Investigation of neurovascular changes during migraine attacks using BOLD-fMRI

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Abstract

Motivation: Migraine is a disabling neurological condition, associated with an increased risk of cardiovascular disease. Cerebrovascular reactivity (CVR) measurements have the potential to detect cerebrovascular pathophysiology and have been shown to be altered in migraine.
Objective: To elucidate neurovascular mechanisms in migraine by investigating CVR changes during spontaneous attacks (ictal phase) compared to pain-free periods (interictal phase).
Methods: Patients with episodic migraine without aura were studied during ictal and interictal phases. BOLD-fMRI data were acquired during a breath-holding (BH) task, to map CVR, as well as during two brain activation tasks (visual and motor), to map cerebrovascular changes in response to brain activation. Whole-brain activation maps were obtained through a general linear model analysis, and the amplitude (CVR, percent signal change) and time-to-peak (TTP) of the BH BOLD response were computed in each voxel. Next, group-level analysis was performed to identify differences in CVR and TTP, as well as in brain activation in response to sensory stimuli between ictal and interictal phases. Additionally, correlation analysis was performed between the individual mean CVR and TTP values across regions-of-interest and migraine clinical features.
Results: Increased CVR and TTP were found in occipital regions during the attack compared with the pain-free period. No significant correlation was found between CVR and TTP values and migraine clinical features.
Conclusions: These results are consistent with previously reported reduced reactivity of the posterior cerebral circulation in the interictal phase of migraineurs relative to controls, and also with the presence of ischemic-like lesions in the posterior circulation of migraineurs. It also confirms the vulnerability of the occipital lobe and posterior cerebral circulation regarding cerebral infarcts in migraine. These findings contribute with new evidence to the limited literature regarding migraineurs ictally, and this is the second only study evaluating CVR across the whole-brain on a voxel-by-voxel basis using fMRI.
Keywords: episodic migraine, functional magnetic resonance imaging, cerebrovascular reactivity, breath-holding, occipital lobe

1. Introduction

Migraine is a severe and disabling neurological disorder, causing significant individual and societal burden due to pain, resulting disability with lost productivity, and an overall decreased quality of life [1]. In 2016, out of 328 diseases and injuries considered by the World Health Organization (WHO), migraine was the sixth most prevalent, and it was ranked second globally in terms of years lived with disability [2]. This disease is a relevant public health problem in both genders and all age groups, but predominantly affects young and middle-aged females: there is a 3:1 female-to-male ratio, and it is most prevalent between the ages of 15 and 49, a time when most people are highly active in the professional area [2], thus representing a significant financial burden on economies worldwide [3].

Migraine is a clinical syndrome of cyclical nature, where intermittent headache attacks - ictal phase - alternate with attack-free periods - interictal phase. Typical characteristics of the headache are its unilateral location, pulsating quality, moderate to severe intensity, and being variable in duration (between 4 and 72 hours) [3]. Additionally, the headache attacks are usually accompanied by a variety of symptoms such as nausea, depression, irritability, attention deficit, photophobia and/or phonophobia [4, 5]. Frequently, the headache is preceded by a prodromal phase, where symptoms including fatigue, reduced concentration, neck stiff-

ness and photo-/phonophobia appear up to 48 hours before the headache [4]. Most headaches are also followed by up to 48 hours of feeling tired, difficulty with concentration and neck stiffness, called the postdrome phase [4]. In addition, some migraine patients suffer from migraine aura just before and/or during the headache phase. Migraine aura includes a variety of visual (most common), sensory, speech, and/or other neurological symptoms that usually develop gradually [4]. Positive (gain of function) and negative (loss of function) symptoms can take place, such as scintillating lights when affecting the visual cortex or paresthesia and numbness of the face and hands when affecting the somatosensory cortex [6].

It has been observed that migraineurs are at increased risk for cardiovascular disease, including conditions such as stroke, myocardial infarction, and cardiovascular mortality [7, 8]. In particular, it was observed that migraine accounted for 13% of all first-ever ischemic stroke of unusual cause [9] and this disease has been independently associated with a 2-fold increased risk of ischemic stroke [10]. Due to the high prevalence and serious consequences on morbidity and mortality of both migraine and cardiovascular disease [11], a potential association between these two conditions would have a substantial impact on public health. Aiming to investigate this possible connection, special interest has been raised in the role of vasculature in migraineurs. In particular, studying changes of cerebrovascular regulation in migraine holds great potential as a way of identifying endothelial dysfunction and potential consequent vascular events in migraineurs' brain.

