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Absence of haemodynamic refractory effects in patients with migraine without aura – an interictal fMRI study

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Abstract

Background: In healthy controls, haemodynamic refractory effects are observed with blood-oxygenation-level dependent (BOLD) functional MRI (fMRI): the haemodynamic response function (HRF) to the second stimulus in a pair of stimuli with short interstimulus interval (ISI) shows a decreased amplitude and an increased time-to-peak. We hypothesize that there may be interictal haemodynamic abnormalities in migraineurs.

Methods: An event-related fMRI design with paired face stimuli and varying ISIs was used to measure interictal HRFs in the face recognition area of patients with migraine without aura (MwoA) and controls. Net responses to the second stimulus in a pair were calculated and averaged per participant. Several characterizing parameters of the net responses were quantified and examined within each group.

Results: Refractory effects were not observed in our patient group. There are no changes in the net responses compared with the reference situation in patients, irrespective of the ISI, whereas in controls all HRF parameters are decreased or delayed for an ISI of 1 second.

Conclusion: This is the first fMRI study investigating the haemodynamic refractory effects in MwoA patients. Unlike in controls, these effects are not observed in migraineurs. Although currently unclear, it is tempting to speculate that this observation reflects the neurovascular correlate of lack of habituation measured with evoked potentials in migraineurs.

Keywords

Migraine without aura, interictal, BOLD-fMRI, haemodynamic refractory effects

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Introduction

During the interictal phase of migraine, some differences in brain functioning between patients and healthy controls can be demonstrated. In 1995, Schoenen et al. were the first to describe the lack of habituation in patients with migraine, demonstrated with visual evoked potential (VEP) measurements (1). Habituation is a phenomenon of a decreasing response amplitude to repetitive stimulation in healthy controls (2). Migraineurs do not show this phenomenon; on the contrary, even a transient potentiation of their responses has been observed. The lack of habituation of evoked brain potentials (EP) is not exclusively related to VEP measurements, and has been described with other types of stimuli, such as auditory, somatosensory and pain stimuli (reviewed by Coppola et al. (2)). A hyperventilation study indicated that the lack of habituation in patients with MwoA is worsened by hypocapnia (low blood carbon dioxide) (3). Transcranial Doppler (TCD) studies mostly reported increased mean blood flow velocities in cerebral arteries and increased cerebrovascular reactivity to carbon dioxide in migraineurs compared with controls (4–9). Furthermore, patients are more sensitive to nitric oxide, such as

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by infusion of nitroglycerin. Both patients and controls develop a headache immediately after infusion, but patients also develop a delayed headache with migraine characteristics (10). A recent study with ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS) demonstrated interictal reductions in ATP and phosphocreatine (PCr) concentrations in patients with MwoA compared with controls (11). Glucose hypometabolism had been reported in episodic migraine using ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET). This hypometabolism was more pronounced with increasing disease duration and increasing attack frequency (12). Despite these interictal findings, a coherent theory to explain migraine neurobiology, culminating in episodic headache attacks, is lacking.

Haemodynamic changes in response to stimuli can be measured non-invasively with functional magnetic resonance imaging (fMRI) to map brain activity. Externally applied stimuli provoke neuronal activity and local changes in cerebral blood flow (CBF), cerebral blood volume (CBV) and cerebral metabolic rate of oxygen consumption ($CMRO_2$), thereby altering the blood oxygenation level. Information about local changes in oxygenation level are directly captured by MRI pulse sequences sensitive to blood-oxygenation-level dependent (BOLD) contrast (13). BOLD-fMRI is by far the most commonly used non-invasive functional imaging method. The BOLD response to a single stimulus is described as the haemodynamic response function (HRF). Single, short, successive stimuli with an interstimulus interval (ISI) of more than 20 s generate separate HRFs. When the ISI is shortened, the overall HRF is the summed response of the successive stimuli (Figure 1, left panel). This summation is linear to a certain extent: subtraction of a reference HRF (the HRF in response to a single stimulus) from the overall HRF reveals the net HRF to the second stimulus in the pair, which is identical to the reference HRF. However, when the ISI is very short (less than 6 seconds), the net HRF to the second stimulus is not identical to the reference HRF (Figure 1, right panel). The response amplitude gradually decreases and the time-to-peak is delayed with shorter ISIs. Consequently, the net HRF to the second stimulus cannot be predicted based on a single HRF. Such nonlinear properties of the HRF are called refractory effects and the short time frame after a stimulus during which refractory effects will appear in subsequent responses is referred to as the refractory period (14,15). Linear responses recur for an ISI of 6 seconds or more.

To date, only a few fMRI studies have been published investigating differences between migraineurs and healthy controls. The ictal phase of visually triggered migraine has been investigated with fMRI by Cao et al. (16,17) to make inferences about changes in the oxygenation of the occipital cortex and brainstem structures during the attack. Moulton et al. reported interictal brainstem abnormalities in migraineurs: the nucleus cuneiformis, part of a modulatory circuit in the brainstem, was found to be hypofunctional compared with controls (18). Recently, work from Stankewitz et al. suggested that the migraine attack might be predictable with fMRI measurements because of increasing preictal activation in spinal trigeminal nuclei following nociceptive stimulation (19), and interictal resting-state fMRI measurements revealed abnormal connectivity in several brain networks (20). To our knowledge, no fMRI activation studies have been published investigating the cerebrum of patients with MwoA interictally, nor on the haemodynamic response characteristics of such activations.

The aim of this fMRI study was to examine whether there are interictal haemodynamic abnormalities in patients with MwoA and to quantify the degree of the haemodynamic refractory effects or the lack of it. We focused on patients with MwoA, to avoid interference of the aura biology. An event-related fMRI study was set up to measure haemodynamic responses to single and paired face stimuli in the face recognition area of the brain, that is, the fusiform face area (FFA), a small higher-order cortical area in which haemodynamic refractory effects in normal participants have been demonstrated (21). Net HRFs were calculated, fitted and quantified per participant. The analysis procedure was applied on data from patients with MwoA and healthy controls.

