Final Report

Title:	**Primary headache disorders: Structural and functional biomarkers**
NKFI ID Number:	*OTKA PD-104715*
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Start of the project:	*2012.10.01*
End of the project:	*2016.03.31*

Summary

In this three years project we proposed to investigate the the following questions:

1. Are white matter microstructural changes, we found earlier, migraine specific?
2. Does white matter disintegration in migraine come with structural disconnection in the pain network? Alternatively, can we reveal stronger connectivity due to maladaptive plasticity in the pain network?
3. Are white matter microstructural alterations related to functional abnormalities as measured with resting state fMRI?
4. Are repeated painful attacks and chronification of migraine related to macroscopic structural alterations?
5. Are (micro)structural alterations related to neuronal/glial damage?

We showed that microstructural disintegration is identifiable in cluster headache (Szabo et al., 2013), and these are even more extensive than those we have formerly published in migraine (Szabo et al., 2012). In our recent investigation we extended the study to migraineurs whose headache is preceded by aura. It seems that in aura patients the microstructural alterations are the opposite to what we have found in non-aura patients and these changes are more extensive in space as well as in magnitude (Szabo et al., submitted). As a methodological novelty we introduced the use of *Linked Independent Component Analysis* into the analysis of diffusion parameters of the white matter (Kincses et al., 2013).

Macrostructural alterations of the subcortical structures was also investigated in primary headache disorders. In migraine enlarged thalami were found is a smaller group of patients. After a lengthy revision process Pain rejected the work with a further option of resubmission after acquiring more data. After the evaluation of the data from a larger patient population we had to realize that other factors, such as aura, comorbidity age and gender have also to be taken into consideration before resubmitting the work (Szabo et al., in prep). Along those lines we investigated the gender and age dependence of size of subcortical structures in a recently published work (Kiraly et al., 2015).

As a methodological challenge we showed that in healthy there is no correlation between the sizes of the subcortical structures. However in Alzheimer's disease such parallel atrophy develops (Stepan-Buksakowska et al., 2013). Similar correlation develops in cluster headache

patients. Considering the size and average diffusion parameters of the subcortical structures we have been able to separate cluster headache patients with pain on the left and right side and healthy controls. The manuscript was rejected from Pain and now is submitted to Brain (Kiraly et al., submitted).

Functional networks are also examined in primary headache disorders. We introduced a novel approach investigating the amplitude and frequency distribution of the BOLD resting state fluctuations (Farago et al., submitted-b). With this approach we have been able to confirm alterations in the resting state BOLD fluctuations in cluster headache patients (Farago et al., submitted-a). Interestingly, such alteration is restricted to the the key regions of the pain matrix (anterior cingulate cortex) in migraineurs with aura (Farago et al., in prep).

Functional consequences of chronification of pain was investigated in a rat model in collaboration with the Preclinical Imaging Center of Gedeon Richter Plc. CFA injection into the wishker pad of the rat caused increased activation of the anterior cingulate cortex in a long term. This activation is thought to be a representation of allodynia. We also showed, in collaboration with the Department of Nuclear Medicine of the University of Debrecen (Spisak et al., submitted) that the effective connectivity of the cingulate cortex is altered due to the chronification of the pain.

We showed that the pituitary adenyl cyclase- polypeptide-38 (PACAP-38) serum concentration is lower in the interictal phase in migraineurs (Tuka et al., 2013). In our recent work we showed that the interictal PACAP-38 concentration is correlating with the disintegration of the intrathalamic white matter (Vereb et al., in prep). In an attempt to identify the serum markers of the white matter disintegration, we failed to identified altered neuronspecific enolase or S100B concentrations in the serum of migraineurs.

To identify the possible genetic marker of migraine chronification we tested the ApoE genotype of migraineurs. Our preliminary data suggest that the ε2ε2 gentotype and the ε2 allele frequency is higher in migraineurs than it was reported in healthy.

Earlier we showed that children with migraine have difficulty in motion processing (Braunitzer et al., 2012). Following up on that issue we identified the white matter pathway underlying the motion detection in a noisy environment (Csete et al., 2014). With a similar approach we separated the segregated visual white matter pathways (Kaposvari et al., 2015).

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In an earlier investigation we found microstructural alteration in the white matter of migraineurs (Szabo et al., 2012). In that work we found reduced fractional anisotropy in a right frontal white matter cluster of migraine patients. In the same region we also found increased mean diffusivity and increased radial diffusivity.