Cerebrovascular reactivity (CVR) is an intrinsic regulatory brain mechanism whereby blood vessels adjust their calibre in response to a vasoactive stimulus, in order to increase or decrease regional cerebral blood flow (CBF). Therefore, this physiological parameter is an important index of the brain's vascular health. Measuring CVR variations within the brain has the potential to detect cerebrovascular pathophysiology and possible consequent vascular events in migraineurs' brain [12, 13]. Importantly, suggestions arose of reduced CVR being a key link between migraine and stroke [14]. CVR is usually measured by applying a challenge to the vasculature (most commonly, the induction of hypercapnia through vasodilating agents or manipulation of respiratory gases) and assessing the associated CBF changes (through Transcranial Doppler Ultrasound or functional Magnetic Resonance Imaging).

Concerning CVR investigation in migraine, very few studies exist, and these provide inconsistent results, with some showing increased and others decreased reactivity of the cerebral circulation of migraineurs' brain. The majority of studies have used TCD to assess blood flow velocities of the middle cerebral arteries (MCA), the posterior cerebral arteries (PCA) and the basilar artery (BA), thus not providing whole-brain information. fMRI studies hold greater interest by providing whole-brain scans with higher spatial resolution. Nevertheless, to our knowledge, there is only one study in migraine that assesses CVR through the use of fMRI [15]. Another important aspect in CVR studies of migraine is that the majority compares healthy controls versus migraine patients only in the interictal phase, thus not covering the cyclical nature of the disease. Instead, following a longitudinal approach, where brain activity of migraineurs is measured along the migraine cycle, holds great potential because it allows for the investigation of differences across several phases of migraine, as well as neurophysiological mechanisms and even attack-specific alterations that may exist [3].

Having said that, the goal of this project is the investigation of vascular reactivity changes during migraine attacks compared to attack-free periods through the analysis of BOLD-fMRI data. As a secondary objective, we also analysed neurovascular changes in response to brain activation associated with visual stimulation and with the performance of a motor task.

2. Materials and Methods

2.1. Participants and data

The present work used data acquired from a population of 14 female episodic migraine patients without aura (MwoA) in the context of a previous project, which was carried out in accordance with the recommendations of *Comissão de Ética para a Investigação Clínica*. All images were acquired with a 3 Tesla Siemens Verio MRI system using a 12-channel head radio-frequency coil. Each participant was evaluated in two different phases of the migraine cycle. The first session (S1) corresponds to the ictal phase of the migraine cycle. All subjects were experiencing head pain during the scan, therefore, the acquired data corresponds to the headache phase of the ictal phase. The second session (S2) refers to the interictal period, i.e, outside a migraine attack. BOLD-fMRI data in each session were acquired during a breath-holding (BH) challenge, a motor task and visual stimulation. While the BH challenge allowed for investigation of vascular changes in migraine, the rationale for the motor and visual data acquisitions was to investigate neuronal/brain activation differences between the two migraine periods. In particular, if there were differences in activation of the brain regions specific to motor and visual functions between the two phases of migraine. All functional scans were acquired using a T2*-weighted gradient echo-echo planar imaging (GE-

EPI) sequence. The motor protocol comprised 3 cycles of alternating periods of 60s of baseline condition (rest) and 60s of bimanual finger tapping, with a final 60s period of baseline condition, resulting in a total protocol duration of 420s (7 minutes). The visual stimulation protocol comprised 4 cycles of alternating periods of 30s of baseline condition (fixation cross) and 30s of flashing checkerboard visualization, with a final period of 60s of baseline condition, resulting in a total duration of 270s (4.5 minutes). Regarding the BH task, the protocol comprised two 25s periods of self-paced breathing (baseline) at the beginning and end of the protocol, and three 75s cycles of alternating periods of 20s apnea (BH) cued by auditory instructions and preceded by a preparatory inspiration, and 55s of self-paced breathing (total duration of 275s, approximately 4.5 minutes). BOLD data referent to the motor task and visual stimulation were acquired with the following parameters: TR/TE=3000/30ms, and voxel size of 4x4x3.75 mm^3. Only 12 of the 14 participants were able to perform both sessions of the motor task and visual stimulation, therefore, only these were considered for data analysis. BOLD images referent to the BH task were obtained with the following parameters: TR/TE=2500/50ms, and voxel resolution of 3.5x3.5x3.5 mm^3. Only 11 of the 14 participants were able to perform both sessions of BH, therefore only these were considered for further BH data analysis. T1-weighted structural anatomic scans were also collected using a magnetization-prepared rapid gradient echo (MPRAGE) series with TR/TE=2250/2.26ms, and voxel size of 1 mm^3. Lastly, participants' demographic data as well as several clinical parameters regarding the participants' migraine attacks and the S1 in specific were also acquired, including usual attack frequency (number of attacks per month) and duration (in hours), usual headache intensity, the time from the beginning of the migraine attack until data acquisition onset and headache intensity of the ongoing attack.

