Macro- and Microstructural Alterations in Migraine and Cluster Headache

PhD Thesis Summary

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II. **Nikoletta Szabó**, Zsigmond Tamas Kincses, Árpád Párdutz, Eszter Tóth, Gergő Csete, Délia Szok, László Vécsei: White matter disintegration in clustere headache. J Headache Pain. 2013 Jul 24;14(1):64. doi: 10.1186/1129-2377-14-64.

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Abbreviations

AD	Axial diffusivity
CGRP	Calcitonin gene related peptide
CH	Cluster headache
CSD	Cortical spreading depression
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
FDT	FMRIB's Diffusion Toolbox
FSL	FMRIB Software Library
MD	Mean diffusivity
MMP	Metalloproteinase
MRI	Magnetic resonance imaging
PAG	Periaqueductal gray matter
PD	Perpendicular diffusivity
PET	Positron emission topography
ROI	Region-of-interest
SPSS	Statistical Package for Social Sciences
TBSS	Tract based spatial statistics
TFCE	Threshold free cluster enhancing approach

Introduction

Migraine

Migraine is the most common primary headache disorder. The disease commonly presents in the form of intense pulsing or throbbing unilateral headache. The socio-economic importance of the disease is that approximately 20% of the population suffers from disabling attacks of migraine during lifetime. The pain commonly accompanied by nausea, vomiting and extreme sensibility to light, smell and sound. In one quarter of the patients the headache is preceded by transient central nervous system symptoms, mostly visual or sensory, called aura.

The underlying pathophysiological process of migraine is not entirely understood and established indicators of the disease are missing. There are evidences, that the trigemiovascular pathway and hemispheric brain structures also must have a central role in the pathomechanism, which suggest the multilevel origin of the disease. Dural perivascular trigeminal activation and the release of calcitonin gene related peptide (CGRP) and substance P are involved in migraine pathomechanism and that cause neurogenic inflammation. The nociceptive information is then transmitted by the sensory neuron, residing in the trigeminal ganglion, to the trigeminal nucleus caudalis, wherefrom it is mediated to the thalamus and forth to cortical centres. Most of the migraineurs also report allodynia during the attacks when non-nociceptive stimuli cause pain either in the trigeminal or extracephalic areas, which is associated with the disease duration and attack frequency. This process involves the central sensitisation of the secondary and tertiary neurons of the trigeminal system. Trigeminal sensitisation might arise from two distinct but not necessarily exclusive processes: stimulation might arise from the cortical spreading depression (CSD), that stimulates trigeminal endings or alternatively top-

down modulation of the trigeminal system from higher-level cortical systems. The altered excitability of the cerebral cortex in the interictal state seems to be fundamental in the brain's susceptibility to migraine attacks.

To understand the parallel steps of the pathogenesis we should examine the migraine with extended methods. Beyond the investigation of animal models (the relevance of which is limited) and laboratory testes functional and structural imaging techniques have a crucial role to investigate *in vivo* alterations in migraine patient's central nervous system.

The first functional imaging studies were based on positron emission tomography (PET). PET studies found increased activity in visual cortex during migraine attack and photophobia. Functional MRI (fMRI) examinations confirmed the results of the PET studies. The pain-related areas showed altered activation during migraine attack and in interictal period.

Structural magnetic resonance imaging (MRI) studies, despite of the different methodological process, consistently revealed loss of gray matter in regions pain related brain regions, including the frontal cortex, temporal lobe, insula, and brainstem. White matter microstructure changes, as defined by diffusion-weighted MRI, are receiving more and more attention. Diffusion-weighted MRI is sensitive to diffusion of water molecules, which in the brain is largely restricted by the membranes of the cellular particles. Diffusion weighted MRI is tuned to measure diffusion in certain directions and by fitting a diffusion tensor model to the measured diffusion profile, it is possible to calculate diffusion parameters that reflect the microscopic organisation of the measured volume. A number of studies reported altered white matter microstructure in migraine with different methodological approaches. For example, lower white matter mean diffusivity (MD) and increased fractional anisotropy (FA) were found in migraine patients by means of a histogram analysis. Using a voxel-based morphometry style analysis, lower FA was

described in the thalamocortical tract of migraineurs, and similar alterations were found in the trigeminothalamic tract and in the periaqueductal gray matter (PAG) of patients with and without aura, respectively. By using a similar approach, Granziera and colleagues found reduced FA in the visual motion-processing network.