Methods

Subjects

Twenty-one patients with MwoA (1 male, aged 18–53 years, mean 32.9 ± 12.2) and 51 age-matched healthy volunteers (17 males, aged 18–60 years, mean 29.8 ± 11.7) participated in this study. We allowed a gender imbalance between the groups, because a previous study failed to show differences in the HRF between men and women (22). Patients were recruited at the local outpatient headache clinic. They were all diagnosed according to criteria of the International Classification of Headache Disorders (23), had 2–8 attacks per month (mean 3.4 ± 1.1), were not taking any prophylactic treatment and were at least 48 hours attack-free before the scanning procedure. None of the patients experienced a migraine attack within 24 h after the study. Candidates with epilepsy or MRI contraindications were excluded. All participants were generally healthy, had no neurological history (other than migraine in the patient group) and were asked to

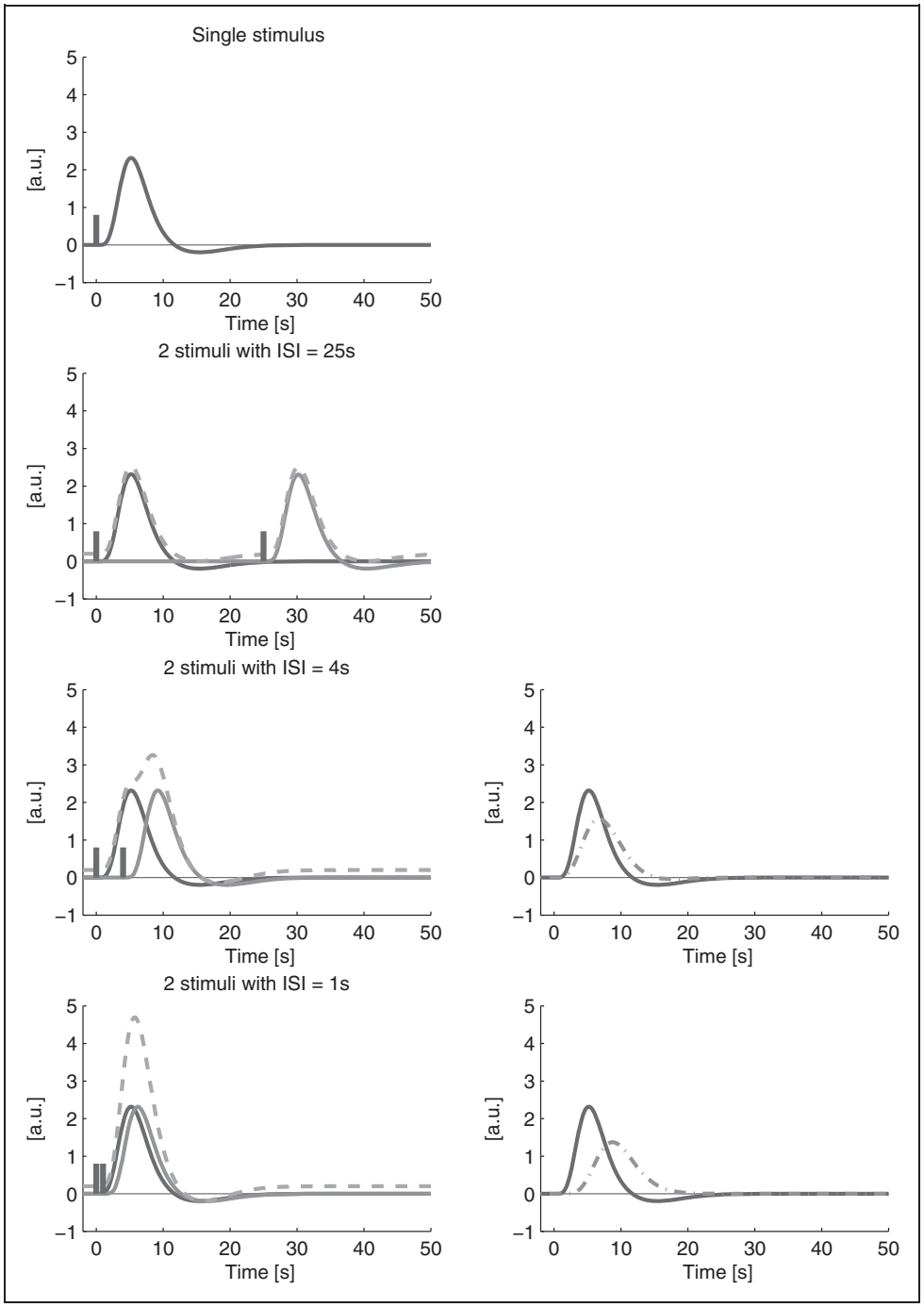

Figure 1. The theoretical BOLD response to one (upper plot) or two stimuli with varying interstimulus intervals is illustrated in the left panel. Two separate stimuli (interstimulus interval >20 s) provoke separate responses (second plot). When the interstimulus interval gets shorter, the responses are summed (third and fourth plot). In the left panel of this schematic representation, the responses are linearly summed (dashed line): refractory effects are not taken into account. Stimulus onsets are depicted with grey bars. For reasons of clarity the summed responses are shifted 0.2 arbitrary units (a.u.) upwards. The right panel illustrates what haemodynamic refractory effects in healthy volunteers are: for short ISIs, the net response to the second stimulus in a pair is decreased in amplitude and delayed compared to the response to a single stimulus.

abstain from caffeine 24 h before the examination. The study was approved by the local ethics committee and all participants provided written informed consent.

