Heterogenous Migraine Aura Symptoms Correlate with Visual Cortex Functional Magnetic Resonance Imaging Responses

Nanna Arngrim, MD,[1]* Anders Hougaard, MD, PhD,[1]* Khazar Ahmadi, Msc,[2]* Mark Bitsch Vestergaard, PhD,[3]* Henrik Winther Schytz, MD, PhD, DMsc,[1] Faisal Mohammad Amin, MD, PhD,[1] Henrik Bo Wiberg Larsson, MD, DMsc,[3] Jes Olesen, MD, DMsc,[1] Michael B. Hoffmann, PhD,[2,4]* and Messoud Ashina, MD, PhD, DMsc[1]*

Objective: Migraine aura is sparsely studied due to the highly challenging task of capturing patients during aura. Cortical spreading depression (CSD) is likely the underlying phenomenon of aura. The possible correlation between the multifaceted phenomenology of aura symptoms and the effects of CSD on the brain has not been ascertained.
Methods: Five migraine patients were studied during various forms of aura symptoms induced by hypoxia, sham hypoxia, or physical exercise with concurrent photostimulation. The blood oxygenation level–dependent (BOLD) functional magnetic resonance imaging (fMRI) signal response to visual stimulation was measured in retinotopic mapping–defined visual cortex areas V1 to V4.
Results: We found reduced BOLD response in patients reporting scotoma and increased response in patients who only experienced positive symptoms. Furthermore, patients with bilateral visual symptoms had corresponding bihemispherical changes in BOLD response.
Interpretation: These findings suggest that different aura symptoms reflect different types of cerebral dysfunction, which correspond to specific changes in BOLD signal reactivity. Furthermore, we provide evidence of bilateral CSD recorded by fMRI during bilateral aura symptoms.

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The classical presentation of migraine aura is the scintillating scotoma-a gradually developing visual field defect bordered by a glittering serrated arc.[1] However, the clinical features of migraine aura are highly variable between patients[2] and between attacks within the same patient.[3] Although visual symptoms are the most common aura manifestation, occurring in 99% of aura episodes,[4] these are often not archetypical scotomas, but rather, for example, wavy lines, small bright dots, or white flashes of light.[2,5] A probable underlying cause of aura is the electrophysiological phenomenon of cortical spreading depression (CSD), which is characterized by a spreading wave of intense gray matter depolarization followed by suppression of neuronal activity.[6] Direct evidence of CSD during aura in patients is still lacking, but previous studies have shown changes in regional cerebral

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Address correspondence to Dr Ashina, Danish Headache Center and Department of Neurology, Rigshospitalet Glostrup, Faculty of Health and Medical Sciences, University of Copenhagen, Nordre Ringvej 57, DK-2600 Glostrup, Denmark. E-mail: ashina@dadlnet.dk

*N.A. and A.H. contributed equally. K.A. and M.B.V. contributed equally. M.A. and M.B.H. contributed equally.
From the [1]Danish Headache Center and Department of Neurology, Rigshospitalet Glostrup, Faculty of Health and Medical Sciences, University of Copenhagen, Glostrup, Denmark; [2]Visual Processing Laboratory, Ophthalmic Department, Otto von Guericke University, Magdeburg, Germany; [3]Functional Imaging Unit, Department of Clinical Physiology, Nuclear Medicine and PET Rigshospitalet Glostrup, Faculty of Health and Medical Sciences, University of Copenhagen, Glostrup, Denmark; and [4]Center for Behavioral Brain Sciences, Magdeburg, Germany

blood flow corresponding to those seen in animals during CSD using planar xenon,[7] single photon emission computed tomography (SPECT),[8] and positron emission tomography (PET).[9] Detection of CSD waves by blood oxygenation level–dependent (BOLD) functional magnetic resonance imaging (fMRI) has been validated in vivo in the feline brain.[10,11] One study using BOLD fMRI reported signal changes with several characteristics of CSD during a typical scintillating scotoma in a migraine patient provoked by physical activity.[12] Similar fMRI studies during migraine aura has not been performed, probably because migraine aura is notoriously difficult to study due to the unpredictable and short-lasting nature of attacks, and because no efficient and consistent method for experimental aura provocation has been developed.[13] In the present study, we used state of the art high-field BOLD fMRI during visual stimulation combined with retinotopic mapping to investigate migraine patients during various forms of aura symptoms induced by hypoxia, sham hypoxia, or physical exercise with concurrent photostimulation. We hypothesized that BOLD signal changes characteristic of CSD would occur during aura and that specific visual symptoms would correspond to different BOLD signal changes.

Patients and Methods

Participants

We studied 5 patients (1 male, 4 female, age = 18–46 years; Table) who suffered exclusively from migraine with visual aura with headache, fulfilling the International Classification of Headache Disorders 3 (ICHD-3) beta version criteria[14] with a minimum of 1 attack per month. The characteristics of the patient's usual migraine aura are shown in the Table. We used the following exclusion criteria for all patients: any other type of headache (except episodic tension type headache <5 days per month), any somatic or psychiatric disease, any daily medication apart from oral contraceptives, any smoking or history of mountaineering training (only applied to patients exposed to hypoxia/sham hypoxia). We recruited patients via the outpatient clinic at the Danish Headache Center (Rigshospitalet Glostrup, Copenhagen, Denmark) and via a Danish website for recruitment of volunteers to health research (www.forsoegsperson.dk).

TABLE . Characteristics of Usual Aura of Included Patients

Characteristic	Patient 1	Patient 2	Patient 3[a]	Patient 4	Patient 5
Age, yr	25	27	22	33	46
Years with migraine with aura	12	18	3	1	25
Attacks per month	1	2	1–2	1	1
Scotoma	Yes	Yes	Yes	No	Yes
Positive symptoms	No	Black/white flickering with zigzag border	Black/white flickering with zigzag border	White spots and flickering lines	Black/white flickering with zigzag border
Location in the visual field	Left	Central and peripherally left and right	Right	Right	Left or right
Gradual spreading (min)	Yes (10)	Yes (10)	Yes (30)	Yes (30)	Yes (5)
Total duration, min	20	60	60	60	30
Colors	No	No	No	No	No
Changes with closed eyes	No	No	No	No	No
Normal vision centrally	Yes	No	Yes	Yes	Yes
Onset of migraine headache in relation to aura	Following	Preceded or accompanied	Often preceded	Following	Following

[a]Usual aura was described as sensory symptoms followed/accompanied by visual symptoms. The sensory symptoms were described as first a throbbing feeling in the right hand, then gradually spreading numbness, prickling, and stinging sensation in the right arm.

The patients were part of 2 previously published studies.[15,16] The studies were approved by the Ethics Committee of the Capital Region of Denmark (H-4-2012-182, H-KA-20060083) and the Danish Data Protection Agency. The studies were registered at Clinicaltrials.gov (NCT01896167, NCT01388894). All patients provided their written informed consent to participate in the study after receiving detailed oral and written information about the study in accordance with the Declaration of Helsinki 2013 version.

