Resting State FMRI Frequency Specific Alterations In Primary Headache Disorders

PhD Thesis

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Introduction

Cluster headache

The cluster headache (CH), a trigeminal autonomic headache, is one of the most painful primary headache disorder. The headache is characterized by severe, strictly unilateral, retro-orbital pain, which is associated with autonomic neurological symptoms at the side of the headache. The pain last for 15-180 minutes, appears several times in a day and the headache periods (clusters) can last several weeks or months followed by a long headache-free period. The headache is strictly unilateral, never appears on the other side or both side. The localisation of the symptoms suggests a trigemino-vascular origin.
The headache in the acute phase could be treated by non-steroid painkillers. Pure oxygen inhalation, local or intranasal anaesthetics (e.g.: lidocaine) or triptans are effective in the acute phase of CH. Calcium channel blockers (e.g.: verapamil), ergot-alkaloids, corticosteroids, tricyclic antidepressants, antiepileptic drugs (e.g.: valproate) and also triptans are used for interval therapy.

The hypotheses about the pathomechanism is based on the following features of the disease: (i) circadian rhythmicity, (ii) trigeminal appearance of the pain and (iii) autonomic neurological symptoms. The periodic appearance, the seasonal activity suggests the role of the hypothalamus in the pathomechanism of the CH. Altered fluctuation of melatonin synthesis, the abnormal circadian rhythmicity and hormonal dysfunction also observed.

Neuropeptide serum concentrations were also showed to be altered in CH, similarly to migraine. Calcitonin gene releasing polypeptide (CGRP) and vasoactive intestinal polypeptide (VIP) levels are increased during headache. Decreased pituitary adenylate cyclase activating polypeptide-38 (PACAP-38) level was reported between the headache periods.

Positron emission tomography studies showed higher activation in the ipsilateral hypothalamus during cluster headache attacks (May et al, 1988). FMRI functional connectivity studies also support the higher activation (May et al, 2000, Morelli et al, 2009). Resting state fMRI studies found altered functional connectivity between the hypothalamus and several other brain structures such as the anterior thalamus, ipsilateral basal ganglia, insula, cingulate cortex or

medial frontal gyrus (Rocca et el, 2010, Qui et al, 2013) e.g. the elements of the pain matrix (according to Tracey: "large distributed brain network [activity] during nociceptive processing") (Sprenger et al, 2007, Tracey et al, 2008). Other than these studies, there are still no evidence for altered activity of the resting state networks (RSN) in CH. However this could be expected from those results showing cortical hyperexcitability in CH (Constentino et al, 2015).

VBM studies found increased grey matter density in hypothalamus in CH compared to healthy individuals (Arkink et al, 2017). However there were other investigations, which were unable to replicate these results. These studies also showed loss of the grey matter volume in various structures of the above mentioned pain matrix during pain free periods in CH (Absinta et al, 2012, Yang et al, 2013). Diffusion tensor imaging (DTI) investigation revealed an extensive white matter microstructural alterations in interictal periods. In our earlier study we proposed that these alterations in the grey as well as in the white matter might be the result of parallel maladaptive plasticity and degenerative changes (Szabo et al, 2013).

Migraine

Migraine is the most common primary headache disorder and also the most common disabling headache disease. The incidence of the disease is about 10% of the whole adult population, and it is two or three times more common among women. The headache mostly starts in young adulthood. The migraine attacks could be provoked by environmental factors, such as specific foods or drinks, smoking or strong smell, stress situation and anxiety. Menstruation is a very common provoking factor among women. While the disease is not life threatening it significantly influences the quality of life and has an enormous economic burden.

The migraine attack can be separated into four phases: prodromal phase, aura phase (not all cases), headache phase, postdrome phase.

Previous studies hypothesised, that the abnormal function of the trigemino-vascular pathway has the key role in the migraine pathomechanism (Pietrobon and Moskowitz, 2013). The dural perivascular afferent fibres are part of the trigeminal nerve. The origin of the sensitisation of the dural afferent is not clearly understood. Previous animal experiments revealed that the local

afferent nerves could be activated by inflammatory mediators, called “inflammatory soup”. During this activation local neuropeptide concentration increases, not only in the local dural area, but also in the jugular vein.