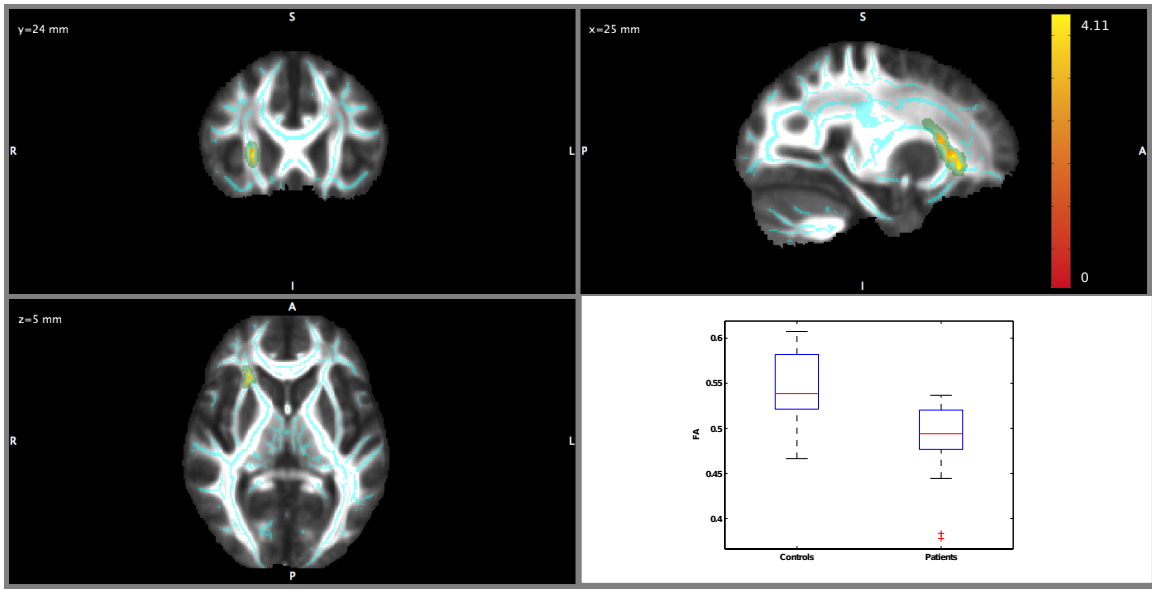

Figure 1. TBSS indicates reduced FA in the right frontal white matter in migraine patients. The mean FA skeleton rendered in light-blue. A thickened version of the significant cluster is used for easier visualisation (green). The t-scores are depicted in red-to- yellow colours within the significant cluster. Mean FA of the two groups within the cluster is depicted on the graph. On the box-plot the central mark is the mean, the boxes represent the 25% and 75% percentiles, outliers are depicted as red crosses.

The probabilistic tractography showed connection of this cluster to other parts of the pain network (orbitofrontal cortex, insula, thalamus, dorsal midbrain). We proposed that these changes are representation of disintegration of the white mater.

In a consecutive analysis using high spatial resolution diffusion measurements we carried out a similar investigation in cluster headache patients (Szabo et al., 2013). There was a significant increment of the mean, axial and perpendicular diffusivity in widespread white matter regions in the frontal, parietal, temporal and occipital lobes. Reduced fractional anisotropy was found in the corpus callosum and some frontal and parietal white matter tracts mainly in the contralateral side of the pain (**Figure 2**).

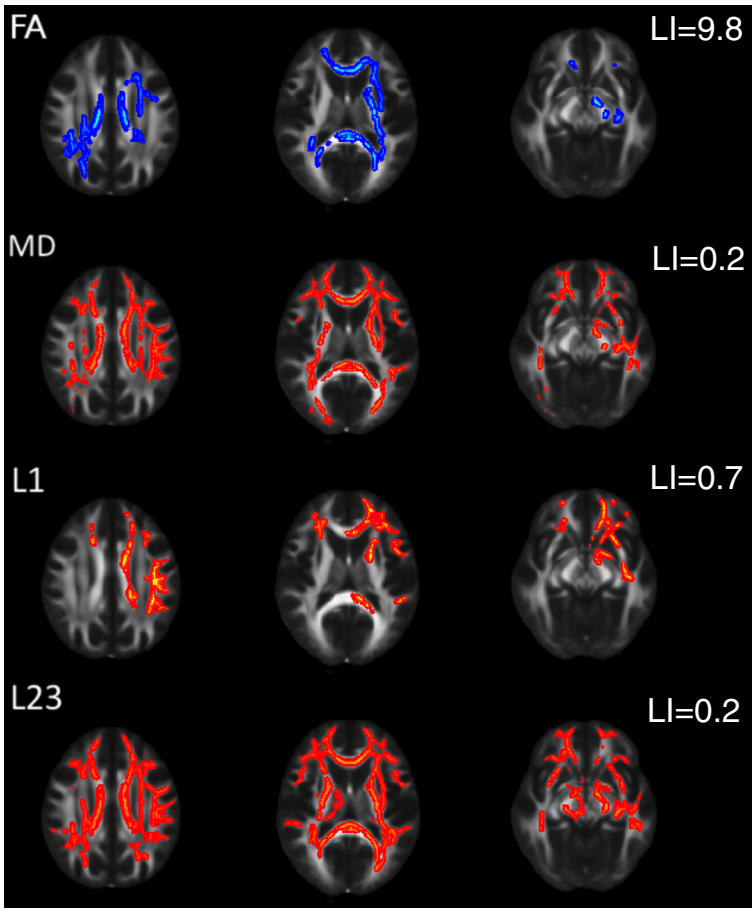

Figure 2. Alteration of diffusion parameters in cluster headache patients. *Blue colors indicate reduction; red-to-yellow colors indicate increment in the given diffusion paramters. The mean FA skeleton is shown in green. A thickened version of the significant cluster is used for easier visualization.*

Axial diffusivity showed negative correlation to the number of the headache attacks. These results indicate that the microstructural disintegration is not only identifiable in cluster headache, but are even more extensive than those we have formerly published in migraine.

Interestingly, it seems that in aura patients (MWA) the microstructural alterations are in the opposite direction to what we have found in non-aura patients (MWoA) and these changes are more extensive in space as well as in magnitude (Szabo et al., submitted) (Figure 3). Furthermore some of the diffusion paramerters correlated with clinical measures, such as disease duration or attack number.