Study Design

The patients participated on 2 study days (Fig 1A). On study day 1, they were MRI scanned at baseline and during visual aura. On study day 2, they were MRI scanned in the interictal phase for retinotopic mapping (see Fig 1A). The patients were part of 2 previously published studies. This study includes only a small fraction of patients from these studies (4/15 and 1/27 patients),[15,16] who happened to have an aura attack and could be scanned early enough. Aura was induced by either hypoxia

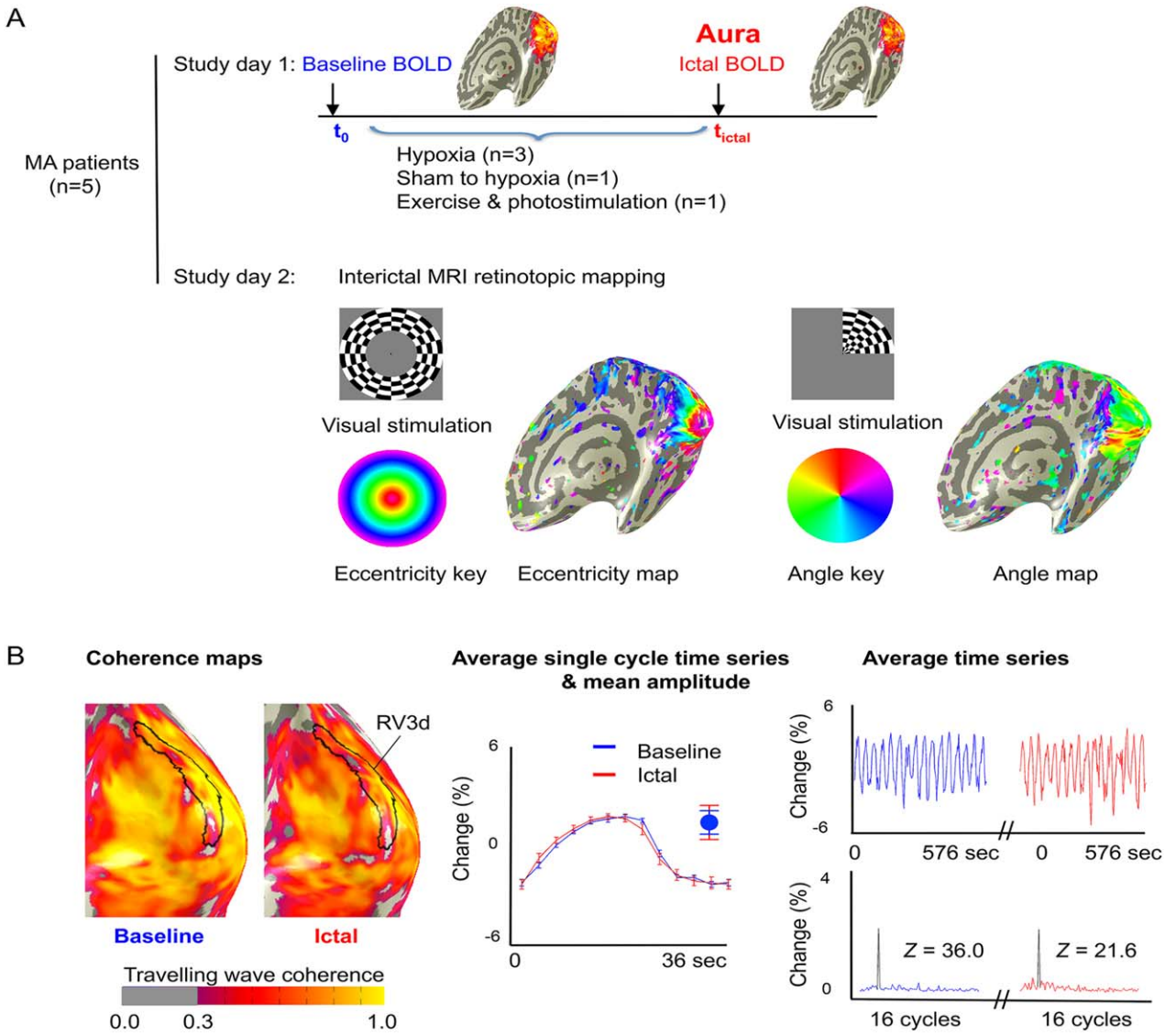

FIGURE 1: (A) Study design. Five migraine with aura (MA) patients participated on 2 study days. On study day 1, patients were scanned using blood oxygenation level–dependent (BOLD) functional magnetic resonance imaging (fMRI) with visual stimulation at baseline and during aura symptoms induced by either hypoxia, sham hypoxia, or exercise combined with photostimulation. Detailed descriptions of study day 1 for each patient are shown in Figure 2. On study day 2, patients were scanned in the interictal phase using BOLD fMRI with visual stimulation for retinotopic mapping of the early visual cortex areas V1 to V4. (B) Data analysis. To search for differences between baseline and ictal scans in cortical responses to visual stimulation measured by fMRI, the presented analysis of BOLD data was performed for V1 to V4 regions of interest (ROIs) in both hemispheres. Coherence maps were superimposed on inflated cortex (threshold = 0.3). Bold signal time series were evaluated as average single cycle time series, mean percentage amplitude changes (*round symbols* with error bars), and average time series. Finally, Fourier transforms were performed on the time series from each ROI, and resultant spectra with associated *z* scores for the stimulus frequency (16 cycles per scan) were shown. Data shown are from Patient 1, right visual cortex area V3 dorsal (RV3d) in the nonictal hemisphere.

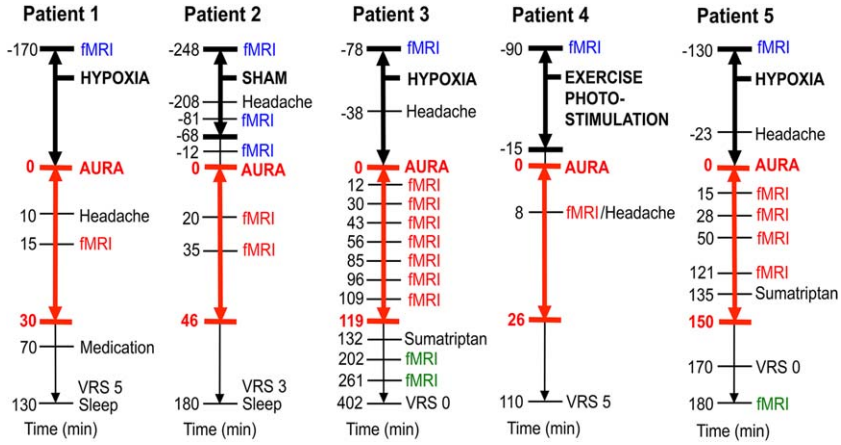

FIGURE 2: Experimental paradigm for study day 1 for Patients 1 through 5. fMRI = functional magnetic resonance imaging; VRS = verbal rating scale for headache intensity score (0–10).

(Patients 1, 3, and 5), sham hypoxia[15] (Patient 2), or physical exercise with concurrent photostimulation (Patient 4).[16]

HYPOXIA AND SHAM HYPOXIA. Patients 1, 2, 3, and 5, in a randomized double-blind, crossover design, were exposed to inhalation of 180 minutes (or until occurrence of aura symptoms) normobaric hypoxia resulting in a capillary oxygen saturation of 70 to 75% or atmospheric air (sham) on 2 separate days with a minimum of 7 days between.[15] Data from the experimental day (sham or hypoxia) where the patients did not develop aura were not included in this study, and aura fMRI data were not included in the previous publication.[15] Hypoxia and sham were induced by an AltiTrainer system (SMTEC, Nyon, Switzerland) through a 7m tube, a 1-way valve, and a tight fitting full-face mask (Hans Rudolph, Shawnee, KS). Capillary oxygen saturation (SpO_2) was measured continuously using a fingertip pulse oximeter (Veris Monitor, Medrad; Bayer HealthCare, Whippany, NJ).[15]

EXERCISE AND PHOTOSTIMULATION. Patient 4 was exposed to strenous physical exercise on an exercise bike for 1 hour, reaching a heart rate >80% of calculated maximum (220 beats per minute subtracted age) in combination with photostimulation (a 10,000-lux therapeutic lamp positioned 70 cm from the patient [15 minutes], a xenon arch flash photo stimulator flickering at 20 Hz [15 minutes], and a 1,500W stroboscope flickering at 20 Hz [15 minutes]) as described in detail in a previous publication.[16] These photostimuli were optimized for the provocation of migraine aura based on patients' reports of migraine trigger factors. The visual stimulation by checkerboard during fMRI scans was not expected to elicit migraine attacks.[16]

All patients were instructed to report immediately to the investigators if they developed any visual disturbances. When aura symptoms were verified by the investigator, the patients were immediately placed in the scanner and the experimental day was defined as study day 1 (as specified below). After study day 1, all patients participated on an additional interictal study day 2 (as specified below), where retinotopic mapping was performed (see Fig 1A).