Imaging and experimental setup

A 3 T Siemens Trio Tim system (Siemens AG, Erlangen, Germany) with an 8-channel head coil was used for image acquisition. Anatomical images were acquired using a T1-weighted sequence (MPRAGE) with isotropic voxels (voxel size $0.9 \times 0.9 \times 0.9\,mm^3$, TR = 1550 ms, TE = 2.39 ms, matrix 256×256, 3D slab with 176 slices). F r functional imaging, a T2*-weighted echo planar sequence was used, which is sensitive to BOLD contrast (voxel size $3.5 \times 3.5 \times 5.0\,mm^3$, TR = 1000 ms, TE = 27 ms, matrix 64×64, 19 slices).

Total protocol scanning time was 45 min 57 s and included two functional measurements: a functional localizer and the main experiment. The block-designed functional localizer comprised three conditions (looking at faces, scenes and a fixation cross hair as rest) and was necessary to determine the subject-specific FFA. The main experiment was an event-related study. The global paradigm design and its four conditions are shown in Figure 2. We acquired five consecutive sessions with 19 trials per session. Each trial belonged to one of the four conditions and consisted of one or two male faces with blurred contours. The faces were presented for 500 ms. A single face followed by 26 seconds rest served as reference condition (REF); for the three other conditions faces were shown in pairs with 1, 2 or 6 seconds ISI (onset-to-onset; called 1S, 2S and 6S, respectively) and a fixed post-stimulus interval of 20 seconds rest before a new face was presented, to allow the HRF to return to baseline. Within one session, each condition was randomly displayed four times and between them three random 'oddball' trials of type REF, 1S, 2S or 6S were added to keep the subject attentive. One session took 7 min 38 s ($4 \times (26\,s + 21\,s + 22\,s + 26\,s) + 3 \times$ max. 26 s). The difference in trial lengths is related to the subtraction step in the analysis (see below). Participants were instructed to look at the face images or the cross hair, without moving the head, and to press a button if the present face was identical to the previous one (oddball). The order of appearance of the trials, including the oddball trials, was randomly assigned and different for each participant to avoid a potential ordering effect. All stimuli were supplied by Mark D'Esposito and are cited in Rissman et al. (24).

Analysis

All data were preprocessed in SPM5 (Wellcome Trust Centre for Neuroimaging Functional Imaging Laboratory, London, UK) comprising slice timing, realignment, co-registration of anatomy to functional images, spatial normalization to 1 and 3 mm isotropic voxels for anatomical and functional images, respectively, and spatial Gaussian smoothing (8 mm kernel).

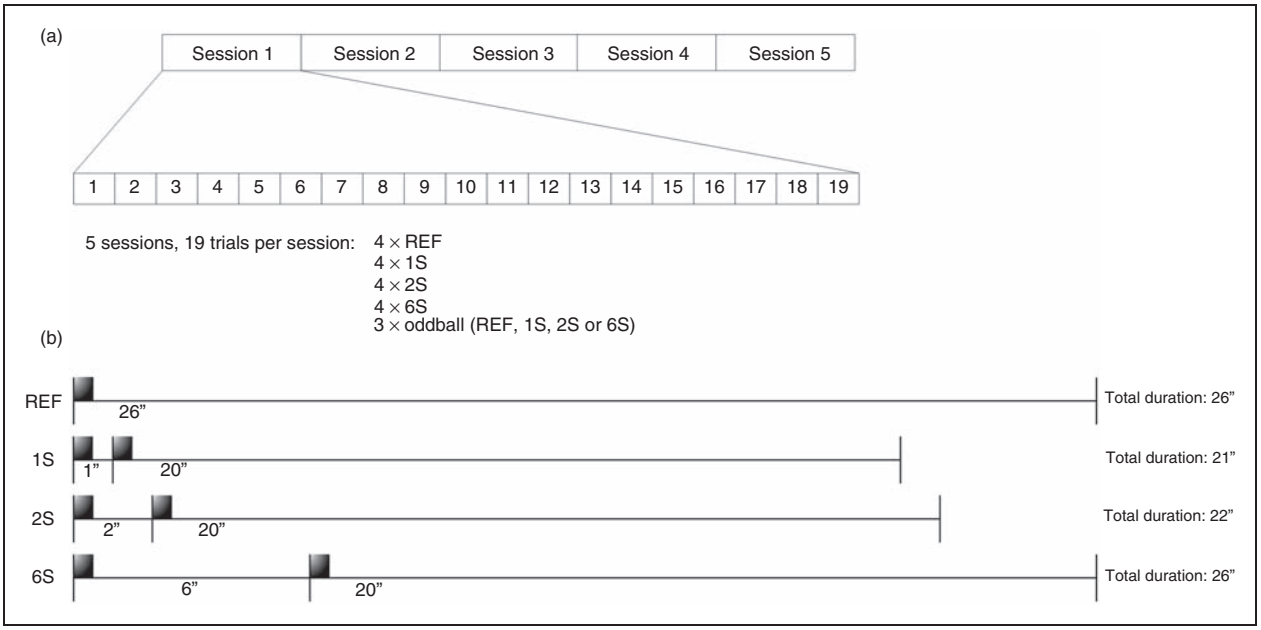

Figure 2. The global paradigm design (a) and a detail of the four conditions (b). One reference condition (REF) and three paired trial types (1S, 2S, and 6S respectively). Each stimulus is presented for 500 ms. Interstimulus (1S, 2S, and 6S) and poststimulus intervals (20S, 26S) are measured onset-to-onset.