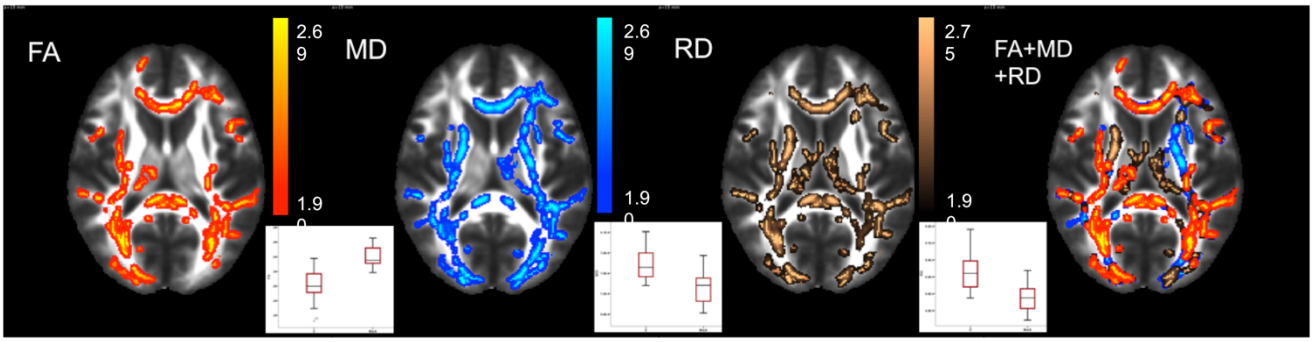

Figure 3. White matter alterations in MWA compared to controls. Axial slices show the diffusivity parameter changes from TBSS. The FA increased, MD and RD decreased in MwA compared to controls. AD showed no alteration. The affected areas are mainly overlapping. Colorbar shows the z-scores of the corrected p-values. Boxplot shows the mean diffusivity parameters depicted from the affected areas, central mark is the mean, the boxes represent the 25% and 75% percentiles.

As a methodological novelty we introduced the use of *Linked Independent Component Analysis* into the analysis of diffusion parameters of the white matter (Kincses et al., 2013). Linked ICA automatically balances the information content of different modalities, finding subject loadings that produce statistically independent and non-Gaussian spatial maps across the modalities (Groves et al., 2011). The novelty of our analysis is that we applied the approach to diffusion paramters only to identify a motive of diffusion parameters that is describing the disease.

Macrostructural alterations of the subcortical structures was also investigated in primary headache disorders. In migraine enlarged thalami were found is a smaller group of patients. The size of the right ($p<0.047$) as well as the left ($p<0.04$) thalamus was significantly larger in group of 17 patients and 17 controls when corrected for total brain volume. This augmentation was localised to the ventral aspect in case of the right thalamus that received fibres from the dorsal midbrain and project to the premotor and prefrontal cortex with the highest probability (Figure 4). The size of the thalami were correlated with the attack frequency (left: $p<0.022$; right: $p<0.043$).

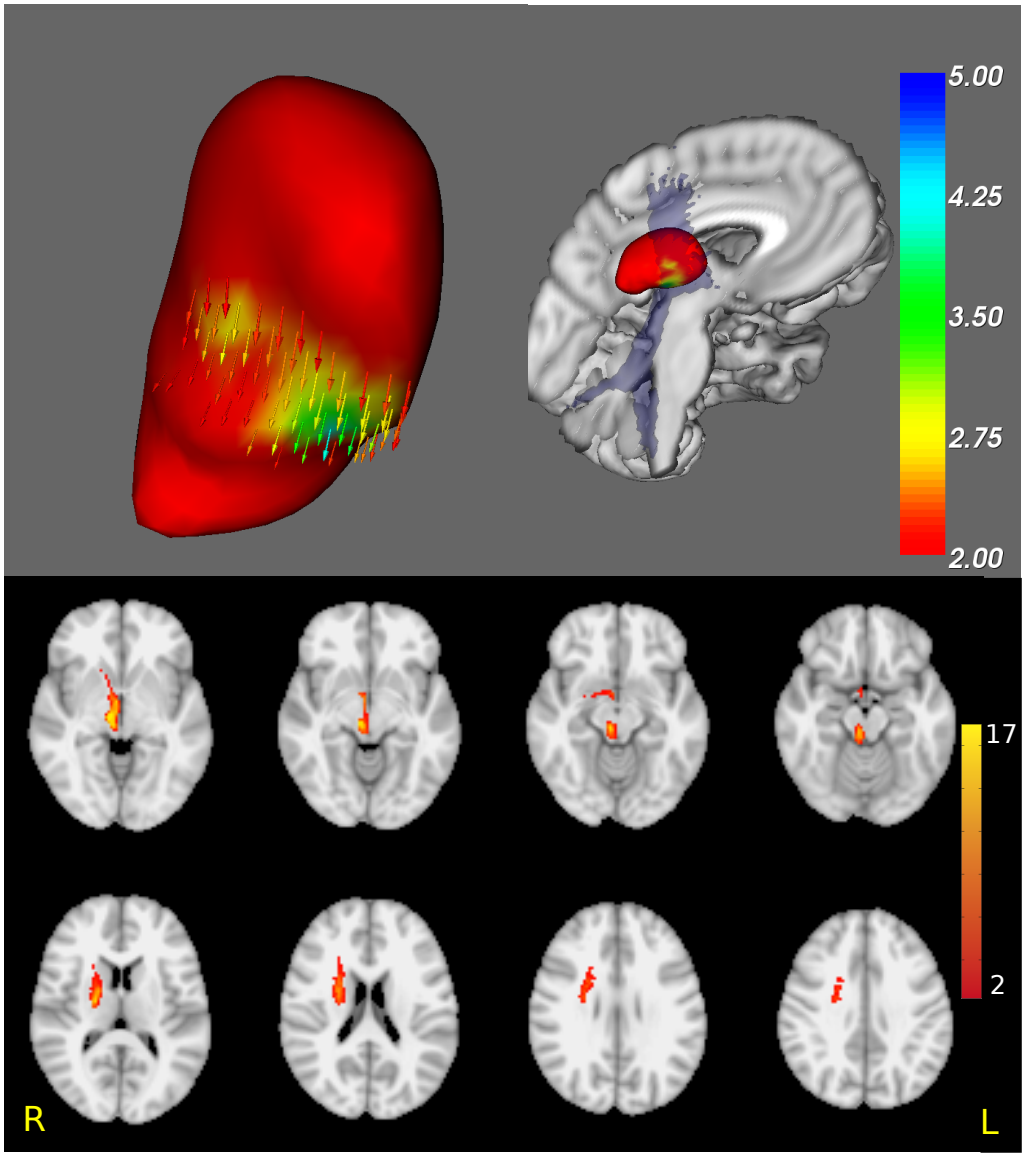

Figure 4. Focal size augmentation of the right thalamus and the connectivity of that region. In the top left corner the right thalamus is depicted from the anterior aspect and slightly below. Yellow to blue colors (color bar on the right represent F-values) represent the location of the size augmentation in patients. Arrows show the direction of movement of individual vertices across groups. On the 3D image the same right thalamus is depicted with the result of the probabilistic tractography in transparent blue. On the lower image the downstream (upper row) and upstream (lower row) connectivity of the affected thalamic region is shown. Images were registered to standard brain, individual tractography results were thresholded at 1000 particles (20%), binarised and summed over subjects. The red to yellow scale shows super-thershold connectivity values present in two patients.