Cluster headache

Cluster headache (CH), a primary headache disorder within the group of trigeminal autonomic cephalalgias, is characterised by paroxysmal hemicrania and ipsilateral craniofacial autonomic symptoms. Cluster periods may last from weeks to months, usually followed by remission periods when the headache attacks stop completely. The prevalence of the CH is about 0.1%.

The pathomechanism of CH is not fully understood but involves both central and peripheral mechanisms. Due to the periodic appearance of the attacks, there has been numerous studies suggesting the role of the hypothalamus. PET studies showed that the anterior cingulate cortex, the contralateral thalamus, the ipsilateral basal ganglia and both insulae were activated in CH. Nevertheless, the activation of the hypothalamus seems to be a specific feature of cluster attacks, indicating its pivotal role in the pathogenesis and pain regulation in CH. Since the hypothalamic activation can influence the pain-matrix these findings point to the multifocal origin of the CH, the dysfunction of the pain-matrix. Structural MRI studies found grey as well as white matter alterations in CH similar to those found in migraine. A recent diffusion tensor imaging (DTI) study in CH found reduced fractional anisotropy (FA) in the pain matrix. Contrarily, another study found no microstructural alterations (investigated FA and MD) in CH. While these results may be contradictory, imaging markers could be a powerful tool to describe disease progression and reveal important clues on the pathomechanism.

Objectives

The aim of our study was to seek biomarkers of primary headache disorders and examine the microstructure of the white matter in migraine and cluster headache. Furthermore, thalamic morphology was examined in migraine using structural MRI methods.

Methods

Participants

Twenty-one female migraine patients and thirteen patients with episodic CH were chosen. All patients underwent a clinical interview. Headache-related allodynia was also evaluated. MRI scans were acquired in the interictal period. As controls, seventeen age-matched, right-handed, healthy female individuals and sixteen controls, with no history of neurological or psychiatric diseases were included in the migraine and CH study respectively. The study was approved by the local ethics committee (authority number: 87/2009.).

Image acquisition

MR imaging was carried out on a 1.5T GE Signa Excite HDxt MRI scanner. 3D spoiled gradient echo and 60 direction diffusion weighted images with 6 non-diffusion-weighted reference volume were acquired for all the subjects.

Image analysis

Processing of diffusion data

Correction for eddy currents and movement artifacts by 12 DOF affine linear registration was done to the first non-diffusion-weighted reference image. Diffusion tensors at each voxel of the brain were fitted by an algorithm of FMRIB's Diffusion Toolbox (FDT) in FSL (v. 4.0, www.fmrib.ox.ac.uk/fsl;). FA, MD, and diffusivity parallel

(λ1) and perpendicular ((λ2 + λ3)/2) to the principal diffusion direction were calculated for the whole brain. In CH study, images were mirrored to the midsaggital axis according to the side affected by the headache. We used the tract based spatial statistical (TBSS) method. Modeling and inference using standard general linear model design set-up was accomplished using permutation-based cluster analysis (5000 permutation) as implemented in FSL. The design encoded for group membership and clinical variables.

In the migraine study clusters were formed according to a defined threshold ($t = 2.3$) and corrected for multiple comparisons (across space) within the permutation framework by building up the null distribution of the maximum cluster size for each permutation ($p < 0.05$). We also carried out a region of interest analysis on the white matter regions whose FA values differed significantly between groups. We tested for differences in FA, MD, longitudinal, and perpendicular diffusivity.

Connectivity of the identified differences in white matter integrity was defined by probabilistic tractography (FDT, part of FSL: www.fmrib.ox.ac.uk/fsl/fdt/) in migraineurs. We fitted a multifibre diffusion model. Probabilistic tractography was then performed from any brain voxel by tracing streamline samples through these probabilistic distributions on fibre direction. We generated 5000 streamline samples from each seed voxel to build up a connectivity distribution. Cluster-masks of the TBSS analysis were used as a binary seed masks.

In CH study after using permutation test (5000 permutation), statistical images were thresholded by the novel threshold free cluster enhancing approach (TFCE). Similar analysis were carried out for the MD, perpendicular (PD) and axial diffusivity (AD). The diffusivity parameters were extracted from the affected regions and these parameters were correlated with the number of cumulative headache days using Statistical Package for Social Sciences. Laterality index

was calculated for every diffusion parameter from the number of suprathreshold voxels.