Another key element in migraine pathomechanism is the cortical spreading depression (CSD). The CSD is a slow cortical depolarization wave on the surface of the brain lasting 1-2 minutes (Leao, 1947). Trigeminal sensitisation can be induced by CSD (Hadjikhani et al, 2001), and local inflammation caused by the mediators (Levy et al, 2012). The connection between CSD and migraine (dominantly MWA) seems to be proved, however the origin of the CSD is still not clear in patients with migraine. One possible explanation is the increased level of sensitivity of migarines’ cortex (Afra et al, 2000b).

The presence of CGRP is notable in the trigeminal afferents, trigeminal ganglion and in the locus coeruleus (Tajti et al, 2001, Uddman et al, 2002). Migraine connected structures, such as periaqueductal gray matter (PAG) or raphe magnus, show increased VIP concentration (Tajti et el, 2001). In the last decades another peptide, the PACAP-38, showed important role in migraine pathomechanism (Tuka et el, 2013).

The patomechanism of MWA and migraine without aura (MWoA) subgroup may have a different background (Manzoni and Tortelli, 2008). It was suggested, that the CSD is responsible for the aura symptoms (Hadjikhani, 2001). Increased cortical hyperexcitability, as measured by visual evoked potentials and transcranial magnetic stimulation (TMS) studies (Connolly et al, 1982), was also mentioned as a potential trigger factor of migraine (Coppola et al, 2015), predominantly for MWA. PET and fMRI studies also strengthen this theory (Vincent et al, 2003).

Only few studies investigated separately the structural parameters in the two sub-groups namely MWA and MWoA. Up to now, only one study revealed white matter microstructural integrity differences between the two sub-groups, ~~, or the s~~studies did not ~~separated~~separate the subgroups for investigation (Szabo et al, 2012, Granziera et al, 2014, Rocca et al, 2014). In our recent work, we found extensive disintegration of the white matter in migraine patients with aura, but not in patients without aura (Szabo et al, 2018). Resting state fMRI studies revealed various connectivity changes in migraine. Some of the changes referred increased (Tedeschi et

al, 2016), in some comparison revealed decreased functional connectivity (Xue et al, 2012, Tessitore et al, 2013). Several previous studies investigated activity changes in migraine and found altered activity in regions connected to pain processing (Maniero et al, 2011, Yuan et al, 2013). Only a few of previous studies separated the two sub-groups and analysed the RSN separately.

Non-steroid painkillers are the first choice to eliminating headache. Triptans and other medications such as ergo alkaloids or octreotide proved to be useful in acute migraine therapy. Beta-receptor blockers, antidepressants, Ca^{++} channel blockers' or antiepileptic drugs are used for prophylactic therapy. In chronic form, the local injection of botulinum toxin proved to be an effective therapy.

Basics of MRI

The basics of MRI imaging are based on the impaired number of protons in molecules. The clinical or research MRI scanners use hydrogen ion (proton) as mediator. The angular velocity of the precession is specific for the substance and only dependent on the magnetic field the substance experience. If we put this material (test structure, human body, etc.) into a high magnetic field, the spins of all molecules are regularized along the main magnetic field. This regularisation does not happen immediately, and the natural characteristics of the measurable material can also influence it. This phenomenon is called T1 relaxation.

Another MRI phenomenon is the T2 relaxation, what describes the transversal magnetisation. If we apply a 90-degree electromagnetic field, the spins move along with the transversal axis. In homogenous magnetic field the spins start a coherent precession, and a receiver coil in the transversal plane can measure a sinus wave. However, in the measured materials the local magnetic field is inhomogeneous, which is due to local molecular-molecular interactions, the local spin precession is gradually becoming incoherent, therefore the amplitude of the sinus signal is exponentially decreasing.