Preprocessed data from the functional localizer were further analysed in SPM5 and provided information for defining each individual's FFA as a region-of-interest (ROI). The ROI was the intersection of the fusiform gyrus, selected with the WFU PickAtlas (25,26), and the subject-specific spmT image, which contains the *t*-values for the contrast '*faces* > (*scenes & rest*)'. Only those voxels with a *t*-value higher than 80% of the maximum *t*-value within this mask image were selected for further analysis. The number of voxels in the ROI of patients and controls were compared with a two-tailed two-sample *t*-test.

Processing of the smoothed images from the main experiment was as follows: first, trials were excluded from analysis when participants had pressed during a trial, indicating oddball detection, to prevent confounding effects caused by the motor responses. If the subject had not pressed at all during a particular session, the subject was considered inattentive and the entire session was also excluded from further analysis. Remaining responses from all voxels in the ROI above the threshold were averaged per condition. The response to the single stimulus (REF) was subtracted from the overall responses to the paired stimuli (1S, 2S and 6S). To execute this subtraction, the two arrays with data points from REF and the paired condition must have the same length, otherwise an error due to different array length will prevent the software from continuing. This is why the REF duration equals the duration of the longest paired condition (i.e. 26 s). For the 1S and 2S conditions the last time points of REF are not necessary, and were thus omitted.

Net haemodynamic responses to the second stimulus in the pair were semi-automatically fitted using a recently developed algorithm (27), which uses the linear combination of three inverse logit functions – sigmoid curves, in fact – followed by a validation of the fits by calculating the R^2. Fitted responses were used to determine four characterizing parameters of the haemodynamic responses: peak amplitude, time-to-peak, time-to-half-peak and peak width (measured at full-width-at-half-maximum (FWHM)). These absolute values were used to calculate relative differences, defined as a percentage in relation to REF, because these percentages are more intuitive for assessing the refractory effects.

Statistical analysis was performed with SPSS 18 (IBM SPSS Inc., Chicago, IL, USA). A repeated measures analysis of variance (ANOVA) – including Levene's test to assess equality of variances – was carried out separately on the absolute and relative values. Each of these ANOVAs had a fixed factor with four levels (the ISI) and a random factor (the participants), to correct for subject-to-subject variability. As separate ANOVAs were calculated for migraineurs and controls, a total of sixteen ANOVAs was carried out: one each for the four parameters (peak amplitude, time-to-peak, time-to-half-peak, peak width), separately for absolute and relative values in each group. Sixteen Bonferroni tests were used for triple comparisons in the post hoc analysis, where the ISI is under investigation, to determine which means differ.

Spearman's rank-correlation coefficients were calculated for each parameter to test whether patient results are correlated with the available clinical data (attack frequency).

Finally, mean absolute values from patients and controls of the REF condition were additionally compared for every separate parameter using Student's *t*-tests (uncorrected, two-tailed two-sample *t*-tests). These comparisons are helpful, for example to check whether the reference amplitude is equal in patients with MwoA and controls.

For all statistical tests, results with *p*-values less than 0.05 were considered to be significant.

Results

Screening of the high resolution T1-weighted anatomical images by a neuroradiologist revealed no clinically relevant abnormalities.

The comparison of the subject-specific ROIs between patients with MwoA and controls revealed no differences for the number of voxels in the ROI (mean ± SEM: 192 ± 39 and 299 ± 82 voxels, respectively, $p > 0.05$) and the maximum *t*-values (mean ± SEM: $t_{max} = 8.678 \pm 0.344$ and $t_{max} = 8.587 \pm 0.323$, respectively).

For some participants, not all HRFs could be fitted. Table 1 summarizes the total number of fitted HRFs per condition in migraineurs and controls. Eleven patients out of 21 (52.4%) had a full dataset (four well-fitted HRFs), in seven patients (33.3%) one HRF was missing (a 1S, 2S or 6S response), and from three patients (14.3%) we obtained no useful data. In the control group, comparable ratios were observed: all four HRFs from 29 participants out of 51 (56.9%) were fitted and partial results were obtained for 15 participants (29.4%). For seven participants (13.7%), fitting was impossible for any HRF. The mean R^2 of the fitted HRFs to assess the goodness-of-fit was high for both groups (0.954 ± 0.006 and 0.974 ± 0.002 in migraineurs and controls, respectively).

An example of the four plotted HRFs from a patient and a healthy control is given in Figure 3. There, refractory effects are seen in the net responses for shorter ISIs in the healthy participants, but not in the patient with MwoA. The four parameters which characterize the HRFs are averaged per condition and per group. These mean values are presented in clustered bar

Table 1. List with the number of fitted HRFs per condition for the 21 patients and 51 controls

Condition	Patients	Controls
REF	21 (100%)	46 (90.2%)
1S	15 (71.4%)	37 (72.5%)
2S	15 (71.4%)	38 (74.5%)
6S	17 (81.0%)	39 (76.5%)

diagrams (Figure 4). Whether amplitude, latencies and width change significantly as a result of the varying ISIs is investigated with the ANOVA. For each parameter, results from the ANOVA and post hoc Bonferroni test of the absolute values are shown in Table 2. For 1S, all parameters were significantly different from REF in controls, but this was not the case for the patients, except for the 1S time-to-half-peak.

The relative values of the four parameters are averaged and shown in Figure 5. ANOVA and post hoc Bonferroni test results of these percentages are comparable to the absolute ones (Table 3). Those values that were significantly different from REF in the ANOVA with absolute values are also significantly different from REF in the ANOVA with relative values, and vice versa, except for three values out of the 24, where there is no agreement with the absolute values. In the migraine patient group, the time-to-peak (1S) and the time-to-half-peak (2S) are additionally significantly different from REF; in the controls, the width in 1S is not significantly different from REF, whereas with absolute values it is.

There is no relationship between the available clinical information of the patients (the attack frequency) and the values of the parameters characterizing the haemodynamic responses ($p > 0.05$ for all percentages per parameter and per ISI, data not shown).