Volumetric analysis

Volumetric analysis of the thalamus was carried out using tools of FSL (FMRIB Software Library, http://www.fmrib.ox.ac.uk/fsl). A deformable-model-based segmentation-registration tool employing a Bayesian Appearance Model (FMRIB's Integrated Registration Segmentation Toolkit), FIRST was used, which can automatically segment the thalami. Given our primary is interest of volume changes related to the sensitization of the tertially, thalamic neuron, our prior hypothesis was centered on the thalamus. The other subcortical structures were also segmented. Volumetric comparison of the segmented thalami across groups was performed using the Statistical Package for Social Sciences.

Shape changes of the subcortical structures

A surface mesh model was fit to the individual thalami. Group comparisons of corresponding vertex locations were performed by calculating vertex-wise F statistics to investigate localized shape differences. Since, this was only performed as a post-hoc localisation of the significant changes, identified with the volumetric analysis, uncorrected p-values were used.

Connectivity of focal thalamic volume changes

Probabilistic tractography was started from the described focal thalamic shape changes (thresholded at $p<0.05$, uncorrected). We fitted a multi-fiber diffusion model that estimates probability distributions on the direction of one or more fiber populations in each seed brain voxel. Individual tractography results were thresholded at 1000 particles (20%), binarised and summed over subjects to represent the group level connectivity pattern.

Results

Migraine

Focal white matter microstructure alterations

As evaluated by group level voxelwise FA differences in the centre of white matter fibre bundles, the white matter microstructure was significantly changed in migraine patients as compared to controls. FA was lower, while MD and perpendicular diffusivity were significantly higher in patients than in controls ($p < 0.0088$ and $p < 0.0002$, respectively). Longitudinal diffusivity was not different between groups ($p > 0.101$). In a whole brain analysis, neither MD nor the longitudinal/perpendicular diffusivity showed any significant difference between patients and controls. No correlation was found between the observed FA and disease duration or attack frequency.

Connectivity of focal FA changes in migraine

The probabilistic tractography indicated that fibres of the right frontal white matter showing the FA alteration were connected to the ipsilateral prefrontal cortical regions, insula, thalamus, dorsal, and ventral midbrain. Fibres were proceed in the direction of the occipital cortex through the putative inferior fronto-occipital fasciculus. Some fibres also crossed the midline through the corpus callosum.

Subcortical structures' volume in migraineurs and controls

The size of the left ($p<0.04$) as well as the right ($p<0.047$) thalami was significantly larger in patients. There was no significant difference between the size of the left and right thalamus either in case of the patients ($p<0.467$) or the healthy subjects ($p<0.299$). The volume of the other subcortical structures was not difference between patients and controls ($p>0.05$).

The relationship of thalamic volume changes and clinical features

The number of attacks within one year significantly correlated with the size of the left ($R=0.550$; $F(1,15)=6.491$; $p<0.022$) and right thalamus ($R=0.496$; $F(1,15)=4.881$; $p<0.043$) (Figure 5.). We found no significant correlation between the number the total attacks and the size of the thalami (right thalamus: $R=0.104$; $F(1,15)=0.164$; $p<0.691$; left thalamus: $R=0.161$; $F(1,15)=0.398$; $p<0.538$). There was no significant correlation between the duration of the disease and the size of the thalami (right thalamus: $R=0.176$; $F(1,15)=0.480$; $p<0.499$; left thalamus: $R=0.137$; $F(1,15)=0.286$; $p<0.601$). Significant correlation was shown between the allodynia score of the patients and the volume of their left thalamus ($R=0.528$; $F(1,15)=5,805$; $p<0.029$). There was no significant correlation between the size of the right thalamus and the allodynia score ($R=0.233$; $F(1,15)=0.859$; $p<0.369$).

Surface changes of the thalami in migraineurs

Focal shape changes showed a local augmentation of size in the ventral region of the right thalamus in patients of the right thalamus. Probabilistic tractography of this enlarged area showed consistent connectivity to the dorsal brainstem. In the other direction, the highest connectivity was found to be to the premotor and prefrontal cortices.