T2* is a special T2 imaging technique. In this case not only the molecular-molecular interactions influence the T2 relaxation, but there are more specific differences of the magnetic field. These differences can occur in areas, where the susceptibility is high. These areas are for

example the air-body border or around veins, or because of the paramagnetic nature of the deoxy-hemoglobin.

Functional MRI

The fMRI based on a T2* EPI technique, where a special signal, as called blood oxygen level dependent (BOLD) signal is measured. The blood, more precisely, the deoxyhaemoglobin has a light paramagnetic nature. This changing paramagnetism due to the alteration of local deoxyhemoglobin concentration can be measured during fMRI scans. During a specified task (e.g.: finger tapping or visual checkerboard stimuli) the local neuronal activity increasing, the neurons' need for oxygen and nutrients are increasing. The local O_2 extraction is increasing, the deoxyhemoglobin concentration rising. After 3-6 s, the local cerebral blood flow becomes elevated, the deoxy hemoglobin is washed out, the oxy-deoxy hemoglobin ratio increasing. The EPI sequence measures this decreasing deoxy- (increased oxy-) hemoglobin phase.

The paradigm could be block design or event related fMRI. The main purpose of the task based fMRI to separate the areas, where we can detect task dependent changes of BOLD signal. The neuronal activity in task free state showed a natural fluctuation (Biswal et al, 1995).

Resting state brain activity, resting state fMRI

The human brain does not rest in task free state, continuous neuronal activity can be measured. This neuronal activity showed fluctuation, the activity is not constant. A study proved relatively high metabolism in healthy in the posterior and anterior cingulate cortex, in task free state.

The resting state fMRI, not measure directly the local cortical metabolism, but the local hemodynamic changes, respond to the local neuronal activity. This resting state BOLD response show coherent fluctuation between regions of the brain, which have no strict anatomical connections. These coherent fluctuation give us an opportunity to investigate coherent activity, called functional connectivity, between remote brain areas (Greicius et al, 2004, Beckmann et al, 2005).

There are two commonly used approaches for resting state fMRI investigations: seed based approach and the independent component analysis. As the traditional task-based fMRI, in the

seed based method, correlation between the activity of a predefined area and the rest of the brain is calculated. Other option if the model free analysis, resulting resting state networks (RSNs) (Beckmann et al, 2005, Mantini et al, 2007). There are other 8-12 known resting state networks based on the method or the selection.

Resting state activity in primary headache disorders

Several studies investigated the activity of the resting state functional networks in migraine and found various alterations of networks that are implicated in pain processing (Maniero et al, 2011, Xue et al, 2012, Tessitore et al, 2013, Tedeschi et al, 2015). Up to now there are only a few studies investigated patients with MWA and MWoA separately.

In CH former studies agreed on the altered functional connectivity of the hypothalamus, and also on the alteration of the connectivity of other regions, part of the pain matrix, such as the medial frontal cortex and the cerebellum, sensorimotor network (Rocca et al, 2010, Yang et al, 2014).

One of the key feature of migraine as well as of CH is the cortical hyperexcitability. In migraine, cortical hyperexcitability, as a potential trigger factor of migraine, is often mentioned. Importantly, cortical hyperexcitability more robustly represented in MWA as demonstrated in visual evoked potential (VEP), TMS, PET and fMRI studies (Sand et al, 2008, Brigo et al, 2012, Coppola et al, 2015).

While there is no direct evidence up to date that resting state fMRI has a connection to cortical excitability, but it seems plausible that because of the tight interconnection between the BOLD signal and the underlying neural activity, one or another feature of resting fMRI is related to the excitability alterations measured by electrophysiological approaches.

Most of the resting state fMRI studies investigated the connectivity between various regions and therefore build on the coherent activity in spatially distributed networks. The variation in the frequency and the amplitude of the resting state BOLD signal is usually neglected. The BOLD resting state fluctuation is a low frequency fluctuation. To filter out the non-neural noise from the raw BOLD time courses most of the studies apply filters (Xue et al, 2012, Schwedt et al, 2013, Jin et al, 2013). However, neural signal could be detected also in the higher frequencies

(Boyacioglu et el, 2013). Furthermore, a few recent studies started to analyse the amplitude of the low frequency fluctuation of the resting state signals offering a unique insight into the resting brain activity in various diseases but not in migraine. The frequency and the amplitude changes of the activity of key pain processing regions is even more important in disorders such as migraine and CH, in which cortical hyperexcitability was reported.