STUDY DAY 1. A detailed description of study day 1 for each patient is given in Figure 2 and in the Results. Aura was induced by either hypoxia (n = 3), sham hypoxia (n = 1), or exercise combined with photostimulation (n = 1; see Fig 1A). Patients were scanned with BOLD fMRI with visual stimulation at baseline (t_0) and during aura (t_{ictal}). The ictal scan was initiated as immediately as possible after the onset of aura. Some variance of the onset of the ictal scan occurred (range = 8–20 minutes) due to different practical issues, for example, removal of hypoxia/sham mask, distance to MRI scanner, positioning of the patient in the MRI scanner, positioning of goggles for visual stimulation, and preparation of MRI scanner. At onset and in between scans, if possible, patients were briefly asked about aura location and characteristics. After the last scan, the patients were systematically interviewed about aura location and characteristics and asked to draw their aura symptoms and the temporal development, if possible. This implied a certain recall bias, as we have previously shown.[17] The typical visual migraine aura consists of positive visual symptoms in the form of flickering lines, dots, or flashing lights that gradually progress spatially in the visual field, often leaving behind a scotoma, that is, negative visual symptoms.[4] There is a large variability of aura symptoms between patients, and visual symptoms may be purely negative or positive.[2]

In hospital, patients were interviewed about occurrence of headache, headache intensity (recorded on a verbal rating scale for increasing headache intensity from 0 to 10), and characteristics every 20 minutes as previously described.[15,16] After discharge, the patients were instructed to complete a validated headache diary until sleep or 12 hours after baseline. The patients were allowed to treat headache with common analgesics and their usual migraine medication if needed according to the ethical approval of the study. As a consequence, 2 patients (Patients 3 and 5) were scanned before and after sumatriptan treatment (see Fig 2).

STUDY DAY 2. In the interictal phase (at least 5 days after an attack), patients were scanned with BOLD fMRI using the

traveling-wave method with visual stimulation (ring and wedge stimuli) for retinotopic mapping of the early visual cortex areas: $V1_{ventral}$ (V1v), $V1_{dorsal}$ (V1d), V2v, V2d, V3v, V3d, V4v′, and V4d′.

All patients arrived headache-free at the laboratory on each study day. Patients were not allowed to consume tea, coffee, alcohol, cocoa, or other methyl xanthine–containing foods or beverages for at least 12 hours prior to the start of the study days. Patients did not have migraine attacks within 5 days or any headache 48 hours prior to the study days. Female patients did not menstruate within 2 days before or after the study days.

MRI Procedures

MRI was performed on a 3.0T Philips Intera Achieva scanner (Philips Medical Systems, Best, the Netherlands) using a 32-element phased-array receive head coil.

ANATOMICAL SCAN. Anatomical images were acquired using a T1-weighted 3-dimensional turbo field-echo sequence (170 sagittal slices of 1mm thickness, in-plane resolution = 1 × 1mm, repetition time = 9.9 milliseconds, echo time = 4.6 milliseconds, flip angle 81°).

FUNCTIONAL MRI. Functional imaging was performed using a gradient-echo planar imaging sequence (32 slices of 4.0mm thickness, slice gap = 0.1mm, field of view = 230 × 230mm, in-plane acquired resolution = 2.9 × 2.9mm, repetition time- = 3.0 seconds, echo time = 35 milliseconds, flip angle = 90°, SENSE [SENSitivity Encoding] factor = 2). Dummy scans (2 volumes) were applied to ensure steady-state longitudinal magnetization. Visual stimulation was presented using OLED video goggles (NordicNeuroLab, Bergen, Norway; super video graphics array, 800 × 600 pixels, refresh rate = 85Hz, field of view = 30° horizontal, 23° vertical, stimulus luminance = 70–110cd/m^2). A fiber optic cable connected the system to a control computer outside the scanner room. The onset of visual stimulation was triggered by the scan acquisition. The patients were instructed to fixate on a central fixation point during the entire scan. The stimulus was generated using the freely available MATLAB-based VISTADISP toolbox (https://github.com/vistalab/vistasoft).

BLOCK-DESIGN VISUAL STIMULATION. Block-design visual stimulation was applied during baseline, ictal, and postictal fMRI scans. It consisted of an alternation of visual stimulation and rest blocks each comprising 18 seconds. A salient high-contrast motion stimulus was used to drive large expanses of visual cortex,[18,19] that is, a moving black and white dartboard pattern (diameter = 22° [circular aperture], ring width = 0.6°, spoke width = 15°; patterns in each spoke moved in opposite directions, alternately inward and outward, with random changes of the motion direction approximately every 2–3 seconds). An entire scan comprised thirty-two 18-second blocks and thereby lasted approximately 10 minutes (576 seconds); 1 or more (Patients 2, 3, and 5) scans were collected for baseline and ictal conditions (see Fig 2).

RETINOTOPIC MAPPING VISUAL STIMULATION. Standard retinotopic mapping was performed to localize the early visual areas V1 to V4.[20–22] Two repetitions of both polar angle and eccentricity mapping fMRI scans were obtained for each patient and were averaged for subsequent analysis (see below). Within a circular aperture of 11° radius, a high-contrast motion dartboard stimuli stepped either through the polar angles as a rotating wedge for polar angle mapping or through the eccentricities as an expanding ring for eccentricity mapping. The wedge was 6 segments and 90° wide, rotating counterclockwise around the center of the visual field. The ring comprised 3 checks, 0.6° wide. Both stimuli were repeated for 7 cycles, each lasting for 36 seconds, that is, a total duration of 252 seconds per scan.

Data Analysis

The T1-weighted anatomical image was segmented using the recon-all function in FreeSurfer (https://surfer.nmr.mgh.harvard.edu). Manual editing was performed to minimize the error in white and gray matter segmentation.[23] Afterward, the cortical surface was reconstructed based on segmented T1-weighted images and rendered as a smoothed inflated surface.[24] The MCFLIRT function of FSL (https://www.fmrib.ox.ac.uk/fsl) was used for motion correction of the fMRI data. Motion corrected data were then analyzed using mrVista (https://github.com/vistalab/vistasoft). After aligning the anatomical image to the functional scans, functional time series were high-pass filtered (cutoff = 36 seconds) to remove the linear trend. Time series for the same conditions of the retinotopic mapping session were averaged across repetitions for each patient to increase the signal-to-noise ratio. For each voxel, a Fourier analysis was applied to the time series to obtain the phase and amplitude values at the stimulus fundamental frequency ($f_0 = 7$).[25] The strength of the stimulus driven activity in each voxel during baseline and ictal scans was determined by calculating the coherence, defined as the Fourier amplitude of the BOLD signal at the fundamental stimulus frequency ($f = 16$/scan), divided by the sum of amplitudes of each Fourier component.[26,27] For retinotopic mapping, the phase values obtained correspond to the visual field regions represented by the voxels and were subsequently projected onto the cortical surface to visualize the eccentricity and polar angle maps, to subsequently delineate the early visual areas (V1, V2, V3, and V4).

We determined the cortical part of the early visual areas V1 to V4, which exhibited activity above a coherence threshold of 0.30, corresponding to an uncorrected probability value of 0.000012 (see Fig 1B).[28] V1 to V3 were subdivided into a dorsal and ventral part for a separate analysis of the lower and upper visual field. The resulting regions of interest (ROIs) were labeled accordingly (eg, LV1d for left dorsal V1, corresponding to the lower right quadrant of the visual field). It must be noted that although-for consistency-the parts of V4 corresponding to the upper and lower visual field were also termed V4v′ and V4d′, respectively, this does not correspond to the anatomical locations of these V4 ROIs. To identify differences between baseline and ictal scans in cortical responses to visual stimulation measured with fMRI, BOLD signal time series in ventral and dorsal V1 to V4 in both hemispheres

were evaluated as (1) the mean main stimulus parameter estimates (ie, beta values), expressed as percentage changes from baseline periods to visual stimulation periods; (2) average single cycle time series; (3) average time series; and (4) Fourier transforms from each ROI's time series and resultant frequency spectra with associated z scores (stimulus frequency = 16 cycles per scan; see Fig 1B).