The results of the Student's *t*-test for the additional comparisons of REF values reveal no significant differences between patients and controls. The REF-amplitude difference between patients and controls has a borderline *p*-value of 0.07 (uncorrected).

Discussion

In this study, the interictal haemodynamic refractory effects in patients with MwoA were investigated using BOLD-fMRI. Refractory effects have been extensively studied in healthy controls, but to date no studies have examined these effects in migraineurs. We hypothesized refractory effects to be altered in migraine, based on several observations in the interictal migrainous brain, such as lack of habituation (EP), altered haemodynamics (TCD), ATP and PCr reductions (^{31}P-MRS), glucose hypometabolism (FDG-PET), abnormal brain network connectivity (resting-state fMRI) and anomalous brainstem activations (fMRI). The nonlinear properties of the HRF in controls appear when short ISIs are applied in the fMRI paradigm, that is, less than 6 s, and gradually decrease when the ISI increases. We demonstrate that these refractory effects are not present in our well-selected group of patients with MwoA.

In this section, we comment on the paradigm set up and the analysis steps, elaborate on the results, discuss the relevance of our findings and relate them to the current knowledge.

Faces were chosen as ecologically valid stimuli to provoke haemodynamic refractory effects. The face stimuli were smoothly blurred along the contours, so that only the internal features of the face were visible, to make sure that the face-selective areas were activated (24) and the subject was not focusing on hair style, face shape or background. The face recognition area was selected to investigate haemodynamic refractory effects, because it is a higher-order cortical area in which haemodynamic refractory effects have already been observed (21,28) and its neurons have size- and position-invariant properties to any face in the visual field (29). This means that neurons remain in an adapted state when new stimuli have a slightly different size or position.

The visual task 'looking at faces' first provokes BOLD changes in primary visual areas. The same visual stimulus activates different brain regions involved in processing visual information. Huettel et al. investigated the characteristics of haemodynamic responses and refractory effects in different regions with different visual stimuli (15,21). They reported regional differences in both the haemodynamic responses and the refractory effects and stipulated that their characteristics are stimulus-specific. This means that haemodynamic responses to a basic alternating checkerboard are more pronounced in primary visual cortical areas, whereas faces provoke more pronounced responses in the face recognition areas. In other words, haemodynamic responses to certain stimuli should be investigated in the corresponding stimulus-specific region of activation. Consequently, for the present study comparisons with different regions are rather pointless. We decided to focus on one region, the FFA, to investigate haemodynamic refractory effects in patients with MwoA and healthy controls because faces were used to provoke HRFs. Volumes acquired during motor responses were omitted because of the possible confounding effect. The oddball task might in theory introduce an additional confounding effect, a difference in working memory load, owing to the difference between the 1 s ISI and 6 s ISI. The connectivity between the hippocampus and the FFA and its potential dynamic