Cluster headache

White matter microstructural alterations

The whole brain TBSS analysis showed decreased FA ($p < 0.02$, corrected for multiple comparison) in the corpus callosum, bilaterally in the forceps minor and major, right corona radiata, left internal and external capsule, left cerebral peduncule, frontal portion of the left corona radiata, right parietal juxtacortical white matter, left inferior fronto-occiptal fascicle. MD was found increased ($p < 0.01$, corrected for

multiple correlations) in regions where FA alterations were found, but the alterations were more extensive involving more frontal, parietal and temporal juxtacortical white matter. Axial diffusivity was also found be increased in widespread white matter regions ($p < 0.02$, corrected for multiple correlations) similar to those of FA changes, but no significant alteration of axial diffusivity was found in the right parietal lobe in the juxtacortical white matter and the posterior corona radiate. Augmented perpendicular diffusivity ($p < 0.01$, corrected for multiple correlations) was the most extensive among the different diffusion parameters, involved essentially all major white matter fibre bundles, except the right external capsule. Laterality indices of all measured diffusion parameters showed left dominancy (LI_{FA}: 9.8, LI_{MD}: 0.2, LI_{AD}: 0.7, LI_{PD}: 0.2). There was a significant correlation between the number days with cumulative headache days and axial diffusivity in regions showing significant differences in AD ($p < 0.022$, r: 0.626, corrected for multiple comparisons, Figure 9.).

Discussion

In this thesis MRI detected structural alterations are presented in migraine and CH patients: (1) in migraine patients the right frontal white matter microstructural alterations were found and (2) increased thalamic volumes were identified, which is correlated with the attack frequency and the level of allodynia, while (3) in cluster CH similar pattern of diffusion parameter alterations were detected, but more widespread in the white matter and (4) correlation was found between the disease burden and axial diffusivity.

Migraine

Microstructural alterations in migraine

Our results are in line with previous reports describing

structural and functional alterations of the frontal cortex of subjects with migraine. Frontal cortical atrophy was reported in migraine patients and frontal cortical gray matter density reduction was correlated to T2-visible lesion load. In migraineurs altered cognitive shift was correlated with the reduced frontal gray matter. In a recent investigation gray matter atrophy was found in the left medial prefrontal cortex, the dorsal anterior cingulate cortex, the right occipital lobe, the cerebellum and brainstem. The volume of the anterior cingulate cortex showed correlation with disease duration. In migraine patients increased functional connectivity was detected between left dorsolateral prefrontal cortex, the bilateral middle temporal lobe, orbitofrontal cortex and the left anterior cingulate cortex. However, there is a potential that alterations of the frontal lobe might not be specific to migraine. In other chronic pain syndromes gray matter loss was frequently reported in the prefrontal cortex, cingulate, parahippocampal gyrus, insula amygdalae, hippocampi, postcentral gyri, anterior cingulate gyri, and superior frontal gyri.

Thalamic volumetry

The thalamus has a cardinal role in pain perception as well as in migraine pathomechanism. It is where the second order neurons project (trigeminothalamic tract) and were the third-order neurons of the thalamocortical tract emerge. Thalamic neurons, which are responding to dural stimulation, also were shown to be sensitised by ipsilateral cephalic and extracephalic chemical stimulation. The role of the thalamus in migraine and trigeminal neuralgia was also indicated by the abnormal balance of metabolite levels, detected by MR spectroscopy, in the thalamus. Thalamic activation was described during migraine attacks. Interestingly, in another trigeminal pain disorder, in temporomandibular disorder thalamic and sensory cortical gray matter enlargement was found and shown to correlate with disease duration. In

contrast, localised thalamic atrophy was detected in trigeminal neuropathy, but not in patients that were classified as having trigeminal neuralgia or temporomandibular disorders. A further study found reduced thalamic volume (contralateral ventral postero-lateral nucleus) in limb amputees with phantom pain. However, this atrophy most probably was related to the loss of sensory input than the pain, since the time since amputation was correlating with the thalamic volume and not the pain index describing the pain intensity and frequency. Contrary, in neuropathic pain in ankylosing spondylitis increased gray matter volume in the thalamus and putamen was observed.

The relation of our finding to migraine pathomechanism

In our view there can be alternative interpretations for the white matter alterations and the thalamic enlargement in migraine patients. Degenerative changes and maladaptive plasticity might co-occur in the disease.

(1.) In localized white matter diffusivity alteration (reduced FA) might reflect degenerative process in migraineurs. One hypothesis states that the depolarization wave progressing through the cortex has a central role in migraine pathomechanism. The excessive activation might well be enough to induce in cellular damage, kindle neuroinflammation and consequently cause pain. There seems, therefore, to be some evidence for biochemical changes potentially involved in the disintegration of white matter fibre bundles that might be reflected by reduction of FA, increase of MD, and augmented perpendicular diffusivity. Similar patterns of DTI abnormalities are most frequently reported as a consequence of neurodegenerative processes. Reduction of FA and longitudinal diffusivity reflect axonal loss, while increased perpendicular diffusivity seems to be a sign of demyelination.