Statistics

We tested the amplitude of the power spectra peaks against each other (baseline vs aura scan) with variance estimated from the rest of the spectrum using an independent test (n = 16 cycles). We used MATLAB version 2014a for all statistical analysis. Data were tested with Bonferroni correction for multiple comparisons, that is, a significance level of $p < 0.0016$ was accepted. For patients with > 2 scans, we tested baseline versus the first aura scan. For Patient 2, we used the last of the baseline scans (scan 3) as reference.

Results

Five migraine with aura patients were MRI scanned at baseline and during visual aura on study day 1 (see Fig 2) and MRI scanned on study day 2 in the interictal phase for retinotopic mapping (see Fig 1A). Patients 1 and 2 experienced a visual scotoma and flickering, and a decrease of BOLD response was observed (Fig 3, rows 1 and 2). Patient 3 experienced a cluster of black and white spots and flickering, and similar to Patient 1 and 2, a decrease of BOLD response during aura was observed (see Fig 3, row 3). In contrast to the other patients, in Patient 4 and 5, who only experienced positive symptoms, an increase of BOLD response was observed during aura (see Fig 3, rows 4 and 5). All aura attacks fulfilled the migraine with aura criteria according to the International Classification of Headache Disorders (ICHD)-3 beta[14] and were accompanied or followed by headache, fulfilling the migraine without aura criteria.[14]

Coherence maps, BOLD signal plotted as functions of time and frequency spectra for each patient, are shown in Figures 4 to 7.

Patient 1

After exposure to 170 minutes of hypoxia (mean SpO_2 = 71.1%, standard deviation [SD] = 2.8%), the patient developed visual aura (see Fig 4). Hypoxia was discontinued, and the patient was scanned 15 minutes after onset of aura. The aura began with a scotoma (a semicircle central in the right hemifield) that spread gradually over 15 minutes to the upper right quadrant and disappeared after 30 minutes. The scotoma had a flickering black–white zigzag border. There were no changes with eyes closed. During the ictal scan the patient experienced both scotoma and flickering, and 10 minutes after onset of aura the patient developed headache.

The mean amplitude of the BOLD response to visual stimulation showed decreased amplitudes during aura in the left visual cortex in dorsal and ventral V1 to V4 (see Fig 3, row 1; Fig 4). These changes matched the location of the visual symptoms in the right visual field. The difference was most pronounced in V2 to V4. In the right visual cortex, there was no ictal decrease in amplitude.

Patient 2

The patient developed visual aura 1 hour after exposure to 3 hours of inhalation of sham hypoxia (atmospheric air; see Fig 6A). Due to MRI preparation procedures (see Patients and Methods), the patient was scanned 20 and 35 minutes after onset of aura. The aura began with a central expanding scotoma with a flickering black–white zigzag border. The flickering spread gradually from the center and lower right quadrant to upper right quadrant and upper left quadrant. There were no changes with eyes closed. The aura disappeared after 46 minutes. During both ictal scans, the patient experienced both scotoma and flickering.

The mean amplitude of the BOLD response to visual stimulation showed decreased amplitudes during aura in the left ventral ROIs and right dorsal and ventral ROIs (see Fig 3, row 2; Fig 6A). There were no differences between baseline and ictal scans in the dorsal ROIs in left visual cortex (ie, right lower visual field quadrant). The affection of both hemispheres corresponds with the central location of scotoma, whereas the patient did not have flickering in the left lower visual field quadrant.

Patient 3

After 78 minutes of hypoxia (mean SpO_2 = 73.0%, SD = 1.7%), the patient simultanously developed visual and sensory aura and subjective slurred voice (which could not be confirmed objectively by the investigators; see Fig 7). Hypoxia was discontinued and the patient was scanned 12 minutes after onset of the visual aura. The durations of the visual and sensory aura symptoms were 119 minutes and 40 minutes, respectively. Afterward, the patient received sumatriptan (100 mg intramuscular [i.m.]) and was scanned again.

The visual symptoms were black and white spots and black/white flickering initially in the right upper quadrant, gradually spreading centrally to both upper quadrants. The black and white spots clustered into a zigzag figure. There was no change with closed eyes. The sensory aura was a gradually spreading numbness, prickling, stinging sensation in the right arm. The aura was preceeeded by a 20-minute throbbing feeling in the right hand.