Objectives

The aim of our investigations to seek functional changes in resting state fMRI networks with specific attention to the amplitude of the resting state activity in various frequency bands in primary headache disorders. Furthermore, the changes connection to clinical data, such as disease duration or cumulative headache days, were also tested.

Methods

Participants

Seventeen episodic CH patients and fifty-one episodic migraine patient were recruited from the Headache Outpatients Clinic of the Department of Neurology, University of Szeged and Semmelweis University. All patients were diagnosed with episodic headache and were scanned during interictal phase. From migraine patients the type of headache (MWA, MWoA) was also acquired.

As control group twenty-six age and gender matched right handed participants were recruited for comparison to CH patients. For the MWA and MWoA groups, thirty-two healthy volunteers were recruited as controls. The study was approved by the ethic committee of the University of Szeged (authority number: 56/2011).

Image acquisition

The MRI imaging was performed on a 1.5 T GE Signa Excite HDxt scanner. For every participants' high resolution T1 weighed images and resting state fMRI protocol with echo planar imaging technique were acquired.

Image analysis

Data preprocessing

Non brain tissues were removed from all T1 high resolution images. The brain extracted structural images were registered to standard space brain image for each participant. The result of the brain extraction and registration was checked manually and the brain extraction mistakes and misregistartion were corrected manually.

The preprocessing of the fMRI data were carried out by FEAT. The first two non steady state images were wiped out for all images. The non-brain tissues were removed. The images were motion corrected spatially smoothed with Gaussian kernel of 6 m FWHM. A high pass filter with 100 s cutoff was also applied to all functional images. Because in the further analysis we were to investigate the higher frequencies, low pass filter was not applied. All preprocessed images were registered to their own T1 structural images with linear registration. The functional images were registered to a standard image (MNI152 T1 image, 2mm isovoxel size) using the matrices and warp-fields previously generated. In the last step all standard space registered images were resampled to 4mm isovoxel to ease the computational burden.

Additionally, in CH patients, because the headache is strictly unilateral, according to the previous studies, we normalized the data to the headache side creating three subgroups. (1) The patients, who had headache in the left side, were inverted along the midsaggital axis. With this method, we created a group that have only headache in the left side. The side inversion was applied on the high resolution T1 images and also on the resting state images. (2) We repeated this method on the other sided (right headache sided) images, created a left headache sided group. (3) As a third group non-inverted dataset was also analysed.

Independent component analysis

Large scale neuronal RSN were identified by independent component analysis. The group specific networks were identified using multi session temporal concatenation approach. The spatial maps of the networks were thresholded to $p<0.5$ variance under the Gaussian-Gamma mixture model. Based ~~on these~~previous articles (Qui et al, 2013, Yang et al, 2013) we ~~the~~ selected eight networks for further analysis: default mode network, right attention network, left attention network, medial visual network, lateral visual network, cerebellar network, somatosensory network and salience network.

Frequency specific amplitude of the resting state activity

The frequency specific modulation of the amplitude of resting state activity and the frequency specific connectivity were analysed by discrete wavelet decomposition. By this method the network's time courses were divided into five consecutive frequency bands (band1: 0.16-0.08 Hz; band2: 0.08-0.04 Hz, band3: 0.04-0.02 Hz; band4: 0.02-0.01 Hz; band5: 0.01-0 Hz).

To measure the mean amplitude of the resting state activity in the various frequency bands, an envelope was fitted to the absolute maximum values of each frequency band. The envelopes are averaged over time for each network to describe the mean activity of a given RSN in a specific frequency band.

Furthermore, not just the networks mean fluctuation went under frequency decomposition and envelope fitting, but also the voxel-wise preprocessed data for each participant. The voxel-wise decomposition and envelop fitting were performed by a house-made Matlab script.

After each processing step visual check was performed.

