Introduction

Migraine is a common primary headache disorder, yet the exact cause and processes underlying the disease are not fully clear. Neuroimaging markers have proven useful in tracking the disease course and acquiring information about the pathomechanism, yet the underlying biological mechanisms often remain unclear, and inconsistencies in methodology can lead to conflicting results. In this work, we investigate and build upon two promising lines of research into structural and functional neuroimaging markers of migraine.

Firstly, microstructural alterations of the white matter have been reported by an increasing number of studies mostly employing diffusion tensor imaging (Szabó, Kincses et al. 2012, Chong and Schwedt 2015, Szabó, Faragó et al. 2017). These changes usually affect visual and pain-related pathways, and though their exact origin remains unclear, microstructural alterations were hypothesized to be a sign of maladaptive plasticity or degeneration in response to the continuous allostatic burden migraineurs experience in the form of headache

attacks (Granziera, DaSilva et al. 2006, DaSilva, Granziera et al. 2007, Rocca, Pagani et al. 2008, Szabó, Kincses et al. 2012). One way to disentangle confounding results in imaging markers is to examine them in a broader context, e.g. in relation to neurochemical alterations in the patients. This could also lead to novel insights about the pathomechanism, and how neurochemical and structural aspects of migraine interact. PACAP38 as a migraine-related neuropeptide is a viable candidate for this because its serum levels approximately represent intracerebral PACAP38 metabolism: the peptide is able to traverse the blood-brain-barrier via a saturable transport mechanism (Banks, Kastin et al. 1993). Since PACAP38 acts as a functional molecule in several migraine-related systems (Zhang, Malmberg et al. 1996, Engelund, Fahrenkrug et al. 2010), it is possible that changes in the peptide's metabolism in migraine might be expressed as or co-occur with microstructural alterations of the white matter. Since PACAP38 alters levels of markers that indicate glial or neuronal damage (migraine patients exhibited altered levels of S100B in the plasma after an infusion of PACAP38 (Guo, Vollesen et al. 2017)), and levels of these

markers reportedly increases during migraine attacks in migraine without aura (Yilmaz, Karaali et al. 2011), it is possible that PACAP38 induces degenerative microstructural changes in the white matter of migraine patients that might be detectable with diffusion tensor imaging. Alternatively, neurotrophic and neuroprotective effects of PACAP38 have also been described (Ogata, Shintani et al. 2015, Shioda and Nakamachi 2015), which might engender plastic changes in the brain that diffusion-weighted MRI sequences are able to detect.

Secondly, functional MRI studies investigating migraine have found altered connectivity during the headache attack and in the interictal period as well, which provides a different perspective on migraine pathomechanism (Chong, Schwedt et al. 2019). Stemming from methodological differences in the estimation of functional connectivity and the use of mixed groups, however, functional connectivity studies report conflicting results in the interictal term. A possible reason for this could be that functional connectivity between brain regions changes over time, on several time scales, as asserted by a host of

recent studies (Chang and Glover 2010, Poldrack, Laumann et al. 2015). In migraine, different thalamocortical network dynamics were found compared to controls (Tu, Fu et al. 2019), and based on dynamic functional connectivity, a study was able to determine whether a participant suffered from a migraine headache with a sensitivity of 0.7 and a specificity of 0.76 (Lee, Park et al. 2019). These findings correspond to a recent model of increased sensory gain in brain networks affected by migraine, which would suggest more flexible intra- and internetwork connections that come with an altered response or adaptation to extrinsic stimuli (Brennan and Pietrobon 2018). Altered levels of excitatory and inhibitory neurotransmitters were described in migraine patients (e.g. glutamate and GABA, see (Younis, Hougaard et al. 2017) for a review), which, accompanied by metabolic disturbances, could manifest as imbalance in network function. Temporal dynamics of intrinsic and between-network connectivity might be suitable markers of this imbalance, which is possibly more emphasized in migraine patients with aura, due to a more frequent or emphasized occurrence of disease features like cortical

hyperexcitability or cortical spreading depression (Charles and Baca 2013). The salience network could be a promising target to capture a network level expression of altered excitability. Regions belonging to this network exhibit temporal changes of connectivity that scales with disease duration in temporal lobe epilepsy, another disorder where cortical hyperexcitability occurs and which shares pathophysiological features with migraine (Luo, Yang et al. 2014, Morgan, Abou-Khalil et al. 2015, Zarcone and Corbetta 2017, Mantegazza and Cestele 2018). Therefore, we hypothesized that migraine patients, especially those with aura symptoms, also show altered temporal features of connectivity in the salience network even in the interictal term, which possibly affect connectivity between large-scale brain networks and appear as a function of the patients' clinical condition.