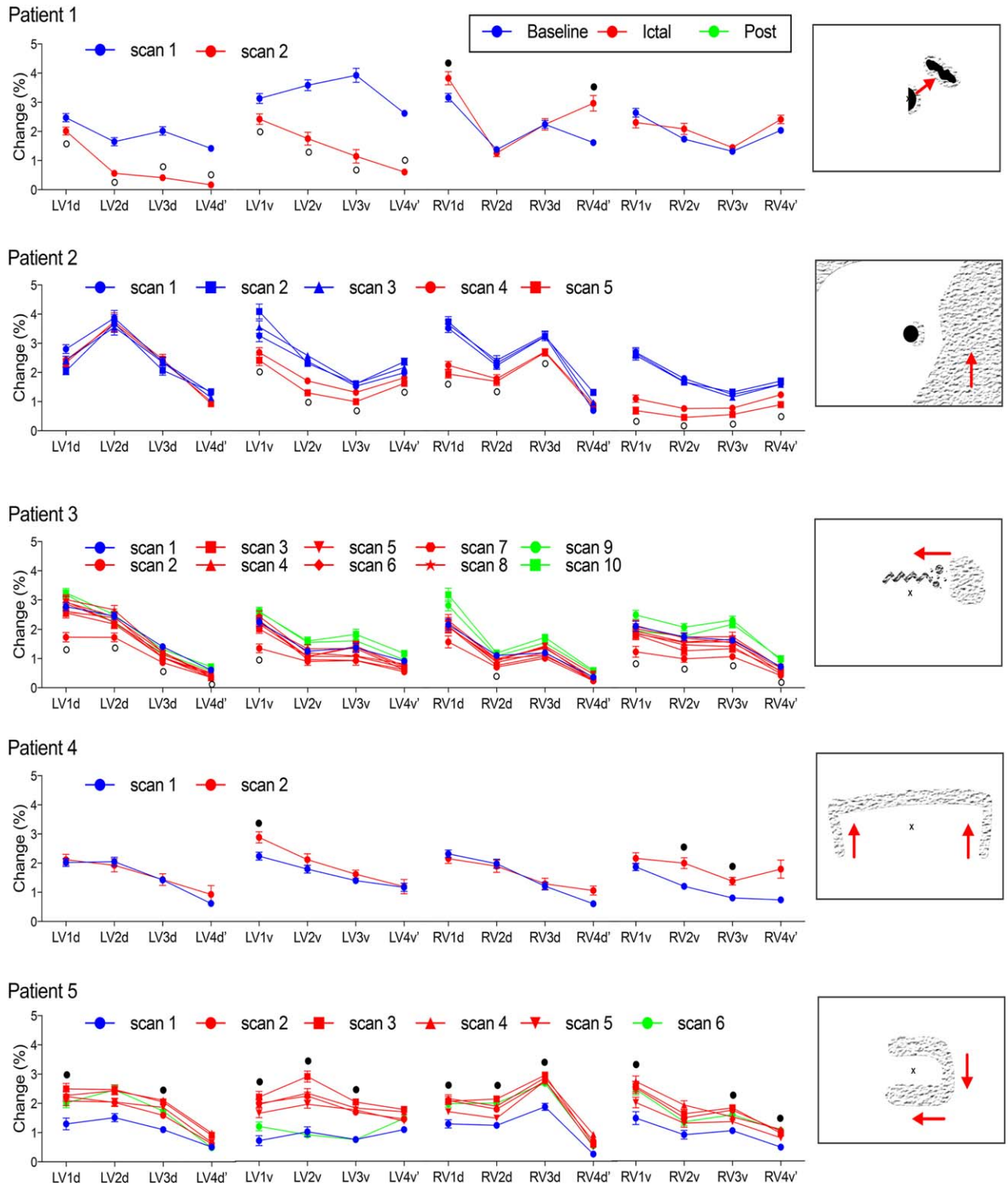

FIGURE 3: Blood oxygenation level–dependent (BOLD) response and aura drawings from study day 1 for Patients 1 through 5. Average percentage amplitude changes in BOLD response in the early visual cortex areas $V1_{ventral}$ (V1v), $V1_{dorsal}$ (V1d), V2v, V2d, V3v, V3d, V4v′, and V4d′ and a schematic drawing of the location of the patients' aura symptoms are shown. Arrows indicate direction of spreading of symptoms. Two patients had a visual scotoma and flickering (rows 1 and 2). One patient had a cluster of black and white spots and flickering (row 3). Two patients only experienced positive symptoms (Patient 4: white spots, flickering lines; Patient 5: flickering; rows 4–5). L = left, R = right. Probability values < 0.016 for comparison of baseline versus first aura scan are indicated with empty circles (decrease) and filled circles (increase). For Patient 2, the last of the baseline scans (scan 3) was used as reference.

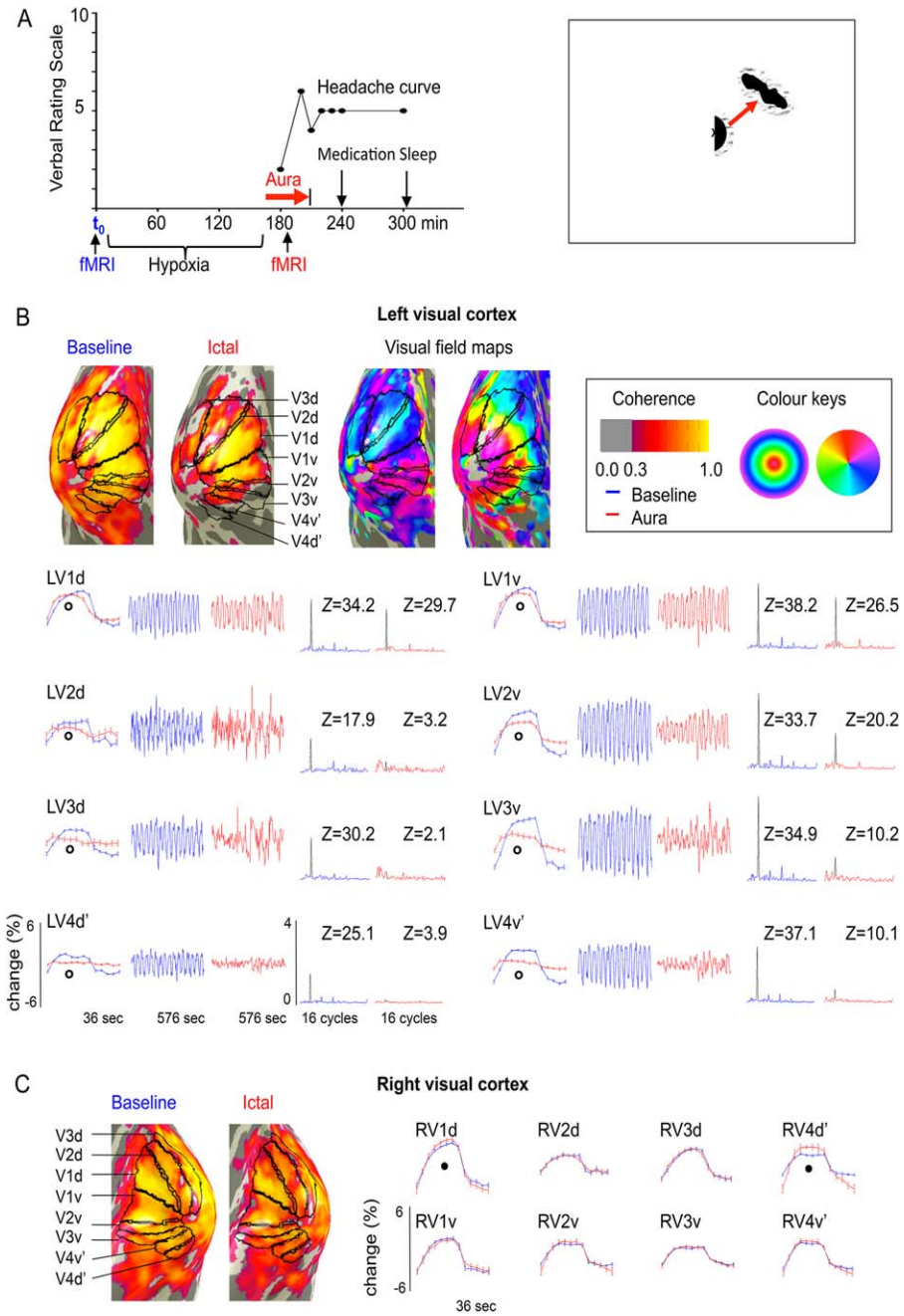

FIGURE 4: Patient 1. (A) Study day 1: experimental paradigm, headache curve, and schematic drawing of the location of aura symtoms (scotoma in black, flickering in pattern). Arrow in inset indicates direction of spreading of symptoms. After functional magnetic resonance imaging (fMRI), the patient received paracetamol (1g), ibuprofen (400mg), and primperan (20mg) at 240 minutes. Headache characteristics included left side, pressing, aggravation by movement, nausea, photophobia, no phonophobia, and mimicking his spontaneous migraine. (B) Left visual cortex. The coherence maps (threshold = 0.3) revealed a clear difference in response to stimuli during the ictal scan compared to baseline scan, with lower coherence. The blood oxygenation level–dependent signal plotted as a function of time (average single cycle time series and average time series) showed decreased amplitudes in left dorsal and ventral V2, V3, and V4′ (LV2d, LV2v, etc). Fourier analysis of the average time series showed no peak signal at the stimulation frequency (z scores < 4.0) in left dorsal V2 to V4. In ventral V2 to V4, the spectra showed peaks at the stimulation frequency with lower amplitude and z scores during ictal scan compared to baseline scan. Furthermore, the average time series plots of ventral V2 to V4 revealed a partial recovery of signal amplitude at the end of the scan. (C) Right visual cortex. In the right visual cortex, there were no differences between the baseline and ictal scan, except in the RV4d′, where the amplitude was higher during the ictal scan. Probability values < 0.016 for comparison of baseline versus aura scan are indicated with empty circles (decrease) and filled circles (increase).