After a lengthy revision process *Pain* rejected the work with a further option of resubmission after acquiring more data. After the evaluation of the data from a larger patient population we had to realize that other factors, such as aura, comorbidity age and gender have also to be taken into consideration before resubmitting the work (Szabo et al., in prep). Along those lines

we investigated the gender and age dependence of size of subcortical structures in a recently published work (Kiraly et al., 2015).

As a methodological challenge we showed that in healthy there is no correlation between the sizes of the subcortical structures. However in Alzheimer's disease such parallel atrophy develops (Stepan-Buksakowska et al., 2013). Similar correlation develops in cluster headache patients (Figure 5).

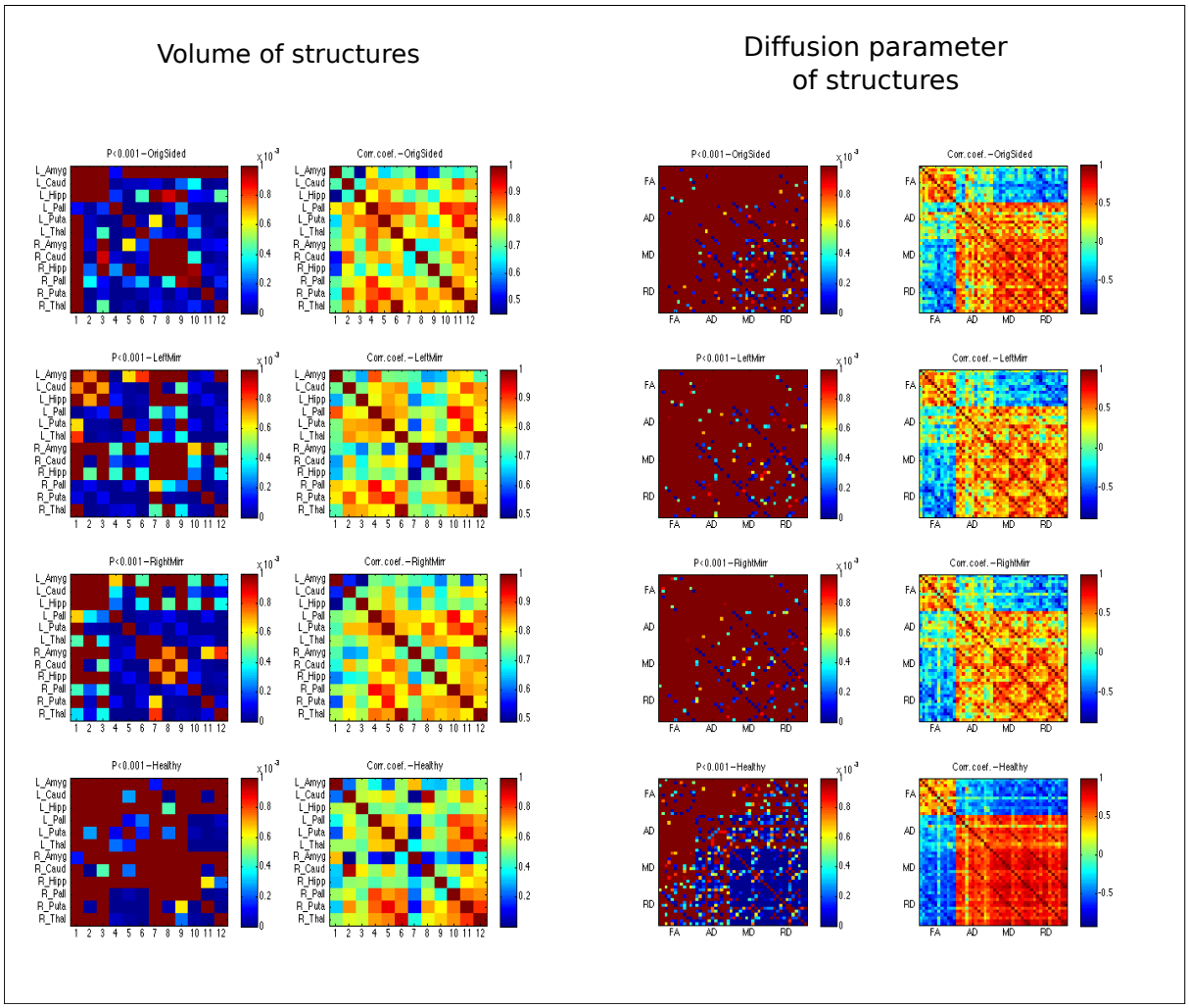

Figure 5. Intra-subject cross-correlations. 1st and 3rd column. P<0.001, uncorrected (blue), 1-6 = LEFT Amyg, Caud, Hipp, Pall, Puta, Thal; 7-12 = RIGHT Amyg, Caud, Hipp, Pall, Puta, Thal. 2nd and 4th column: R = Pearson's correlation coefficient (all positive). ***Rows:*** *OrigSided, LHS-CH, RHS-CH, Control groups.* ***Volume correlations:*** *12x12 matrix (12 structure),* ***Diffusion correlations:*** *48x48 matrix (12 structure x 4 diffusion parameter).*

Considering the size and average diffusion parameters of the subcortical structures we have been able to separate cluster headache patients with pain on the left (LHS-CH) and right side

(RHS-CH) and healthy controls (Figure 6). After a lengthy review process manuscript was recently rejected from *Pain* and now is submitted to *Cephalgia* (Kiraly et al., submitted).