2.2. BOLD-fMRI data analysis

Data analysis was conducted using both the FMRIB Software Library (FSL, `https://fsl.fmrib.ox.ac.uk/`) and the MATLAB software (`https://www.mathworks.com/products/matlab.html`). The first step consisted in applying preprocessing methods. These included brain extraction, distortion and motion correction (using a rigid body transformation with 6 degrees-of-freedom - 3 translations and 3 rotations), nuisance regression of motion parameters and motion outliers, spatial smoothing (Gaussian kernel, 5 mm FWHM), and high-pass temporal filtering (0.01 Hz cutoff frequency). Functional images were co-registered to the subject's structural image using FLIRT (`https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT_BBR`) and to the Montreal Neurological Institute (MNI) standard space using FNIRT (`https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT`).

2.2.1. Subject-level data analysis

Next, a general linear model (GLM) was defined to model the BOLD response to the motor task, visual stimulation, and BH challenge in each voxel. Regarding the sensory stimuli (motor and visual), the explanatory variables were obtained in the standard way: convolving a square box function at the timing of the stimulus presentation with the canonical double gamma HRF, and adding its temporal derivative. As for the BH data, the regressors consisted of sine and cosine waves at task frequency (75s) and their first and second harmonics [16]. Each GLM was fit to the data in each voxel using FILM, and cluster thresholding (cluster $p<0.05$ and voxel $Z > 2.3$) was then performed on the maps of the parameter estimates (PEs) (for motor and visual data) and F-test (for BH data) across the GLM estimated coefficients in order to identify brain regions with stimulus-related activation.

The following step of the analysis was to obtain whole-brain maps describing CVR. CVR was characterised quantitatively in terms of amplitude and temporal responses. In terms of amplitude, BOLD percentage signal changes (PSC) were computed in each voxel as the amplitude of the model's maximum relative to the average signal during the baseline periods multiplied by 100 (% BOLD). Outlier voxels were identified as having PSC values 2 standard deviations above the mean value and were removed from further analysis. In terms of temporal responses, time-to-peak (TTP) values were also computed in each voxel as the time of the model curve maximum relative to the onset of the BH. Grey matter (GM) masks were obtained individually by tissue segmentation of the structural images using the FAST tool (`https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET`) and co-registered to the functional images in each subject/session. The CVR and TTP maps were obtained within GM for each subject and session. The rationale for this was that GM is a very much vascularized area that receives several times more flow than white matter (WM) [17], and in BH-CVR studies greater BOLD signal change is usually seen in GM while nonsignificant changes are usually observed in WM [18].

2.2.2. Group-level data analysis

To perform group-level analysis, non-parametric permutation testing was applied using the *randomise* tool from FSL (`https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise`). Ictal versus interictal differences were investigated in a voxel-wise

approach, meaning that every single voxel was individually tested for significant differences between S1 and S2. Statistically relevant voxels were the ones surviving to a significance threshold of 0.05, FWE-corrected with the TFCE method.

This investigation was performed in the CVR and TTP maps, as well as in the motor and visual whole-brain activation maps. Next, ictal versus interictal differences in CVR and TTP were assessed within particular brain regions, i.e. a region-specific group-level analysis was performed. This analysis was done by creating binary brain masks of the specific brain regions and including them as an input to the *randomise* command, which investigated S1 versus S2 CVR and TTP differences only in the voxels included in the masks. The "masked" brain regions were the four cerebral lobes (frontal, occipital, temporal and parietal lobes) and the flow territories of the internal carotid arteries (ICA) and the vertebrobasilar artery (VBA). Additionally, in order to assess if there was a particular region included in the VBA flow territory that presented ictal versus interictal CVR and TTP differences, group-level analysis was performed within four additional masks representing the regions supplied by the VBA: the brainstem, the cerebellum, the occipital lobe, and the thalamus.