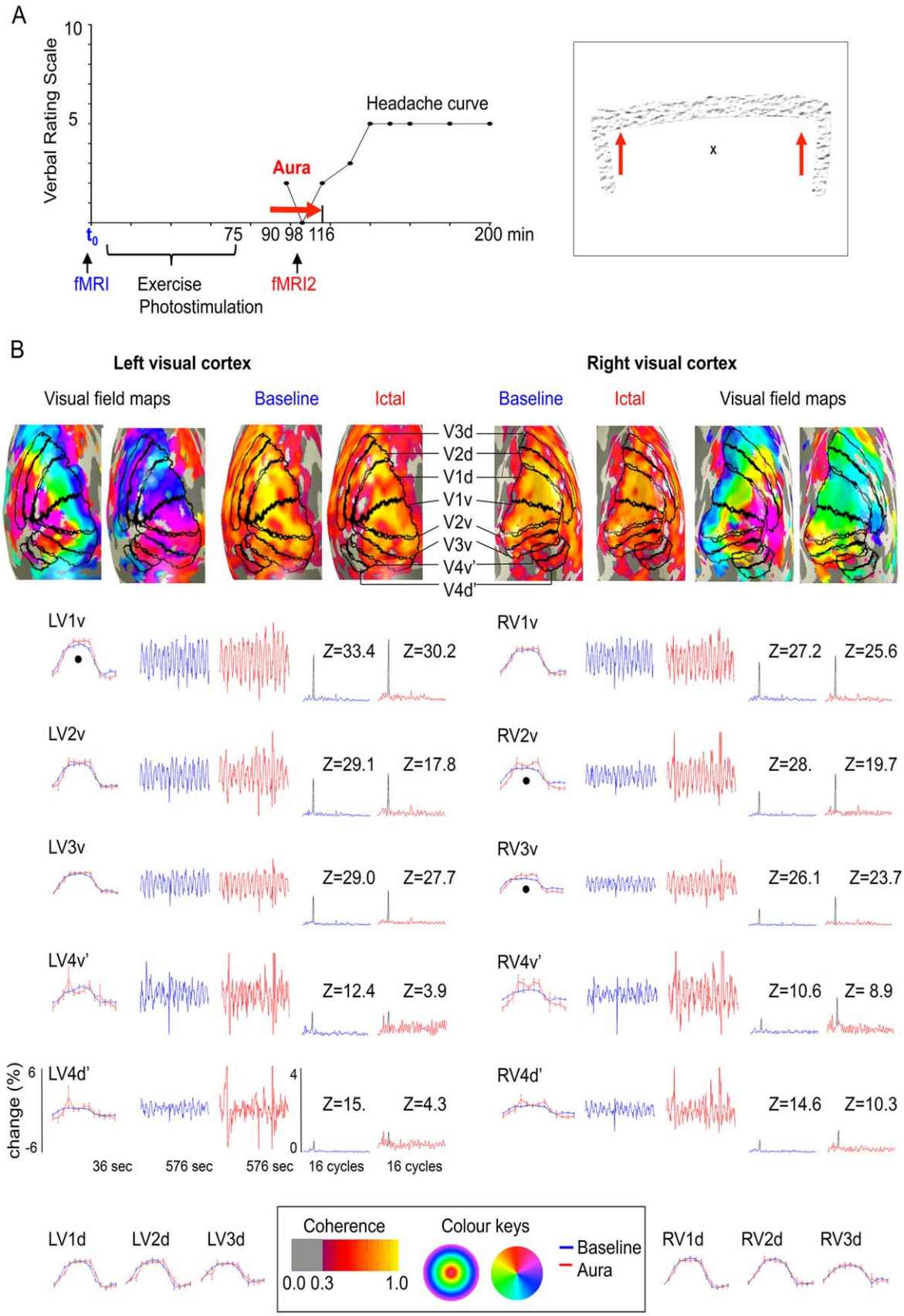

FIGURE 5: Patient 4. (A) Study day 1: experimental paradigm, headache curve, and schematic drawing of the location of aura symtoms (white spots and flickering lines in pattern). Arrows in inset show the overall direction of progression of the visual symptoms. (B) The coherence maps (threshold = 0.3) revealed lower coherence in dorsal and ventral V4′ in the left hemisphere (LV4d′ and LV4v′) during the ictal scan compared to baseline. The blood oxygenation level–dependent signal plotted as function of time (average single cycle time series and average time series) showed increased amplitudes in left ventral V1 to V2 and right ventral V2 to V4. In the dorsal regions of interest (ROIs), there were no differences in the average single cycle time series bilaterally. The frequency spectra showed low amplitudes at the stimulation frequency in left ventral V4′ (z score = 3.9) and left dorsal V4 (z score 4.3). The average time series plots revealed no changes in signal amplitude during the aura scan in any ROIs. Furthermore, the average time series revealed motion artifacts. Probability values < 0.016 for comparison of baseline versus aura scan are indicated with filled circles (increase). fMRI = functional magnetic resonance imaging.

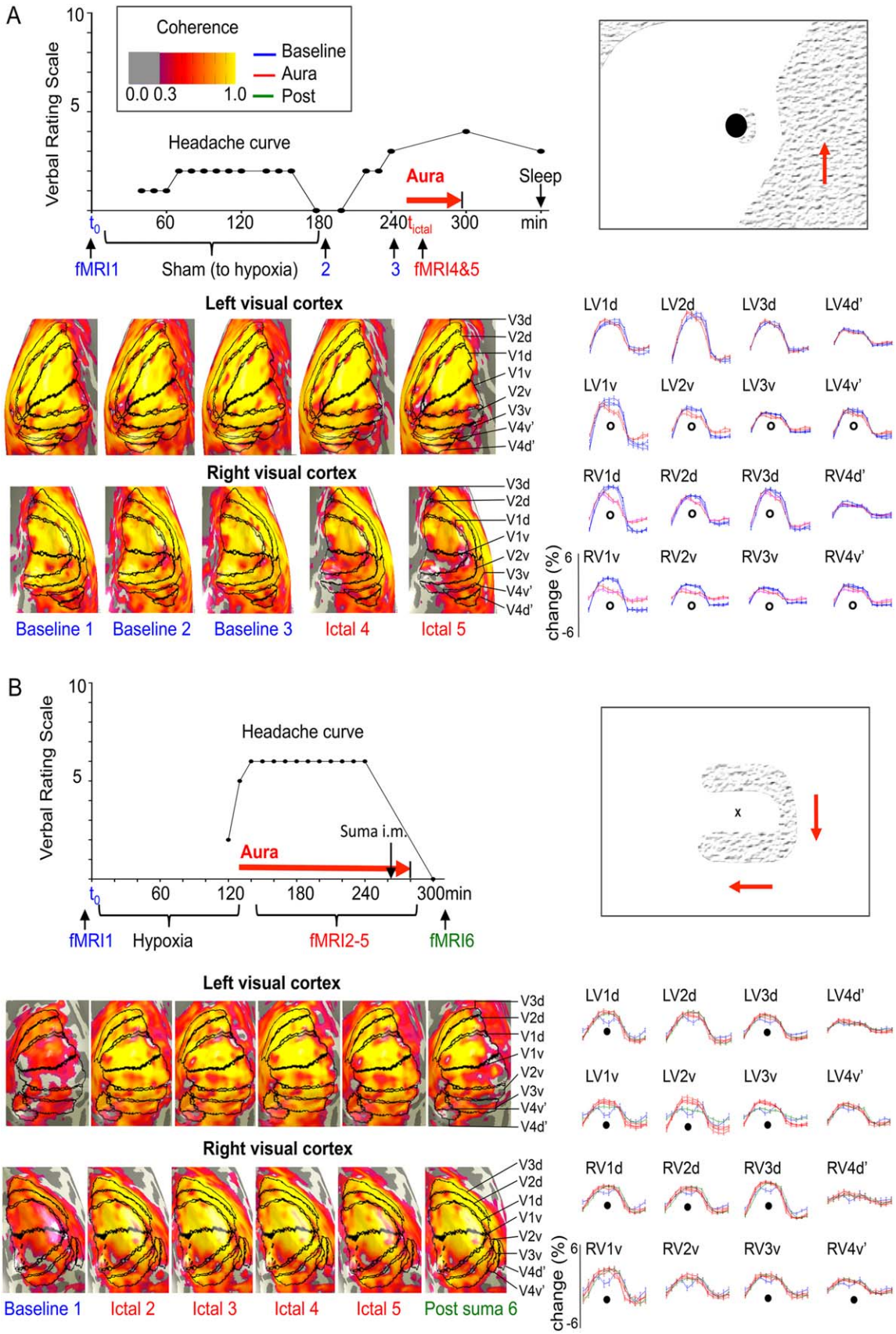

FIGURE 6.