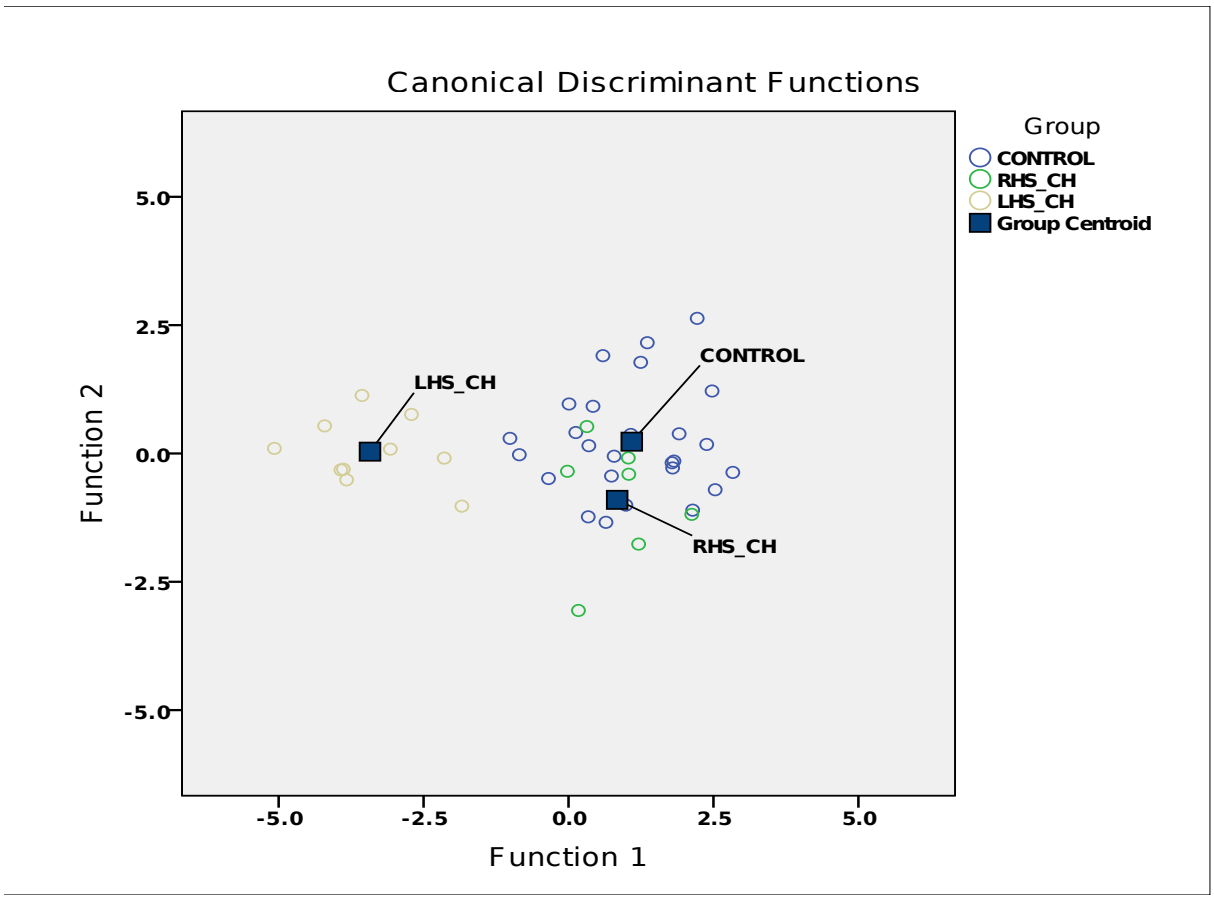

Figure 6. Discriminant analysis. Results from SPSS 20.0. Controls are labelled with blue circle, RHS-CH group with green circle and LHS-CH group with yellow circle. Blue squares indicate the centroid of the groups.

Functional networks are also examined in primary headache disorders. We introduced a novel approach investigating the amplitude and frequency distribution of the BOLD resting state fluctuations (Farago et al., submitted-b). The novelty of our approach is that the decomposition of the timecourses to frequency bands is made by wavelet analysis. With this approach we have been able to confirm alterations in the resting state BOLD fluctuations in cluster headache patients ipsilateral to the pain (Farago et al., submitted-a). The amplitude of the resting fluctuation is correlated with the cumulative disease burden (Figure 7).