Finally, a region-of-interest (ROI) analysis was performed. The spatial maps of the ictal versus interictal differences identified with the group-level analysis were used to define masks representing the ROIs within which the values of CVR and TTP were averaged for each subject. In order to investigate paired differences between the ictal and interictal phases, an analysis of variance (ANOVA) test was performed over the within ROI-averaged CVR and TTP values, for each ROI. Next, Pearson correlation analysis was performed between the within-ROIs individual mean CVR and TTP values and several migraine clinical features. For testing the null hypothesis (no correlation) against the alternative hypothesis of a significant correlation, a significance threshold of 5% was applied. The usual headache intensity reported by each participant was tested for correlation with CVR and TTP values from both S1 and S2, while the clinical features characterising the ongoing migraine attack (attack/pain duration, headache intensity, and photophobia) were correlated with CVR and TTP values only from S1.

3. Results

3.1. Subject-level data analysis

Regarding the CVR and TTP maps, as referred, these were only obtained (for each individual and each session) within the voxels belonging to each individual's GM. For all subjects and for both sessions, virtually the entire GM presented significant activation in response to the BH task. All the individual motor and visual brain activation maps (for all subjects and sessions) showed clusters of activated voxels located in the brain regions specific to motor and visual functions, i.e. motor and visual cortices.

3.2. Group-level data analysis

3.2.1. Whole-brain analysis

Regarding the motor task, the whole-brain voxel-wise group-level analysis revealed no voxel surviving the significance threshold of 0.05, meaning that no significant group differences between S1 and S2 were found. As for the visual data, group-level analysis revealed significant differences in brain activation between S1 and S2, which are presented in figure 1. It is possible to observe that these differences were not in brain regions involved in visual functions, as they did not occur in occipital/visual cortex regions, as expected.

As for the CVR and TTP maps, the respective group-level analyses revealed a very small cluster of voxels in occipital regions surviving the significance threshold of 0.05.

3.2.2. Region-specific analysis

Concerning the frontal, parietal, and temporal lobes, no voxel has survived the significance threshold of $p<0.05$, meaning that no significant group differences between S1 and S2 were found in any of the three cerebral lobes. As for the occipital lobe mask, the group-level analysis revealed an increased CVR and TTP during the ictal phase compared to the interictal phase of migraine, as shown in figure 2. Specifically, these differences were located in the primary visual cortex (V1), and in the visual areas 2, 3 and 4 (V2, V3, and V4).

Regarding the group analysis performed within the ICA territory mask, no voxels survived the significance threshold of 0.05, which means that no differences in CVR and TTP between S1 and S2 were found in this region. As for the VBA territory mask, group-level analysis revealed increased CVR and TTP within this region during the ictal compared to the interictal session, as presented in figure 3. Group-level analysis performed within the four masks representing the brain regions supplied by the VBA revealed statistically significant differences only within the VBA flow territory overlapped with the occipital lobe mask, that can be seen in figure 4.

3.2.3. Region-of-interest (ROI) analysis: correlation with clinical features

The boxplots representing the distributions of the mean CVR and TTP values for each session and ROI are represented in figure 5. Median of mean ictal CVR across subjects in the occipital lobe, VBA territory, and VBA territory + occipital lobe