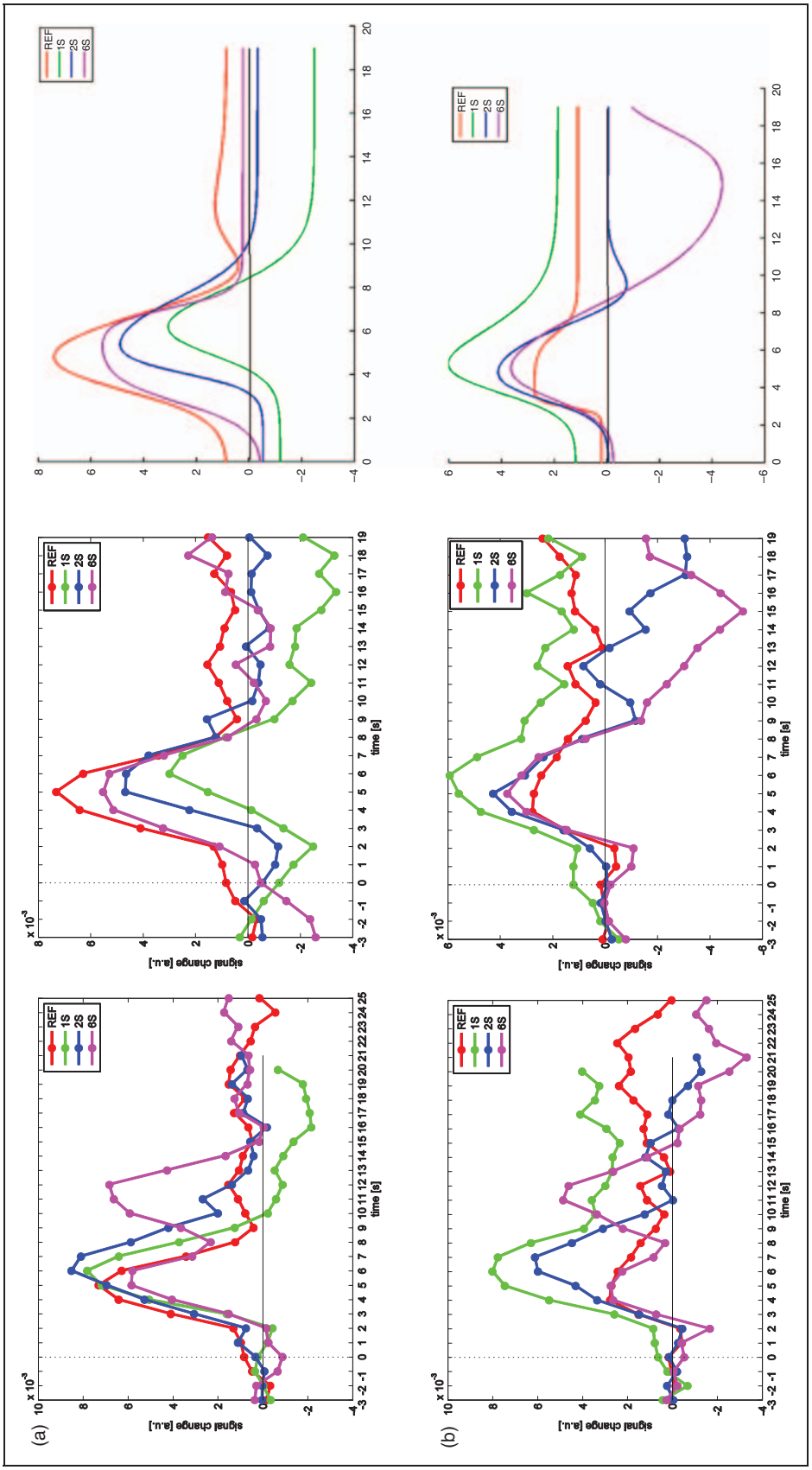

Figure 3. Time-courses from one healthy volunteer (a) and one patient (b), with responses to the four conditions (REF, 1S, 2S, 6S) both before (left) and after (middle) subtraction of the reference response. The fitted responses (right) were used to derive the characteristic parameters (amplitude, time-to-peak, time-to-half-peak and peak width). Parameters are quantified at the single-subject level. Afterwards, the data are put together to perform an ANOVA. The first three data points are used to calculate the baseline signal and are omitted in the fitted plots.

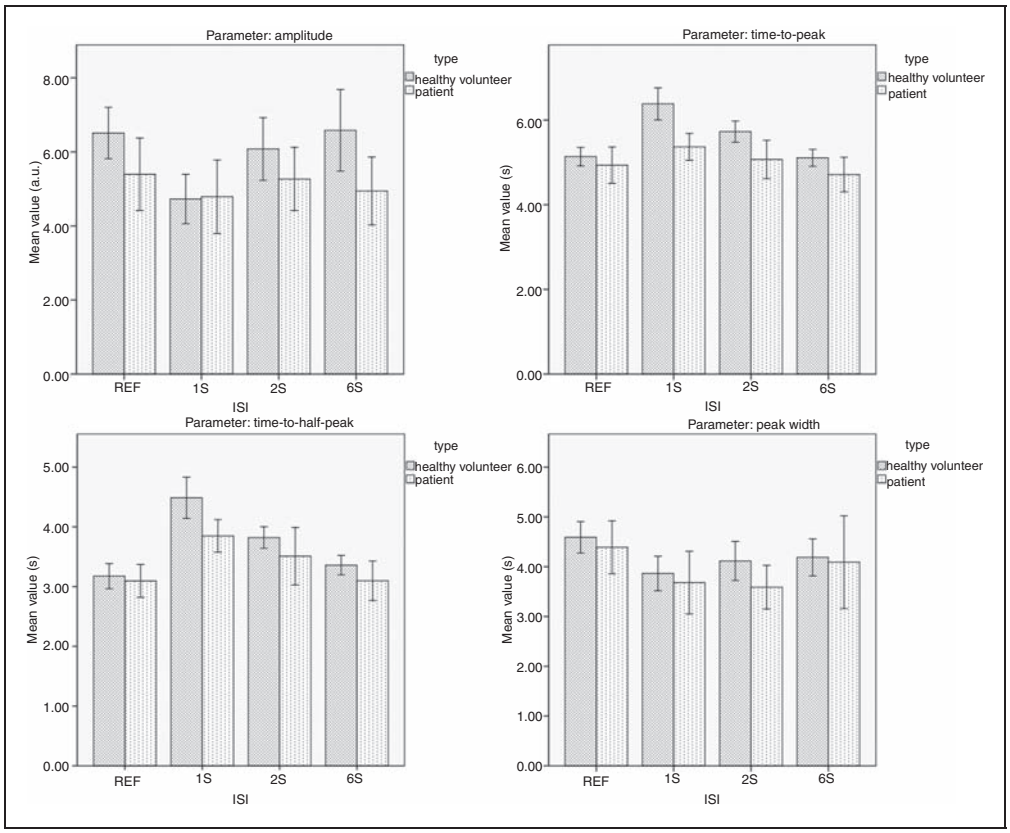

Figure 4. Graphs showing the mean absolute values for each parameter (amplitude, time-to-peak, time-to-half-peak and peak width) in patients and healthy controls. Error bars represent 95% confidence intervals. The shorter the ISI, the larger the deviation from the reference condition. Only for controls are all 1S parameters significantly different from REF. The significant differences are listed in Table 2.

Table 2. F-values of the ANOVAs with absolute values and p-values for post hoc comparisons with the reference condition following the ANOVAs

	Patients				Controls			
Parameter	F	1S	2S	6S	F	1S	2S	6S
Amplitude	0.396	n.s.	n.s.	n.s.	4.161*	<0.001	n.s.	n.s.
Time-to-peak	1.829	n.s.	n.s.	n.s.	20.594*	<0.001	<0.001	n.s.
Time-to-half-peak	5.011*	<0.001	n.s.	n.s.	25.730*	<0.001	<0.001	n.s.
Width	1.430	n.s.	n.s.	n.s.	3.095*	<0.01	n.s.	n.s.

Post hoc Bonferroni tests, threshold for significance = 0.05 for both groups. For each parameter, absolute values for at least one ISI are significantly different from REF in healthy controls, whereas nearly all these differences are not observed in patients.

adjustments might affect the remaining HRFs (30). Another point of discussion is the gender imbalance in the groups. For this, we can refer to one study, which failed to show statistically significant differences in the HRF between men and women, $p > 0.10$ (22). However, small sample sizes were used as well as different stimuli and a different brain region. Therefore, we mention the possibility of a confounding effect.