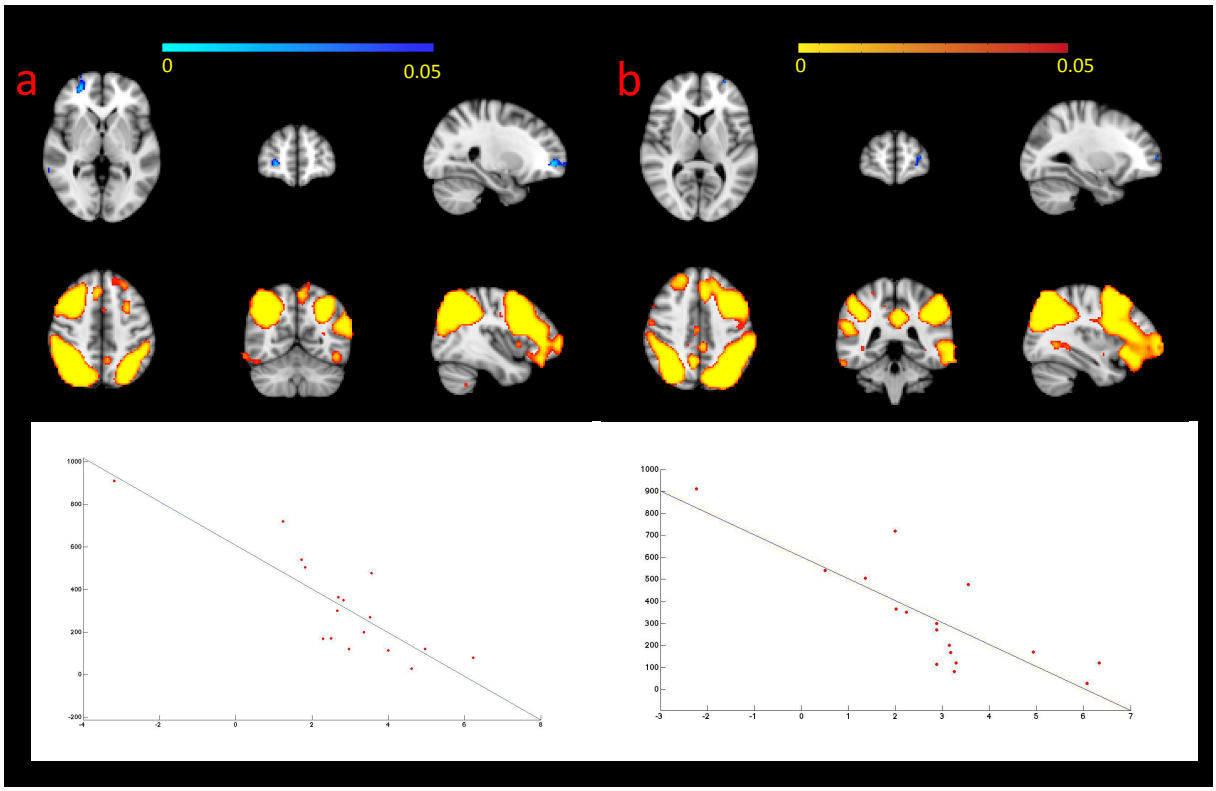

Figure 7. Correlation was found between cumulative headache days and expression of the resting state activity fluctuations. In the left mirrored dataset, the right attention network showed correlation with the cumulative headache days in the right frontal pole (p<0.05). The right mirror dataset showed correlation in the left attention network (b) near the right frontal pole (p<0.05). The colorbar represents p-values.

Interestingly, a similar approach can also separate migraineurs with and without aura symptoms (Farago et al., in prep). Moreover, in the superior parietal lobule and in the anterior cingulate gyrus the amplitude of resting BOLD activity is higher in migraineurs with aura (Figure 8).

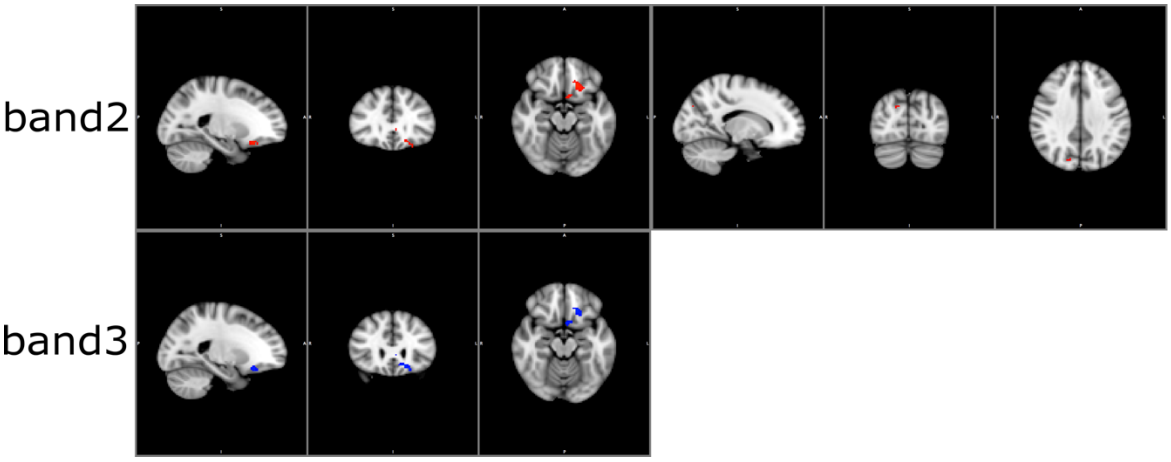

Figure 8. Woxel-wise comparison of the amplitudes of the resting state fluctuation were higher in the MWA group in the 0.04-0.08Hz (band2) and in the 0.02-0.04Hz frequency range.

Functional consequences of chronification of pain was investigated in a rat model in collaboration with the Preclinical Imaging Center of Gedeon Richter Plc. CFA injection into the wishker pad of the rat caused increased activation of the anterior cingulate cortex in a long term. This activation is thought to be a representation of allodynia. We also showed, in collaboration with the Department of Nuclear Medicine of the University of Debrecen (Spisak et al., submitted) that the effective connectivity of the sensory-motor cortex and the anterior cingulate cortex strengthens by the intervention (Figure 9).