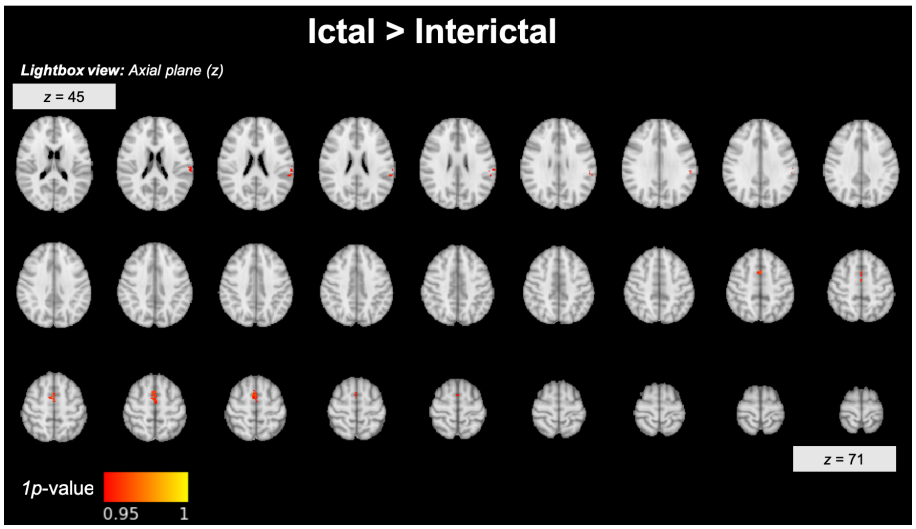

Figure 1: Group-level analysis of brain activation maps in response to visual stimulation: map of statistically significant differences between the ictal and interictal phases. Increased brain activation was found in the ictal vs. interictal phase (p<0.05). The map is represented in the standard MNI152 space. The z coordinate in the standard space is shown for the most inferior and most superior slices in the axial plane.

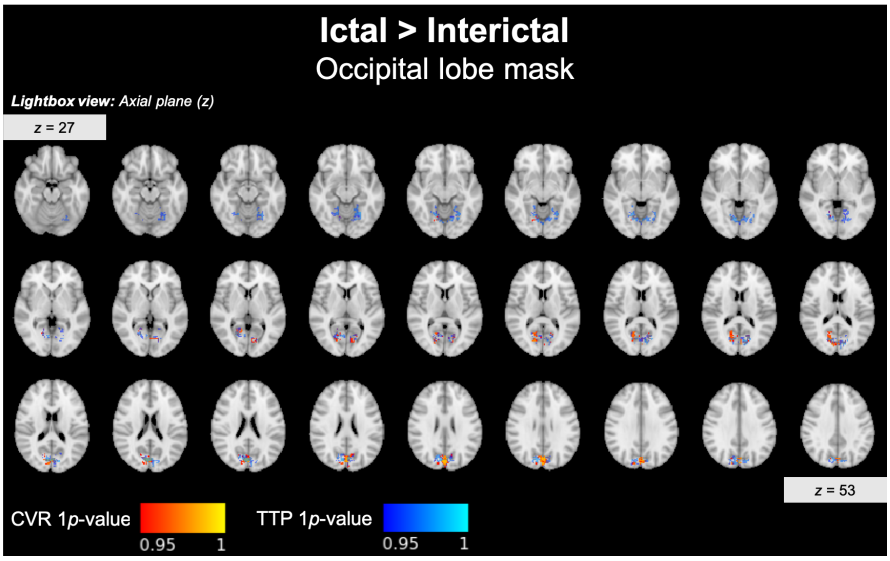

Figure 2: Group-level analysis of CVR and TTP maps performed within the occipital lobe mask: maps of statistically significant differences between the ictal and interictal phases. Increased CVR and TTP were found in the ictal vs. interictal phase (p<0.05). The map is represented in the standard MNI152 space. The z coordinate in the standard space is shown for the most inferior and most superior slices in the axial plane.

was 2.3%, 2.6%, and 2.3%, respectively. Median of mean interictal CVR across subjects in the occipital lobe, VBA territory, and VBA territory + occipital lobe was 1.3%, 1.3%, and 1.3%, respectively. Median of mean ictal TTP across subjects in the occipital lobe, VBA territory, and VBA territory + occipital lobe was 36.8s, 36.8s, and 36.6s, respectively. Median of mean interictal TTP across subjects in the occipital lobe, VBA territory, and VBA territory + occipital lobe was 24.6s, 22.6s, and 24.8s, respectively. The ANOVA test revealed a significant paired difference in the mean CVR and TTP of all ROIs between the ictal and interictal periods (p<0.05). The results of the Pearson coorelation analysis, employed to assess the relationship between the individual mean CVR and TTP within the ROIs and several migraine clinical parameters, showed no p-value below the significance threshold of 0.05, which means that no significant correlations were found between any of the migraine clinical parameters and mean CVR and TTP values.