Differences between groups of the resting state data

The RSNs' connectivity were compared by a modified dual regression approach. The previously defined networks' spatial maps were regressed into all individuals' functional images to obtain individual specific time courses for each network. In the second step the individual network time courses were regressed to the functional image, to get individual specific network distribution. In addition, using the wavelet decomposition mentioned above,

in the second step we used the frequency specific time courses for the second regression to get frequency specific network distribution for each individual.

The frequency specific networks comparison was accomplished across groups using permutation based cluster analysis (5000 permutation). The modelling was accomplished using standard General Linear Model (GLM). For statistical analysis threshold-free cluster enhancement were used (TFCE) and corrected for multiple comparisons, family wise error (FWE), across space within each permutation network.

Similarly, GLM based permutation test was used to investigate the differences of the amplitude of the mean network time courses across groups in the individual frequency bands.

The cumulative headache days and the disease duration, and the degree of networks' coactivation were also calculated. The correlations accomplished in the General Linear Model framework, were calculated with permutation based test for each voxel and corrected for multiple comparisons.

The average time courses of each network were calculated by wavelet decomposition and envelop fitting. The time course data were compared with two sample T-test between groups.

Results

Resting state fMRI alterations in cluster headache

The MELODIC analysis found different number of components in all three investigated CH groups (plus healthy controls). All of the three investigated groups' analysis revealed all eight investigated networks, mentioned above.

Frequency specific expression of the resting state networks

Left sided dataset

The modified dual regression approach revealed alterations of the left attention network in two frequency bands: 0.08-0.04, 0.04-0.02 Hz. The increased coactivation was measured ipsilateral

to the headache side. Increased coactivation was found in CH group compared to controls. The alterations localised within the left superior frontal gyrus and in the left medial frontal cortex.

Significant increased coactivation was found in CH within the cerebellar network in the 0.02-0.01 Hz frequency band, localized in both cerebellar tonsils. There were no other significant results in any RSNs, in any frequency band.

Right sided dataset

Increased coactivation within the right attention network was found in CH compared to controls in the 0.08-0.04 Hz frequency band. The results were found also ipsilateral to the headache side. The alteration localised in the right superior fontal gyrus ($p<0.003$) and in the right medial frontal gyrus. In the cerebellar network, increased coactivation was found in CH group in the 0.02-0.01 Hz frequency band, localised in the cerebellar tonsils. There were no other differences between any other RSNs in any frequency bands.

Non-flipped, original dataset

Increased coactivation was found in the left and the right attention network in the 0.08-0.04 Hz frequency band. The left attention network showed differences in the left superior frontal gyrus. The right attention network showed increased coactivation in the right superior frontal gyrus. The cerebellar network revealed increased connectivity in the inferior cerebellar vermis and in the left cerebellar tonsil. In all case CH group showed greater coactivation.

There were no other differences between any RSNs in any frequency band in any dataset.

Connection between resting state network and clinical data

Correlation analysis between cumulative headache days and resting state functional connectivity revealed correlations in the left and in the right sided dataset.

The left sided dataset revealed negative correlations in the contralateral (right) attention network in the 0.08-0.04 Hz band, localized in the right frontal pole.

The right sided dataset revealed negative correlation between the coactivation and the contralateral (left) attention network. The area of correlation localized in the 0.16-0.08 Hz frequency band, in the contralateral frontal pole.

There were no other correlations between any clinical data and resting state networks' functional connectivity in any frequency band.

Resting state fMRI alterations in Migraine

The MELODIC analysis found 33 independent components in the healthy controls. Components representing artefacts were rejected and finally five networks went under further analysis: default mode network, right attention network, left attention network, medial visual network and lateral visual network.

Alterations in the amplitude of the resting state networks

MWA vs. healthy controls

Our analysis showed that in the highest frequencies (0.16-0.08 Hz) the amplitude of the resting activity was higher in the left attention network ($p<0.051$) and tendentially in the right attention network ($p<0.058$) in MWA as compared to healthy controls. There were no other significant results in any other frequency bands or in case of the non-filtered data.