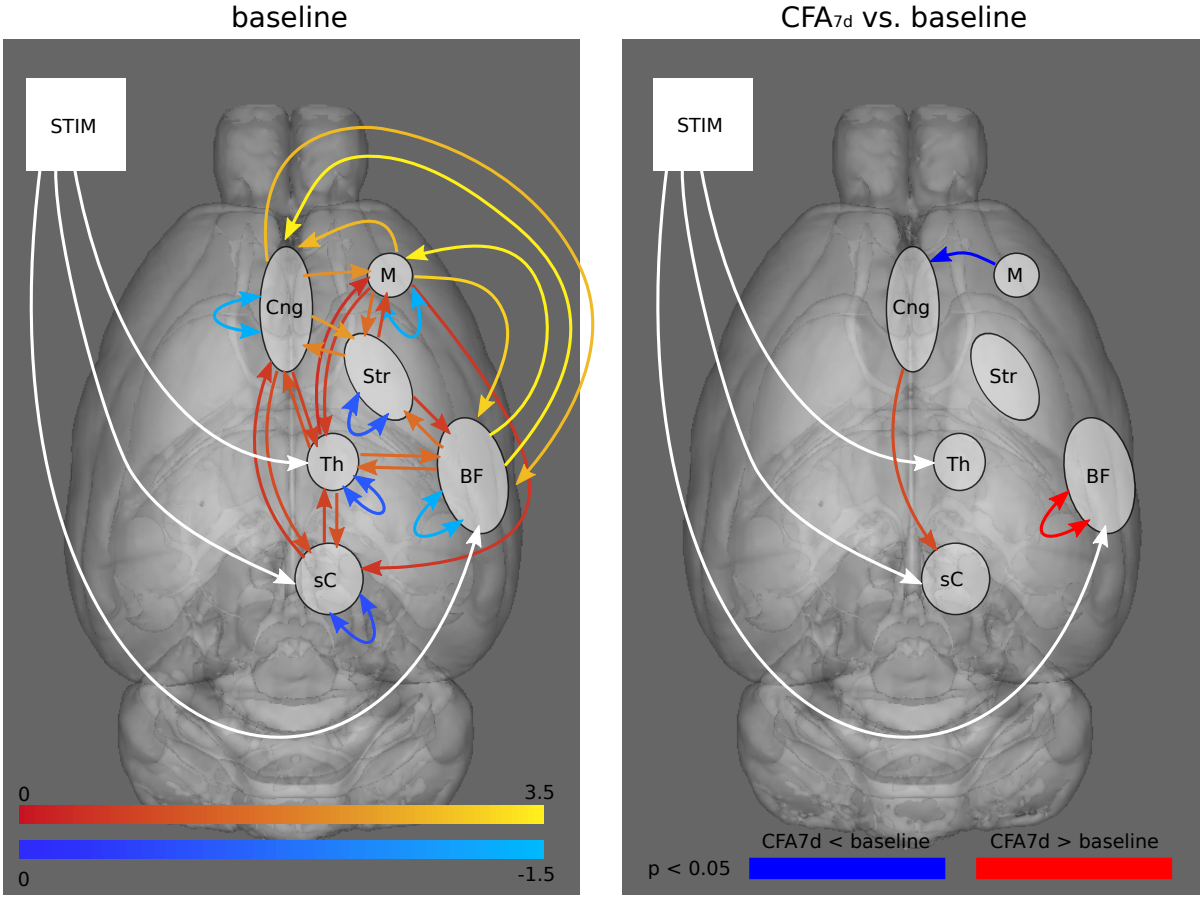

Figure 9. Connectivity strengths estimated by Casual Dynamic Modelling for the baseline measurements (left panel), and connections yielding an optimal and significant model fit for explaining treatment-related changes, obtained with gamma-LASSO variable selectin for logistic regression analysis.

In a further group of analyses we investigated the molecular and genetic markers of migraine.

We showed that pituitary adenyl cyclase- polypeptide-38 (PACAP-38) concentration is significantly lower in the plasma of migraine patients in the interictal period but “normalises” in during headache. The PACAP-38 concentration is negatively correlated with the disease duration (Tuka et al., 2013). In our latest investigation we showed in a TBSS style analysis that the interictal PACAP-38 concentration is correlated with the mean, radial and axial diffusivity in the intrathalamic white matter (Vereb et al., in prep) (Figure 10).