4. Discussion

The fact that brain regions normally involved in and responsible for motor and visual functions showed no differential activation in response to motor and visual stimulation between S1 and S2 allowed us to assume that migraineurs do not present any neu-

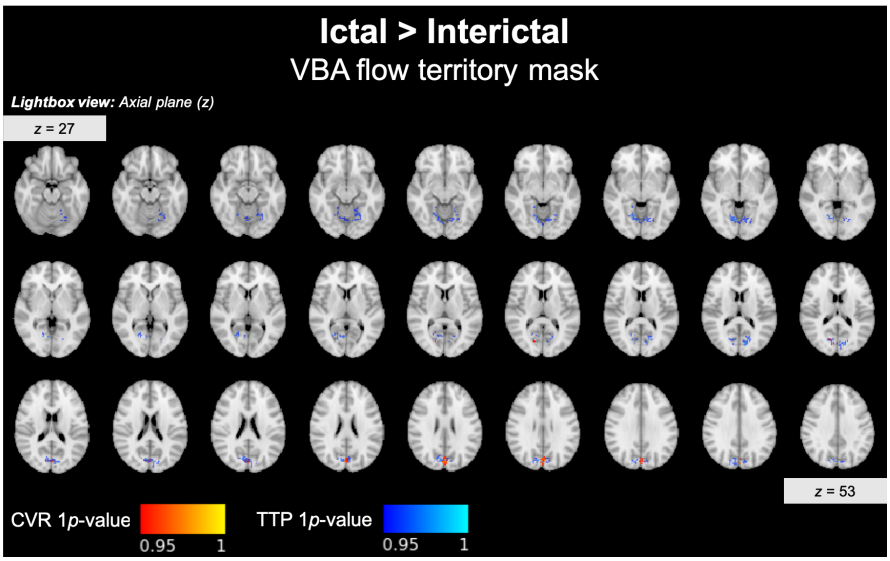

Figure 3: Group-level analysis of CVR and TTP maps performed within the VBA flow territory mask: maps of statistically significant differences between the ictal and interictal phases. Increased CVR and TTP were found in the ictal vs. interictal phase (p<0.05). The map is represented in the standard MNI152 space. The z coordinate in the standard space is shown for the most inferior and most superior slices in the axial plane.

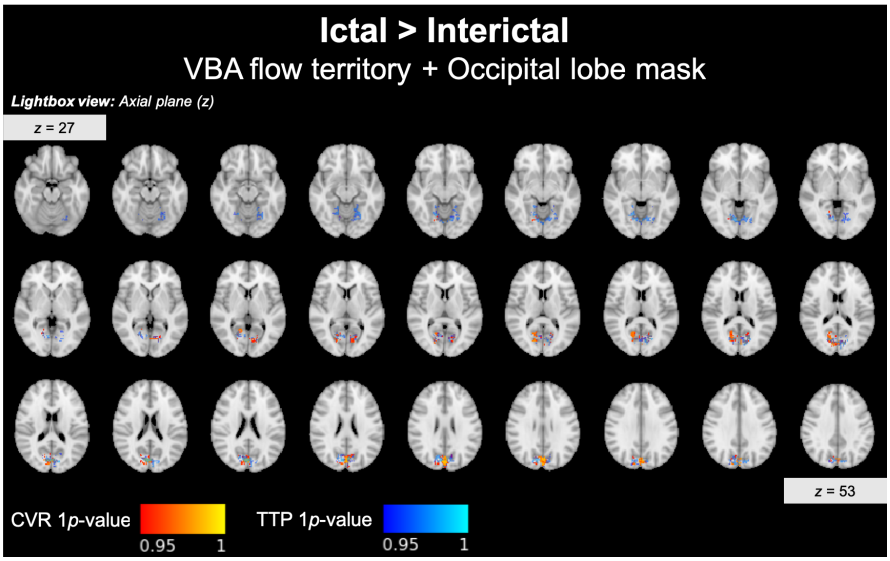

Figure 4: Group-level analysis of CVR and TTP maps performed within the VBA flow territory mask overlapped with the occipital lobe mask: maps of statistically significant differences between the ictal and interictal phases. Increased CVR and TTP were found in the ictal vs. interictal phase (p<0.05). The map is represented in the standard MNI152 space. The z coordinate in the standard space is shown for the most inferior and most superior slices in the axial plane.

ronal alterations between the ictal and interictal phases of the migraine cycle. Regarding CVR, we did not consider the clusters identified in the whole-brain analysis to be relevant due to their very small size. In fact, they were barely detected when thresholding the outpup $1p$-value map at $1p$>0.95 (equivalent to p<0.05).

