely behaving conditions. Therefore, in Chapter 3, we investigated mechanisms underlying CSD susceptibility in freely behaving FHM1 R192Q and WT mice. Long-term cortical DC-EEG recordings revealed an increase in cortical EEG gamma power in both the visual and motor cortex of R192Q compared with WT mice, which suggests that the mutant brain displays an overall enhanced cortical excitability. Notably, R192Q mice were found to display spontaneous CSD events, evidenced by characteristic changes in patterns of electrical activity, that were never observed in WT mice. Unfortunately, an insufficient
number of spontaneous CSD events has been recorded until now to demonstrate whether these events occur at a particular time of day, which may be expected considering reports from patients indicating a circadian component in the occurrence of their attacks. Parallel CSD frequency recordings carried out under anesthesia in the presence of physiological control, at least, did not reveal that the enhanced susceptibility of FHM1 R192Q mice for CSD was different between the start of the day and the start of the night. Our observations provide evidence that cortical hyperexcitability contributes to the enhanced susceptibility to experimentally induced and spontaneous CSD in FHM1 mice.

Many migraine patients report stress as a prominent factor that brings about their migraine attacks. In Chapter 4 we investigated the link between stress and CSD susceptibility, as surrogate for a migraine attack. In FHM1 R192Q and WT mice two paradigms were tested: behavioral restraint stress and administration of stress hormone corticosterone. Whereas subjecting mice to 20 min or even 3 hr restraint stress did not change CSD susceptibility, the administration of a single injection of corticosterone increased CSD susceptibility in R192Q mice, but not WT mice. Our finding suggests that a sudden rise in stress hormone may lead to a migraine attack when this occurs in the context of a brain that is prone to display increased excitatory neurotransmission, like it is the case when specific genetic mutations are present. It remains an enigma why natural stress - such as the restraint stress paradigm used, in which corticosterone levels also rise, does not cause a change in CSD susceptibility. It may be that in response to natural stress a spatiotemporally more complex biological response with multiple modulators is needed before an effect on CSD susceptibility can be detected. Also, it may be that such a response takes longer than the 3 hr paradigm that was used in this experiment.

In Chapters 5 and 6 we investigated effects of CSD on biomolecular profiles in brain using various mass spectrometry (MS) technologies. MS imaging (MSI) was combined with matrix-assisted laser desorption/ionisation (MALDI) for the analysis of brain sections to unravel molecular consequences of CSD in the brain of mice while maintaining spatial resolution of these compounds.

Chapter 5 described the applicability of MALDI MSI in identifying molecular changes in the brains of WT mice that were subjected to CSD. CSD-related differences in metabolite and peptide composition were observed in the hemisphere in which 7 CSD events had been induced by topical application of KCl on the dura. No changes were observed in protein composition, which can be explained by the fact that changes in protein expression take longer than the duration of the experiment. Observed changes in metabolites and peptides were CSD-related as they were absent in sham controls, in whom KCl was replaced by NaCl, which does not induce CSD.

In Chapter 6 we used the applicability of MALDI MSI from Chapter 5 to investigate whether migraine-relevant molecular changes had occurred in the brains of FHM1 R192Q after experimentally induced CSD. CSD events were associated with various changes in the content of three molecular classes (i.e., metabolites, peptides and proteins); molecular changes were observed in cortex as well as subcortical areas. When in future research the identity of the molecules is revealed, these may pinpoint (novel) neurobiological pathways involved in migraine pathophysiology. At the moment,
the findings only demonstrate that CSD events in R192Q and WT mice are associated with different molecular profiles, shown as differences in $m/z$ values.

In Chapter 7, MS technology was combined with capillary electrophoresis (CE) to assess changes in plasma metabolite composition in FHM1 R192Q and WT mice following experimentally induced CSD. We could show that specific metabolite changes can be captured in peripheral body fluid. Compared with WT mice, CSD events induced in R192Q mice were associated with a lower level of lysine and a higher level of pipecolic acid, the by-product of lysine catabolism. The study holds the promise that metabolic changes that occur in the brains of migraine patients may be measured in plasma, which is accessible in clinical research. If proven correct, metabolic changes in plasma of migraine patients could serve as potential disease biomarkers.

Chapter 8 provides a general discussion of the main findings in this thesis.