The mean amplitude of the BOLD response to visual stimulation showed decreased amplitudes during aura corresponding to both hemifields, most pronounced in V1 in both sides and during the first ictal scan. There were no major differences between dorsal and ventral ROIs (see Fig 3, row 3; Fig 7). In contrast, the patient's visual symptoms were mainly reported as present in the upper visual field quadrants. After sumatriptan, the amplitude increased in the left (ventral) and right (dorsal and ventral) visual cortex.

Patient 4

The patient developed visual aura after 75 minutes of strenous exercise combined with photostimulation (see Fig 5A). The patient was scanned 8 minutes after onset of aura. The aura initiated with white spots and flickering lines peripherally in the left and right visual fields and spread gradually to the upper part of the visual fields. The aura disappeared after 26 minutes.

The mean amplitude of the BOLD response to visual stimulation showed increased amplitudes during aura bilaterally in ventral V1 to V3 (ie, upper visual field quadrants) and dorsal V4 ROIs, and no difference in ventral V1 and dorsal V1 to V3 bilaterally (see Figs 3, 5A). These changes match the visual symptoms, which were most pronounced in the upper quadrants.

Patient 5

The patient developed visual aura after exposure to 130 minutes of hypoxia (mean $SpO_2 = 75.0\%$, $SD = 4.7\%$; see Fig 6B). Hypoxia was discontinued and the patient was scanned 15 minutes after onset of aura. The duration of the aura symptoms was 150 minutes. Afterward, the patient received sumatriptan (100mg i.m.) and was scanned again.

The aura initiated as flickering in the upper quadrants that spread centrally and to the right and left lower quadrant, forming an open circle around the middle of the visual field.

The mean amplitude of the BOLD response to visual stimulation showed increased amplitudes during aura in all ROIs in left and right visual cortex. These changes match the widespread location of the visual symptoms (see Figs 3 and 6B). After sumatriptan administration, the amplitudes were still increased compared to baseline, except in left ventral V1 to V3.

Discussion

The important novel finding of the present study is the identification of specific patterns in clinically heterogenous visual aura symptoms and BOLD changes; the BOLD response was reduced in patients reporting scotoma and increased in patients who only experienced positive symptoms. Furthermore, patients with bilateral visual symptoms had corresponding bihemispherical changes in BOLD response. These findings suggest that specific aura symptoms reflect specific types of cerebral dysfunction, which can be detected by specific changes in BOLD response.

We investigated patients with multifaceted aura symptoms: positive and negative symptoms, only positive symptoms, and unilateral and bilateral aura. This clinical heterogeneity of aura symptoms among patients and within the same patient is well known and has been

FIGURE 6: Patient 2 and 5. (A) Patient 2, study day 1: experimental paradigm, headache curve, and schematic drawing of the location of aura symtoms (scotoma in black, flickering in pattern). Arrows in insets show the overall direction of progression of the visual symptoms. Headache characteristics included right side, throbbing/pressing, aggravation by movement, nausea, photophobia, phonophobia, and mimicking her spontaneous migraine. The coherence maps (threshold = 0.3) revealed lower coherence in response to stimuli during the ictal scan compared to baseline in the ventral regions of interest (ROIs) in left and right visual cortex (ie, upper visual field). The blood oxygenation level–dependent (BOLD) signal plotted as a function of time (average single cycle time series and average time series) showed decreased amplitudes in all ROIs except the left dorsal ROIs. The frequency spectra (plots not shown) showed lower amplitudes and *z* scores in all ROIs except the left dorsal ROIs (ie, right lower visual field quadrant). In right ventral V1 and V2 (RV1v, RV2v; ie, upper left visual field quadrant), the *z* scores were < 9 in the second ictal scan, and the average time series revealed a decrease in signal amplitude in these ROIs during the first ictal scan, which did not recover during the second ictal scan (plots not shown). (B) Patient 5, study day 1: experimental paradigm, headache curve, and schematic drawing of the location of aura symtoms (flickering in pattern). The arrows show the overall direction of progression of the visual symptoms. Headache characteristics included right side, throbbing, aggravation by movement, nausea, photophobia, no phonophobia, and mimicking her spontaneous migraine). The coherence maps (threshold = 0.3) revealed a clear difference in response to stimuli during the baseline scan compared to the ictal scans, with lower coherence in all ROIs during baseline. The BOLD signal plotted as a function of time (average single cycle time series and average time series) also showed lower amplitudes at baseline. Fourier analysis of the average time series generally showed lower peak signal amplitudes at the stimulation frequency at baseline with lower *z* scores compared to ictal scan and the scan after sumatriptan (plots not shown). The average time series plots (plots not shown) revealed no changes in signal amplitude during the aura scan in any ROIs. Probability values < 0.016 for comparison of baseline versus first aura scan are indicated with a empty circles (decrease) and filled circles (increase). fMRI = functional magnetic resonance imaging; i.m. = intramuscular; Suma = sumatriptan (100mg).