The region-specific analysis found no statistically significant differences in both CVR and TTP between S1 and S2 in the frontal, parietal, and temporal lobes. This result enabled to conclude that there is no CVR differences in these brain regions between the ictal and interictal phases of migraine. Similarly, the group analysis performed within the ICA territory mask revealed no voxels presenting significant differences in CVR and TTP between S1 and S2. The ICA territory mask represented the anterior cerebral circulation, including the regions supplied by the ICA as well as by the ACA and MCA, i.e. frontal and lateral surfaces of the temporal and parietal lobes. Thus, the fact that no group differences were found within this brain mask is concordant with the previous result of no group differences in frontal, parietal and temporal lobes.

The vast majority of studies have used TCD to evaluate CVR in the MCA. Moveover, most of

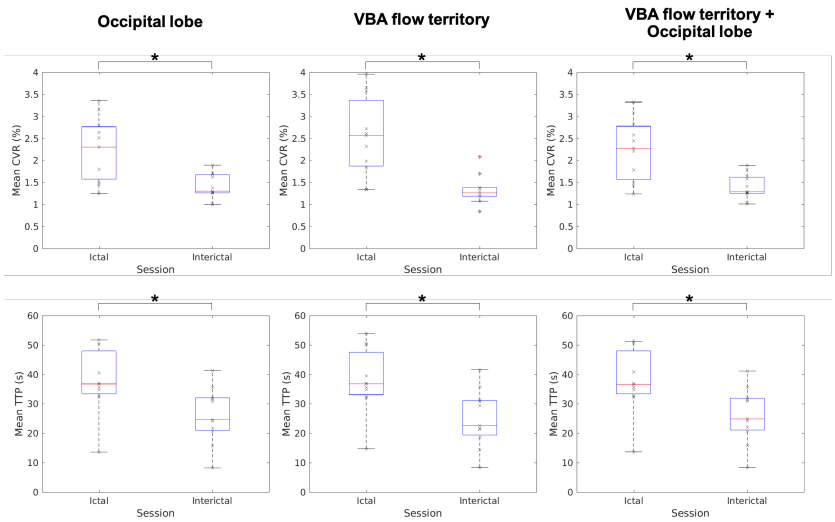

Figure 5: Boxplots representing the distributions across patients of the mean ictal and interictal CVR and TTP values within the ictal>interictal ROIs shown in figures 2, 3 and 4. In the boxplots, the central mark is the median; the edges of the box are the 25th and 75th percentiles; the upper and lower extreme represent, respectively, the maximum and minimum values (excluding outliers). The ANOVA test revealed significant differences ($p<0.05$) for the CVR and TTP differences between the ictal and interictal phases within all the three ROIs, which are marked with an asterisk.

them compared patients in the interictal phase of migraine with matched healthy controls. The results have yielded both hyperreactivity [19, 20, 21] and hyporeactivity [22, 23, 24] of migraineurs' brain compared to controls, but most of them observed no CVR differences between both groups [25, 26, 27, 28, 29]. Thus, it can be declared that the MCA in the interictal phase of migraine are not affected by an abnormal CVR. Regarding the investigation of the MCA' reactivity between the ictal versus interictal phases of migraine, there is evidence of reduced CVR of these arteries during the attack and excessive during the interictal/headache-free phase [30], as well as evidence of no difference in MCA' CVR between both migraine periods [31, 32]. The results of the present work are consistent with these last findings and extent them to the anterior cerebral circulation (not only the MCA), showing similar vascular reactivity (CVR and TTP) of this circulation between the ictal and interictal phases of migraine. Thus, previous studies' results of normal interictal CVR of the MCA in migraine patients (compared to controls), together with this work's result of similar CVR of the ICA flow territory between the ictal and interictal phases of migraine, suggest that the anterior cerebral circulation is not affected by impaired CVR in migraine.

On the contrary, increased CVR and TTP during the ictal phase compared to the interictal phase of migraine were found in the occipital lobe, in the VBA flow territory, and in the VBA flow territory overlapped with the occipital lobe, which showed that patients with episodic migraine without aura have increased CVR of the posterior cerebral circulation restricted to occipital regions during the headache phase of a spontaneous migraine attack compared to the interictal phase.