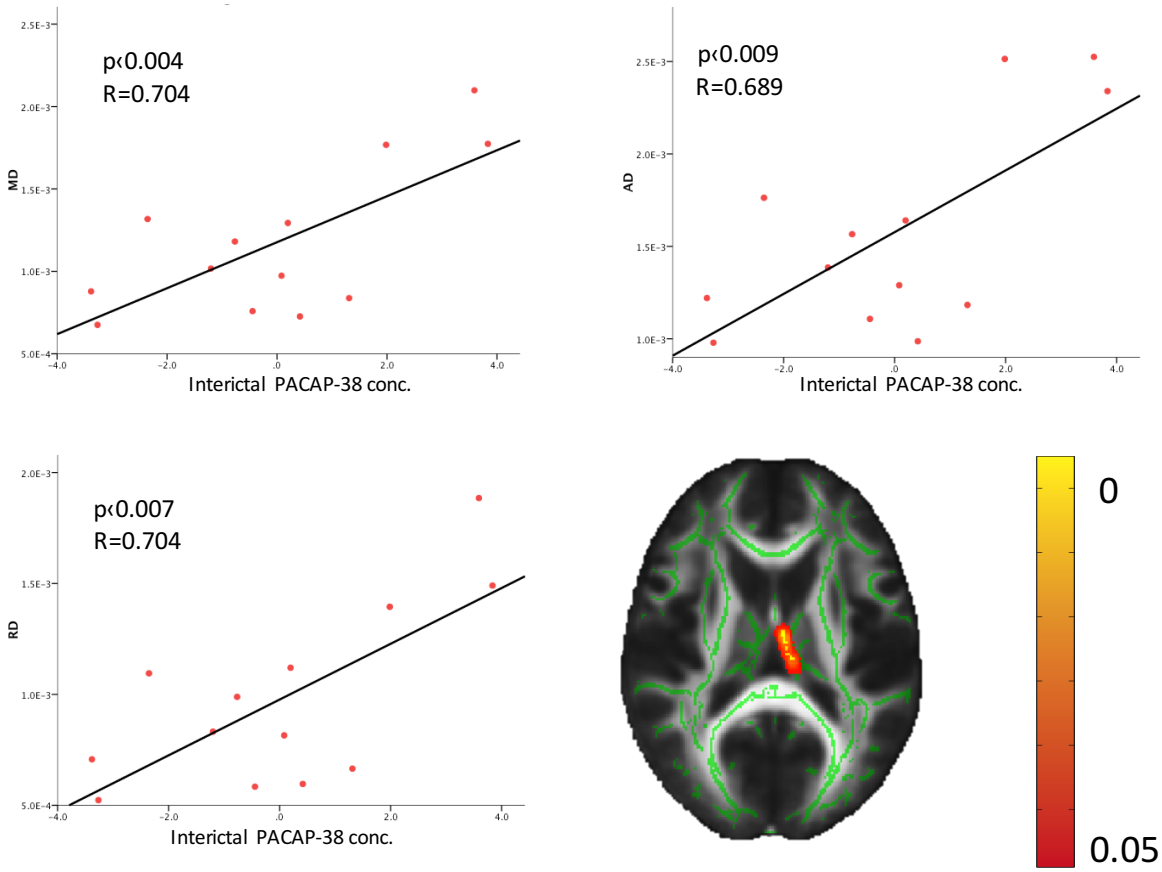

Figure 10. Correlation of interictal PACAP-38 concentration and white matter diffusion parameters. The white matter skeleton is indicated in green. The statistically significant correlation of PACAP-38 concentration and mean diffusivity (MD) is depicted in red-to-yellow. The colorbar represent p values. The scatterplots depict the relationship of PACAP-38 concentration and diffusion parameters (axial diffusivity: AD, mean diffusivity: MD, radial diffusivity: RD) in the intrathalamic white matter.

We have also been interested if the white matter disintegration, what we have found in our MRI study is detectable in the periphery by molecular markers. There were former reports that neuronspecific enolase, a neuronal marker and S100B a glial marker is altered in migraineurs. In our pilot sample of 12 healthy controls and 24 migraine patients we found no significant differences (p=0.067 for NSE and p=0.52 for S100B).

Another aspect of migraine chronification might be the genetic predisposing factors. The relationship of APoE and plasticity is well known. We hypothetised that certain ApoE allels

that makes the brain “more plastic” is more common in migraineurs and especially in those patients who has chronic migraine. In a sample of 102 migraineurs we investigated the AopE genotype (Table 1). Our results showed that the ε2ε2 genotype and ε2 allele frequency is higher than it was reported for healthy controls. Currently the analysis of the local age matched controls is on the way.

	Genotype (%)						Allél frequency (%)		
	ε2ε2	ε2ε3	ε3ε3	ε3ε4	ε2ε4	ε4ε4	ε2	ε3	ε4
Migraine (n=102)	7 (3,5)	8 (8,1)	69 (71)	18 (17,4)	0 (0)	0 (0)	22 (10,8)	164 (80,4)	18 (8,8)

Table 1. ApoE genotypes in migraineurs.

We formerly showed that childhood migraineurs have a deficient motion perception (Braunitzer et al., 2012). To follow up on that issue we investigated the structural background of motion processing performance. A significant positive correlation was found between the motion perception threshold and the local fractional anisotropy in the posterior part of the right superior frontal gyrus, the right juxta-cortical superior parietal lobule, the left parietal white matter, the left superior temporal gyrus and the left optic radiation. Probabilistic tractography identified pathways that are highly similar to the segregated attention networks, which have a crucial role in the paradigm (Csete et al., 2014).

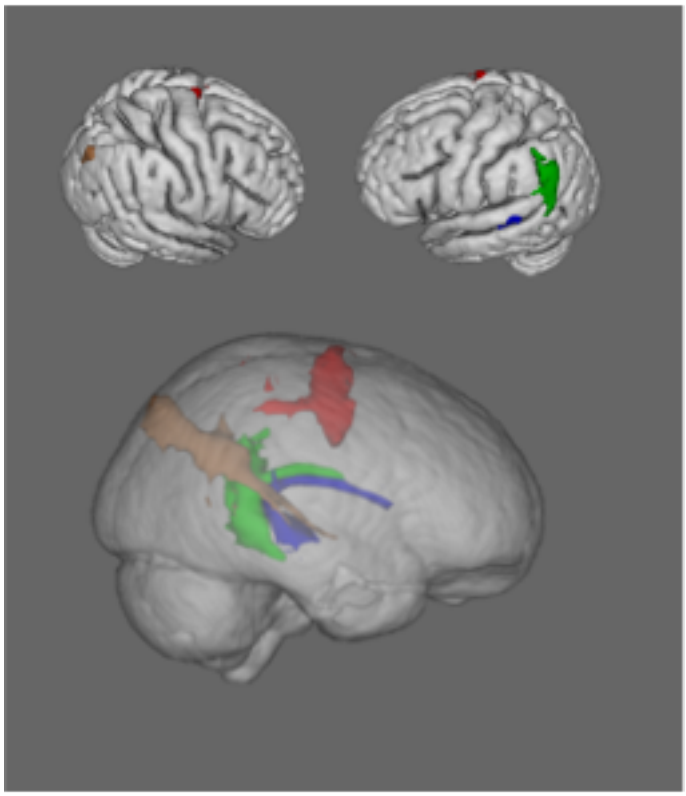

Figure 11. Pathways running through the white matter regions showing a significant correlation between microstructure and behavioural measures. The lower image indicates the pathways; the upper two images (right and left views) show the cortical projections. The inferior and medial branches of the superior longitudinal fascicle on the left are indicated in blue and green. The superior branch of the right superior longitudinal fascicle and part of the cortico-spinal tract originating from the putative frontal-eye field are indicated in red. The connection on the right, between the superior parietal lobule and the superior temporal gyrus, is indicated in brown.

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