One of the crucial steps involved in this study is the curve fitting. We incorporated a recently developed algorithm into the analysis procedure. The choice for the inverse logit model to fit the HRFs is based on the information provided by Lindquist and Wager (27,31). The only criterion for this fitting method is the presence of the three phases of the haemodynamic response: the rise, the fall and the recovery from undershoot (the negative signal in the HRF due to the combination of

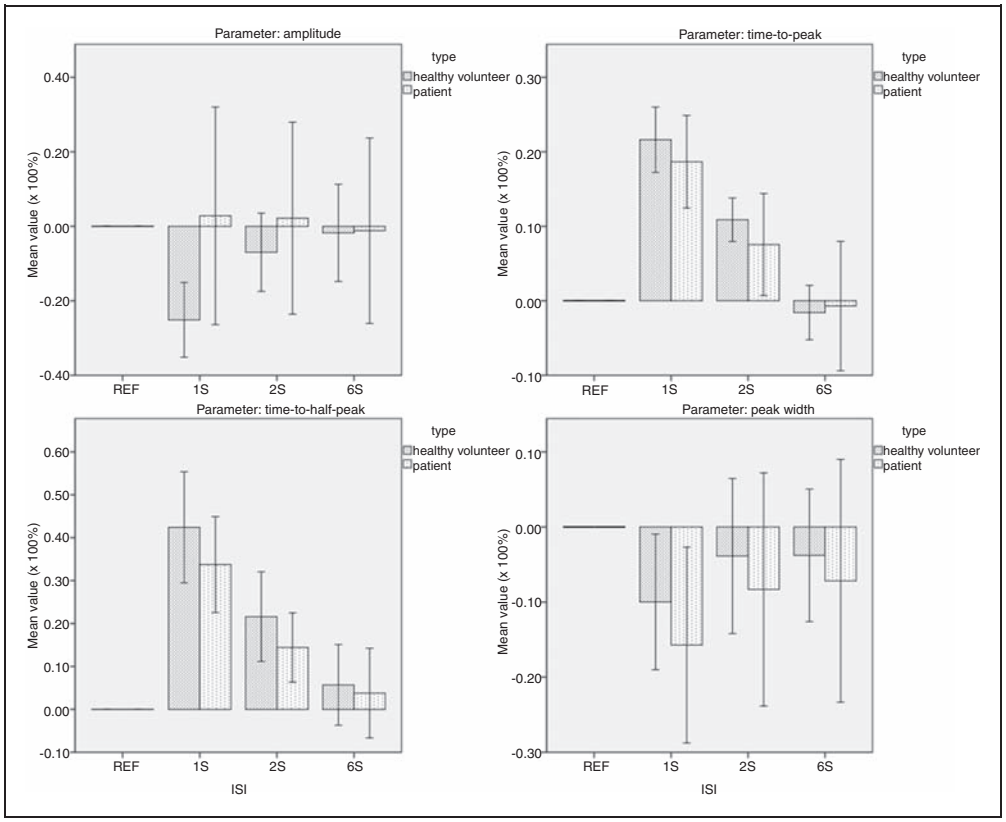

Figure 5. Graphs showing the mean percentages for each parameter (amplitude, time-to-peak, time-to-half-peak and peak width) in patients and healthy controls. The shorter the ISI, the larger the deviation from the reference condition, which is added only for clarity on these graphs. Error bars indicate 95% confidence intervals. A remarkable difference can be clearly observed for parameter amplitude: controls show an amplitude decrease of 25% for the net 1S response, whereas there is no significant difference from REF in patients with this ISI. Only for controls are all 1S parameters significantly different from REF. Significant differences are listed in Table 3.

Table 3. F-values of the ANOVAs with relative values and mean relative differences for the three net responses in relation to REF for the four parameters for both patients and controls

	Patients				Controls			
Parameter	F	1S	2S	6S	F	1S	2S	6S
Amplitude	0.034	2.8%	2.2%	−1.2%	6.388*	−25.1%*	−7.0%	−1.8%
Time-to-peak	9.950*	18.7%*	7.5%	−0.7%	53.114*	21.6%*	10.9%*	−1.6%
Time-to-half-peak	15.831*	33.7%*	14.4%*	3.8%	19.201*	42.4%*	21.6%*	5.7%
Width	1.330	−15.7%	−8.3%	−7.2%	1.252*	−10.0%	−3.9%	−3.8%

Post hoc Bonferroni tests are used with the threshold for significance at 0.05, indicated with an asterisk.

decreased CBF and increased CBV). Contrary to other methods, assumptions about timing and steepness of the phases are not required. This has the advantage that the parameter calculation is based on fitted curves that are as close to the raw data as possible. The algorithm cannot fit the HRF when rise, fall and recovery to baseline are not distinguishable or if the fall of the HRF does not go below half of the rise (i.e. if FWHM becomes infinite). After the subtraction step we may get HRFs with such a shape that the subsequent fitting step cannot be run properly. This may happen when, for example, too many trials had to be excluded for a particular condition. If the criterion is not fulfilled, no fitting was performed and we end up with missing HRFs. The extracted parameters, which are calculated on the fitted HRFs, will also be missing.

The origin of the haemodynamic refractory effects is difficult to trace. Neuronal as well as vascular mechanisms may be involved, and we discuss both below.