MWoA vs. healthy controls

The amplitude of fluctuation in the 0.08-0.04 Hz band was decreased in the MWoA group in the default mode network. There were no other differences between MWoA and healthy group in any other frequency band or in the non-filtered data.

MWA vs. MWoA

The amplitude of the fluctuation was higher in all investigated network in the 0.08-0.04 Hz frequency band ($p<0.05$) in the MWA group. Furthermore, the lateral visual network showed higher amplitude in the 0.16-0.08 Hz frequency band in the MWA group. There were no other differences in any other frequency band, neither in the non-decomposed data.

Voxel-wise comparison of the amplitude of resting activity

The voxel wise comparisons of the amplitude of the resting state activity revealed higher amplitudes ($p<0.05$) in several frequency bands. The left parietal lobule showed higher amplitude in MWA in all frequency bands. Furthermore, in 0.08-0.04 Hz band higher amplitude

was found in the both cerebellar hemispheres, left occipital junction and in the left occipital pole. In 0.04-0.02 Hz band, higher amplitudes were found in the cerebellum and in the anterior cingulate gyrus. In the 0.02-0.01 Hz band, MWA showed higher amplitude in the cerebellum.

Discussion

In this thesis we presented resting state functional MRI alterations in CH and migraine with novel analysis approaches. The summarized results: (I) Our first study revealed increased frequency specific resting state activity in CH patients in the attention network ipsilateral to the headache side and in the cerebellar network bilaterally. (II) Our second analysis showed that in migraine the amplitude of the activity of the RSNs in the 0.08-0.04 Hz frequency range was higher in MWA than in MWoA. On the contrary, in MWoA the amplitude of the activity fluctuation of the default-mode network was lower than in healthy controls. The amplitude of resting state BOLD fluctuation was higher in MWA in the faster frequencies in the medial frontal, anterior cingulate cortex and in the superior parietal lobule compared to MWoA even if RSNs were not considered. This increased activity must be understood as increased frequency specific coherent activity within networks and also increased amplitude of the BOLD fluctuation.

Several studies implicated cortical hyperexcitability in migraine patients (Gawel et al, 1983, Antal et al, 2005, Pierelli et al, 2013). Evoked potential, PET and fMRI studies strengthened that, higher amplitude of visual evoked potentials was shown in migraine patients (Connolly et al, 1982, Oelkers et al, 1999). TMS study also found lower phosphene threshold in migraine (Vincent et al, 2003).

The above mentioned studies showed us important results about the pathomechanism of migraine, however they failed to separate the MWoA and MWA in their analysis. Interestingly VEP studies revealed higher amplitude in MWA (Sand et al, 2008). A TMS meta-analysis pointed out that the cortical hyperexcitability mostly true for patients' with aura, but not for MWoA. The increased BOLD for photic stimuli were higher in MWA compared to MWoA (Datta et al, 2013, Cucchiara et al, 2015).

In CH the primary role of the cortical dysfunction was strengthened by former electrophysiological studies (Casale et al, 2008, van Vliet et al, 2003). A former TMS stimulation study found hyperexcitability in CH patients similar to that in migraine (Constentino et al, 2015). Interestingly, they found increased excitability ipsilateral to the headache side, which is in agreement with our findings.

One possibility to explain our results is that higher cortical excitability (easier to evoke response from the cortex) could be paralleled with higher spiking activity of the cortex as shown by direct current stimulation studies (Bindmen et al, 1964, Antal et al, 2003), which because of the higher energy demand would result in higher amplitude of BOLD fluctuation. Importantly, while it is tempting to propose that the cortical hyperexcitability and the higher amplitude of resting state activation is related, alternative explanations should not be dismissed.

It has to be noted that cortical hyperexcitability is not unanimously reported in migraine even with aura. In a TMS study the prevalence of stimulation-induced phosphene production was lower in MWA compare to healthy individuals, suggesting cortical hypoexcitability in migraine (Afra et al, 1998). One explanation of such controversy is that the cortical response property in migraine is a more complex issue, which is exemplified by the lack of habituation in migraine (Afra et al, 2000).