Most studies regarding CVR of the posterior cerebral circulation in migraine compared patients during the interictal/attack-free period with healthy subjects. Contradictory results have been achieved, ranging from reduced [22, 14, 25] to normal [31, 27] and even exaggerated [20] interictal vascular responses of migraineurs' brain in comparison with controls. Regarding the investigation of ictal versus interictal CVR differences of the posterior cerebral circulation, to the best of knowledge, there are only three TCD studies performing this comparison. Two of them detected no differences in vascular reactivity between both periods [31, 33], while the third observed a lower reactivity during the migraine attack compared to the migraine-free interval [32]. The results of the present work contribute with new evidence to the literature, demonstrating for the first time that migraine patients during spontaneous attacks had increased CVR and TTP of the posterior cerebral circulation restricted to occipital regions compared to the interictal period, suggesting a failure of cerebrovascular regulation and a potential endothelial dysfunction of these areas during the interictal phase of migraine without aura.

Reduced CVR and endothelial dysfunction are hypothesized to predispose migraine patients to an increased risk of ischemic stroke by several manners: firstly, reduced CVR may explain the lower ischemic threshold reported in experimental models of migraine, additively or synergistically with cortical excitability and spreading depolarization [34]; as for endothelial dysfunction, not only it can impair the detection and rapid vasodilatory response

to hypercapnia, but it can also hamper the development of collateral channels in response to ischemia [22]. Having said, the results of this work may suggest that migraine patients are at increased risk of an ischemic stroke during the interictal phase of migraine, which is consistent with the finding of strokes rarely developing during a migraine attack [34].

Additionally, our findings of decreased interictal versus ictal CVR restricted to occipital brain regions of migraineurs are consistent with the increased vulnerability of these areas regarding cerebral infarcts and ischemic-like lesions in migraine [35, 36, 37, 38].

5. Conclusions

The main goal of the present work was the investigation of neurovascular differences between the ictal and interictal phases of migraine. We showed that BH-CVR using fMRI is a promising and practical measure to assess CVR, and one that is sensitive to group differences when comparing migraineurs in different phases of the migraine cycle. It was found that migraine patients without aura have increased CVR and TTP of the posterior cerebral circulation restricted to occipital brain regions in the ictal phase versus the interictal phase, pointing to a cerebral endothelial dysfunction of these areas, which could associate migraine and cerebral infarcts that are more common in the PCA distribution in these patients. This result contributes with new evidence to the limited literature as this was the first study to use fMRI to report on CVR differences between spontaneous migraine attacks and pain-free periods. In addition, to the best of our knowledge, this was the first time that CVR was examined not only in terms of percent signal change, but also in terms of the amount of time to reach the peak of response/CVR.

One of the pitfalls of this study could be the fact that the CVR metrics achieved using BH BOLD-fMRI were relative metrics (expressed in percent change) and therefore mainly qualitative in nature. Furthermore, the direct cause of the vascular response and BOLD signal changes is the change in arterial CO_2 levels associated with the BH challenge. In order to deal with these limitations, the $PETCO_2$ could have been measured and used to normalize the BOLD ratio, which would allow a quantitative interpretation of CVR results. At the same time, $PETCO_2$ measurements could provide a full model of the hypercapnia stimulus and be used as a regressor of the GLM analysis instead of the sine-cosine regressor employed in this study. At the same time, it would increase the reproducibility of the results and reduce inter- and intrasubject variability caused by respiratory and BH-task compliance differences [39]. However, CO_2 tracing requires complex experimental setups that are in general uncomfortable for the participants. Furthermore, it has been shown that absolute BOLD signal intensity changes after an hypercapnic challenge hold better reproducibility and lower between-subject variability than BOLD ratios normalized by $PETCO_2$ [40]. Lastly, the small number of participants strongly limits the statistical power of the findings, possibly limiting the generalisability of the results.

Despite of that, this study holds the great advantage of following a longitudinal approach, i.e. comparing the same migraine patients in the ictal and interictal phases of the disease, which makes the observed CVR and TTP alterations possibly specific to migraine.

It is important to refer that this work did not perform any comparison between migraineurs and healthy controls, thus being impossible to infer whether our results indicate an above normal CVR during the ictal phase or a below normal interictal CVR. Having said, future work should focus on comparing both ictal and interictal phases of migraineurs with healthy controls to test the hypothesis of a reduced interictal CVR that increases during attacks. Furthermore, migraine patients during other phases of the ictal period, such as the prodrome and postdrome, could also be investigated, as a means to increase the limited knowledge on mechanisms involved in these phases. In particular, migraine therapeutic targets and biomarkers could be identified, which would help to predict improvements or worsening in the patients.

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