Objectives

In this work, we aim to place established alterations of white matter microstructure in the context of neurochemical alterations. Furthermore, we set out to identify new potential functional markers for migraine

employing temporal features of functional connectivity that might complement current approaches.

Methods

In the first study, we use tract-based spatial statistics to investigate how microstructural alterations of the white matter relate to interictal serum levels of PACAP38, a neuropeptide implicated in migraine pathogenesis, in a cohort of 26 migraine patients. In the second study, we investigate how temporal features of functional connectivity in the salience network differ between healthy controls (n=32), migraine with (n=20) and migraine without aura patients (n=37), using dynamic conditional correlation. We then proceed to describe the effects of connectivity variability on large-scale network interactions using spectral Granger's causality.

Results

Serum levels of PACAP38 correlated with mean, axial and radial diffusivity in Study 1 ($p<0.018$, $p<0.043$, $p<0.042$, respectively). These findings were located mainly in the left optic radiation and the left posterior corpus callosum,

reaching into the parietal and temporal white matter tracts. When we included disease duration in the regression model, the spatial pattern of findings relocated to the left thalamus (mean and axial diffusivity: $p<0.01$). In Study 2, the temporal variance of correlation was higher in the aura group compared to migraine without aura and healthy controls between the right anterior insula and dorsal anterior cingulate cortex ($p<0.011$ and $p<0.026$) and also higher in the aura group compared to healthy controls between the left prefrontal cortex and dorsal anterior cingulate cortex ($p<0.021$). The sum of causality from the salience to the dorsal attention network in the 0.01-0.05 Hz range was lower in migraine with aura compared to migraine without aura and healthy controls ($p<0.032$ in both cases). In migraine without aura, the variance of right and left prefrontal cortex connectivity and right anterior insula – right prefrontal cortex connectivity diminished with increasing attack frequency ($R= -0.516$, $p<0.003$ and $R= -0.456$, $p<0.012$). Causal interaction power in the 0.01-0.05 Hz range between the default-mode and dorsal attention networks diminished with longer disease duration in the migraine with aura group ($R= -0.525$,

$p<0.036$). We also found a relationship between right prefrontal cortex – dorsal anterior cingulate cortex connectivity variance and salience-default-mode network causality in the migraine with aura group ($R= -0.564$, $p<0.045$).

Conclusions

Although various biomarkers derived from MRI measurements have proven informative and useful in migraine research, questions remain about the underlying pathophysiological mechanisms and how these markers can be integrated with other markers of the disease into a unified framework. In an effort to tie alterations detectable with MRI methods to neurochemical markers, we described a link between measures derived from diffusion-weighted MRI scans and serum levels of PACAP38, an important neuropeptide implicated in migraine pathogenesis. Placing migraine-specific MRI alterations in the context of neurochemical biomarkers of the disease emphasizes the values of both markers and opens up new directions for future research.

In the second study, using recent developments in the estimation of functional connectivity, we found that intrinsic connectivity is more variable in the salience network in migraine patients with aura compared to healthy controls and migraineurs without aura. Altered connectivity variance scaled with clinical parameters, and was associated with reduced interaction between large-scale networks. These results emphasize the importance of regarding the two main migraine subtypes separately in functional connectivity studies, and add to our knowledge about changes in functional brain architecture that underlie disease processes in migraine.

Thesis points:

- During the interictal term, serum levels of PACAP38 correlate with mean, axial and radial diffusivity in migraine mainly in the left optic radiation and the left posterior corpus callosum, reaching into the parietal and temporal white matter tracts.

- When controlling for disease duration, serum levels of PACAP38 correlate with mean and axial diffusivity in the left thalamus in migraine.
- During the interictal term, the temporal variance of resting state functional connectivity is higher in migraine with aura compared to migraine without aura and healthy controls between the right anterior insula and dorsal anterior cingulate cortex and also higher in migraine with aura compared to healthy controls between the left prefrontal cortex and dorsal anterior cingulate cortex.
- The sum of causality from the salience to the dorsal attention network in the 0.01-0.05 Hz range is lower in migraine with aura compared to migraine without aura and healthy controls during rest in the interictal term.
- In migraine without aura, the variance of right and left prefrontal cortex connectivity and right anterior insula – right prefrontal cortex connectivity diminishes with increasing attack frequency.

- Causal interaction power in the 0.01-0.05 Hz range between the default-mode and dorsal attention networks diminishes with longer disease duration in migraine with aura.
- Right prefrontal cortex – dorsal anterior cingulate cortex connectivity variance correlates with salience-default-mode network causality in migraine with aura.