(2.) As regarding the increased size of the thalami in migraine the first scenario is the thalami are genuinely larger that could

kindle the pathomechanism. Longitudinal imaging studies of normal people, who later develop migraine, or genetically stratified imaging studies could be of use.

Alternatively, and more likely, the increased volume of the thalami is the result of the disease. There are evidences that repeated painful stimuli similarly to training lead to plastic changes in the brain.

Apart of the repeated pain in migraineurs, other factors related to the pathomechanism of the disorder might also contribute to maladaptive plasticity: the altered cortical excitability might also lead to such changes. Cortical spreading depression itself may induce neurogenesis in the cortex as well as in subcortical structures.

Explanation of such controversy may lie in a lack of noxious stimulus in chronic pain, chronification of the pain condition, compensatory mechanisms or affective components, and personality traits related to migraine.

Another aspect of our findings that has to be considered is the integration of the thalamic enlargement and the prefrontal white matter alteration in the pain related functional networks. Hadjipavlou and co-workers described the pain network that contains prefrontal cortex, periaqueductal gray and cuneiform nucleus, amygdala, thalamus and hypothalamus, and rostroventral medulla. Our tractography findings are in line with this study; accurately the prefrontal white matter lesion and the enlarged thalamic region are connected to the above-mentioned structures.

The vertex analysis showed that the ventral surface of the thalamus was enlarged in patients. The probabilistic tractography showed that this ventral aspect of the thalamus is the area connected to brainstem structures. These structures cannot be unanimously identified with the current resolution, but might connect thalamus to the PAG or could possibly be the fibres from the putative trigemino-thalamic pathway. Regarding the pathomechanism of migraine each of these pathways could have crucial importance.

We found thalamic volume being related to attack frequency and allodynia reported by the patients. Allodynia is a frequently reported feature of migraine and thought to be a sign of neuronal sensitisation. In a recent parallel rodent electrophysiological and human fMRI investigation extracranial allodynia was associated with the sensitisation of these third order neurons. Furthermore, reduced fractional anisotropy was found in the thalamocortical tracts in migraineurs. It would be tempting to relate the thalamic enlargement to the appearance of extracranial allodynia in our study group also, but almost all the patients having allodynia also reported that as being extracranial, and therefore we do not have appropriate statistical power.

Cluster headache

In the literature there are contradictory results on the diffusion alterations in cluster headache, despite using the same analysis approach as in our study. Teepker and colleagues described reduction of FA in several brain regions, but no other diffusion parameters were investigated. Another investigation on CH patients found no alteration of FA or MD with similar analytical method. One possible reason why we found more widespread changes than in previous studies could be because of the high angular resolution DTI acquisition, what we have used in our study, thereby providing a higher signal to noise ratio.

Correlation between the alteration of diffusion parameters and tissue microstructure is not yet entirely clear. However, the increment of mean and perpendicular diffusivity, which was the most prominent finding of our study, is most probably a sign of increased distances between membranes. This mostly relates to demyelination, but combined axon and myelin loss may also cause a complex change of diffusion parameters. The increased inter-membrane distance may also cause increased perpendicular diffusivity. One could speculate

that the changes in the extracellular space might be related to the sterile inflammation proposed in CH.

It was previously suggested that the lack of correlation of diffusion abnormalities with attack frequency or disease duration point to a phenotypic biomarker of the disease, reflecting a congenital condition rather than a process related to disease progression over time. However in our current investigation we found a negative correlation between the axial diffusivity in and the cumulative headache days. This interesting finding can be explained by observations showing that early stage of axon damage is associated with reduced axial diffusivity. However later, the axial diffusivity will pseudo-normalise again as the axon and myelin debris gradually cleared. This mechanism could potentially explain our findings, nevertheless it should be emphasized that none of the DTI indices are a direct measurement of specific white matter compartments, hence no direct relation can be established between our results and the pathomechanism of CH.

Conclusions

Our findings raise the possibility that diffusion imaging and thalamic volumetry in research settings could be a possible biomarker of the primary headache disorders. The pattern of diffusion parameter changes, what we found in CH is similar to what we have described with identical methods in migraine, but the changes in CH are more extensive. However, specificity to migraine as opposed to other chronic pain conditions has to be investigated. While thalamic enlargement seems to be a clue to the pathogenesis of migraine chronification, further investigations into the different phases of the disease would help to elucidate the importance of our findings.

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