Importantly, our results call attention to investigate patients with and without aura symptoms in separate groups. Only the patients experiencing aura symptoms were the ones who had higher amplitude BOLD fluctuation in our study.

A few studies investigated the amplitude of the resting state BOLD fluctuation in chronic pain conditions and found higher amplitude of resting activity in chronic back pain, irritable bowel syndrome, knee osteoarthritis and complex regional pain syndrome (Ma et al, 2015, Baliki et al, 2014). Only single study investigated the amplitude of the low frequency fluctuations in 24 ~~migraineurs~~migraineous, without grouping the patients based on the aura symptoms (Wang et al, 2016).

Recently, the focus of attention has shifted to investigate the activity of resting state fluctuations in various frequency bands (Gao et al, 2015), especially in pain conditions. The importance of the various frequencies of BOLD fluctuations is not yet known. It was proposed that functional

connectivity of various brain regions are represented in different dominant frequency bands (Salvador et al, 2008). This could be responsible for the alterations found in different frequency bands in the attention network and the cerebellar network. Another option might be that CH and migraine was proposed to be a neurovascular disease, and the altered neurovascular coupling may affect the frequency of the resting BOLD fluctuations (Malinen et al, 2010) by acting as a filter. Furthermore, the group difference in the RSN activity might well be the result of improved signal to noise ratio by filtering out the low and high frequency artefact. Since most of the slow frequency fluctuation in our analysis with a relatively long TR were shown to be neural origin, this hypothesis seems rather unlikely.

Another important aspect of our result is the spatial distribution of differences. As mentioned we found increased amplitude or resting BOLD fluctuation in the cerebellum, cingulate cortex, parietal lobule and frontal cortex. These results are in agreement with further resting state fMRI studies of CH and migraine (Rocca et al, 2010, Chou et al, 2017). These regions are possibly part of the pain matrix elements (Lee and Tracey, 2013). The anterior cingular cortex could be a key area of the central sensation of the pain (Spisak et al, 2017).

Interestingly, in case of CH our result showed no functional connectivity or amplitude changes in hypothalamus. However, areas we found to have altered connectivity are in structural or functional connection with the hypothalamus. Lemaire's research group found structural white matter connection between the prefrontal cortex and the antero-ventral hypothalamus using diffusion tractography (Lemaire et al, 2011). In the same investigation white matter connections were found between cerebellum and the hypothalamus. Similar hypothalamo-cortical (Risold et al, 1997) and hypotahamo-cerebellar (Dietrich et Haines, 2002) connections were shown in previous rodent experiments. The structural connection of hypothalamus and frontal cortex strengthened by postoperative investigations of chronic cluster headache patients. Probabilistic tractography found connection between the effective places of stimulation and the medial lemniscus, frontal cortex, cerebellum and the brainstem trigeminal nuclei. DBS stimulation not just influenced the function of the stimulated structures, but also caused structural changes, in effective DBS stimulation hypothalamus showed increased volume (Akram et al, 2017). Other volumetric studies found atrophy in CH in the frontal areas (Absinta et al, 2012, Naegel et al, 2014). They hypothesized, these regions are the part of the pain processing system, and

therefore the atrophy is the part of pain related pathomechanism. It is important to note that our findings seem to be in accordance with the volumetric studies; even though our analysis did not reveal hypothalamic activity changes in CH patients. While several studies indicated the important role of the hypothalamus in CH (May et al, 1998, Morelli et al, 2009, Qui et al, 2013, Qui et al, 2015), it is still debated if it actively contributes to the attacks or the altered hypothalamic activation is secondary to the cortical and subcortical malfunction of the pain matrix.

Conclusion

Our studies revealed changes in the connectivity of the RSN in specific frequency bands. The amplitude of fluctuation also showed alterations. The amplitude changes in MWA and in CH also suggest cortical hyperexcitability. The area of alteration localized in the elements of the pain matrix, as indicates differences in the pain processing in both headache disorder. In CH according to our finding, the changes attach strictly to the headache side.

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