Neuronal activity contributes to the BOLD signal, albeit indirectly. The BOLD signal directly reflects the blood oxygenation level, which is an indirect measure of neuronal activity. The BOLD signal changes are indisputably correlated with the neuronal compartment, because the initial triggers for the haemodynamic changes are the higher regional oxygen and glucose demand from neurons and astrocytes during neuronal activation. Several studies, including electrophysiological and fMRI studies, have investigated the cortical responsiveness of patients with migraine (32–35). The fMRI studies all included patients with MwA and were focusing on the implications of their findings for the aura phenomenon. The widely accepted phenomenon of habituation has been considered a protective mechanism against cortical hyperexcitability. The assumption is that patients with both MwA and MwoA show increased cortical excitability as they lack this protective mechanism (36). Given that the refractory period of the haemodynamic response is a sign of temporary neural adaptation and believed to be a protective phenomenon (29,37), the lack of habituation in migraineurs could be the basis of the absence of refractory effects. However, the cortical hyperexcitability hypothesis in migraine has been refuted (35). A reduction in the cortical pre-activation level of migraine patients has been suggested as an alternative explanation and it is inferred from the initial lower amplitude of the P100 in the first averaged block of VEP measurements (38,39). The reduced cortical pre-activation level could be due to abnormalities in thalamocortical networks (40) or hypoactivation in subcortico-cortical aminergic pathways (41). This observation might be reflected in the haemodynamic data presented in our study: the amplitude of a single response (REF) in patients seems on average lower than the REF amplitude in controls (Figure 4, parameter amplitude, condition REF). However, the mean difference we have found is only borderline non-significant ($p = 0.07$).

Another point of view to explain the observed lack of haemodynamic refractory effects in patients is that the effects are related to the vascular compartment. Vascular-related parameters (CBF, CBV, oxygen extraction fraction, etc) directly contribute to the BOLD signal. The interictal vascular reactivity in patients might be affected (either increased or decreased). However, a recent near infra-red spectroscopy study demonstrated that the interictal neurovascular coupling in patients with MwoA seems to be intact (42). Simultaneous measurements of BOLD, CBV and CBF with a dedicated MR-sequence could add further strength to this hypothesis (43,44).

Taking into account the fact that successive stimuli applied to induce the measured HRF provoke local electrical changes, it is tempting to see a link between the electrophysiological habituation and haemodynamic refractory effects. However, there are some major differences between these two phenomena. A first involves the different temporal properties. The electrophysiological peak latencies are detected at the millisecond scale after stimulus onset, whereas the peak of the HRF is reached at 5 to 8 seconds after stimulus onset. Furthermore, the stimuli used in electrophysiology are presented at a high rate (typically 2–8 Hz), whereas the stimuli in this BOLD-fMRI paradigm should be considered as single events (viewing faces) presented for 500 ms with limited ISIs (1–6 s onset-to-onset).

Often, inferences in fMRI studies are based on group results, similar to EP data: first, individual data are averaged, followed by the determination of the derived group parameters. One strength of the paradigm applied in this study is that the data have first been analysed and quantified at the single-subject level. A visual evaluation of the net HRFs in individuals may already suggest the lack of haemodynamic refractory effects in individual patients with MwoA, which is illustrated by the examples in Figure 3. However, measuring haemodynamic refractory effects is not suitable as a biomarker for migraine in individual patients. The sensitivity of the measurements is unsatisfactory for discriminating between healthy controls and patients owing to an overlap in observations. Averaging of the extracted data per group is needed to make statistical inferences.

There is no relationship between available clinical information of the patients, that is, the attack frequency, and their values characterizing the haemodynamic responses, lacking refractory effects. Migraine can be considered as a threshold disorder. Lack of haemodynamic refractory effects, decreased ATP concentration (11), genetic susceptibility (45), and so on can all contribute to a lowered migraine threshold. It is impossible to link each of these factors to, for example, the attack frequency. One predisposing factor is not generalizable to the whole population of migraine patients, which is an additional argument why the absence of haemodynamic refractory effects in migraineurs is unsuitable as biomarker for migraine.

Finally, it is impossible to determine the exact link between the two abovementioned signal types (vascular vs. neuronal) when data from simultaneous measurements are missing. Habituation to repetitive stimulation has been investigated in the face recognition region using intracranial event-related potentials (with, for example, significant habituation of the face-specific P350) in patients with medically intractable epilepsy (46). However, habituation in face perception areas in healthy controls and patients with migraine has not been investigated directly, because of the invasive

character of the intracranial recordings. Therefore, we do not wish to state conclusively that the lack of haemodynamic refractory effects in patients caused by a face recognition task also means that they really show a lack of habituation during that task. A subsequent, interesting step in this research would be applying a paradigm to measure combined EEG-fMRI, to define a more precise relationship between the electrophysiological and the haemodynamic observations. From a practical point of view, a basic visual paradigm with MR-compatible surface electrodes is the most realistic experimental set up.

Besides combined EEG-fMRI or simultaneous BOLD, CBV and CBF measurements, it would also be interesting to try to link our findings to the genotype of the participants. Recently, a genome-wide association study identified a minor allele on chromosome 8q22.1 (rs1835740) to be associated with migraine (45). One of the strengths of this study was the selection of a well-defined patient group (only patients with MwoA, no prophylactic treatment, 2–8 attacks per month, 48 h attack free before scan session, and so on). However, genotyping might reveal additional divergences or uncover a sub-group in the patient population, in line with a proposal by Schoenen et al. (47) and a recent ^{31}P-MRS study (11).

Irrespective of the origin of the observations in the present study, one implication is worth mentioning. fMRI is widely used to investigate cognitive functions and physiological brain processes in healthy volunteers, and several exclusion criteria are therefore applied, such as MRI incompatible devices and neurological conditions such as stroke and epilepsy. However, migraine is hardly ever mentioned. Our findings suggest that studies with event-related fMRI paradigms using stimuli with short ISIs should add migraine (without aura) to the exclusion criteria for candidate participants, because including data from these patients may considerably influence the results of these studies.

In summary, this work is the first fMRI study that has measured BOLD haemodynamics interictally in patients with MwoA. The net HRFs of healthy controls have nonlinear properties for very short ISIs, but these refractory effects are not observed in patients with MwoA. Conclusive statements about the origin of the absence of refractory effects are difficult to make without additional, coupled measurements.

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