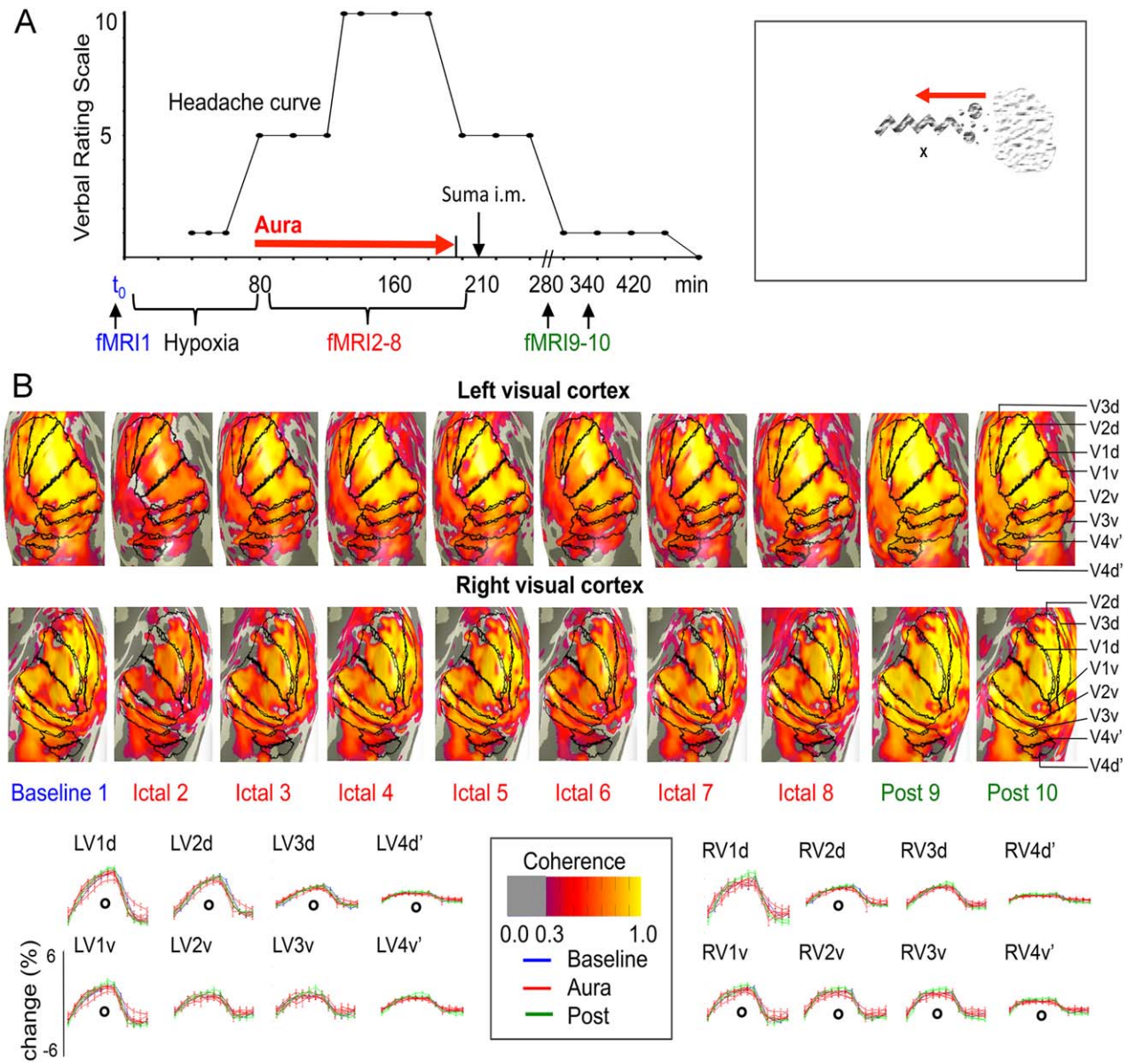

FIGURE 7: Patient 3. (A) Study day 1: experimental paradigm, headache curve, and schematic drawing of the location of aura symtoms (cluster of black and white spots and flickering in pattern). Arrow in inset indicates direction of spreading of symptoms. Headache characteristics included right side, throbbing/pressing, aggravation by movement, nausea, photophobia, no phonophobia, and mimicking her spontaneous migraine. (B) The coherence maps revealed lower coherence in response to stimuli during the ictal scan compared to baseline in all regions of interest (ROIs), most pronounced in the first ictal scan. The blood oxygenation level–dependent signal plotted as a function of time (average single cycle time series) showed lower amplitude, primarily in the first ictal scan in left dorsal and ventral V1 (LV1d, LV1v). The frequency spectra showed generally lower amplitudes and *z* scores during aura (plots not shown). There were no changes in signal amplitude during the aura scan in any ROIs (plots not shown). Probability values < 0.016 for comparison of baseline versus aura scan are indicated with empty circles (decrease). fMRI = functional magnetic resonance imaging; i.m. = intramuscular; Suma = sumatriptan (100mg).

confirmed in prospective studies.[2,29,30] However, although CSD is widely accepted as the underlying mechanism of migraine visual aura,[31] the correlation between the multifaceted phenomenology of aura symptoms and CSD has been sparsely investigated. Not all aura patients experience classic scintillating scotomas, and in some studies patients more commonly report positive symptoms like wavy lines, small bright dots, or white flashes of light.[2,17,30] In the present study, a reduced BOLD response was observed in Patients 1 and 2, who clearly experienced negative symptoms (scotomas) accompanied by 1 positive symptom (flickering), as well as Patient 3, who likely also experienced both negative ("black spots") and positive ("white spots," flickering)

symptoms. In contrast, Patients 4 and 5 experienced only positive symptoms (white spots and flickering lines, and flickering, respectively), which coincided with an increase of the BOLD response. These different BOLD responses during positive and negative symptoms may represent different effects of CSD on the neuronal activity or neurovascular coupling in the human brain. The only previous fMRI study conducted during migraine with aura[12] described 3 patients with visual aura symptoms and provided a detailed description of a classical unilateral scintillating scotoma in 1 patient. The authors reported a reduced BOLD fMRI response to visual stimulation during perception of aura scotomas. Interestingly, this study found an increase in mean BOLD signal (and a decrease of the BOLD response to visual stimulation) during perception of flickering that preceeded the aura scotoma in 1 patient, followed by a decrease in mean magnetic resonance signal and BOLD response corresponding to the perception of scotoma.[12]

The reduced BOLD response during scotomas is most likely caused by the depression of neural activity following CSD. During attacks of migraine aura, a decrease in perfusion (oligemia) has been shown, which most likely reflect the vascular effects of CSD.[32] Interestingly, studies during aura in humans have shown some variability of these perfusion changes, with some patients exhibiting initial hyperemia.[7,32] In addition, different vascular responses (hyperemia/oligemia) to electrophysiologically similar CSD events in patients with subarachnoid hemorrhage, stroke, and traumatic brain injury have been reported.[33] Interspecies variability of the spread of CSD and hemodynamic changes have also been reported.[33] In lissencephalic brains of rats, cats, and rabbits, CSD is followed by a massive hyperemia and later oligemia,[6] whereas in monkeys the propagation of CSD seems limited to a few centimeters and a focal and small hyperemia without post-CSD oligemia.[34] Different effects of CSD on the cerebral hemodynamics may explain the difference in BOLD response during positive and negative symptoms. Remarkably, the positive effect on the BOLD response seen during flickering in Patients 4 and 5 was not observed in Patients 1, 2, and 3, although they also experienced flickering. One explanation for this could be that the positive symptoms of the visual aura typically appear in the border of the scintillating scotoma, and they are believed to reflect the front of the depolarization wave during CSD, whereas the negative symptoms are more prominent and longer lasting, reflecting the electrically silent cortical areas observed in the wake of CSD. Thus, this preponderance of negative symptoms (reflecting silent neurons) would result in a net decrease of the BOLD response in the affected cortical visual areas. However, in the present study Patients 2 and 3 reported positive symptoms to be likely more prominent than scotomas (see Fig 3). An alternative explanation could therefore be that in the case of purely positive symptoms, neurons are hyperexcitable and quickly regain their resting potential following CSD-induced depolarization, thus producing a greater mean BOLD response, whereas in the "classical" case of scotoma following positive symptoms, neurons are unresponsive for several minutes (the duration of aura symptoms) following depolarization, resulting in a negative mean BOLD response, as observed in the present study for Patients 1 to 3. The 2 different types of BOLD signal response might therefore reflect 2 distinct types of neuronal reaction to CSD.

Prospective data showed that up to 35% of aura patients always experience bilateral visual aura.[30] Bilateral regional cerebral blood flow changes have been reported in single subjects using SPECT.[8,35] In the present study, only Patient 1 experienced unilateral aura with corresponding contralateral changes in BOLD response. The other patients experienced bilateral visual symptoms, and we here show corresponding bihemispherical changes in BOLD response. In Patient 4, the symptoms in right and left visual field showed similar temporal and spatial development of positive symptoms, suggesting at least 2 waves of CSD - 1 in each hemisphere. In the other patients, the visual symptoms were unevenly distributed in the left and right visual field and showed spreading over the midline. The mechanism of bilateral CSD is not understood. Propagation of CSD through the corpus callosum has been shown in rabbits.[36,37] This has not been verified in humans. However, pathophysiological propagation over the corpus callosum in humans is well known from epilepsy, where the corpus callosum is the major pathway for seizure generalization.[38] Another possible mechanism may be connectional diaschisis as described after stroke, where unilateral cortical lesions may lead to contralateral reduction of evoked potential, fMRI activation,[39] and regional cerebral blood flow.[40]

In some patients, we found changes in the BOLD response in areas of the visual cortex that did not fully correspond to the reported location of the visual symptoms in the visual field. This may represent recall difficulties in relation to aura symptoms. It is also possible that the patient is only aware of the most pronounced symptoms. However, it may also represent clinically "silent" propagation of CSD. This could also explain why a decrease of the BOLD response was observed in Patient 3, who experienced a cluster of black and white spots and flickering. Interestingly, in support of the concept of "silent" CSD propagation, it has been shown in

some patients that the hypoperfusion during aura spread well beyond the visual cortex, although only visual symptoms were reported.[32] Investigations of migraine patients without aura might help to shed light on this issue.

Methodological Considerations

Some methodological considerations should be mentioned. First, ideally the patients should be scanned continuously from immediately prior to the initiation of aura symptoms. However, this is almost impossible due to the unpredictable nature of aura attacks and the resistance to aura provocation.[13,16] This may explain why we were not able to show temporal changes of the BOLD response. Also, the drawings and description of the aura should ideally have been done during the auras. We have previously shown that this is preferable,[17] but immediate MRI scanning was of higher priority in the present study.

The only feasible way to study aura mechanisms in an experimental setting is to provoke attacks. We managed to MRI scan 5 migraine with aura patients from different migraine provocation studies during the aura phase of their migraine attack. It may be questioned whether a "true spontaneous" aura attack is the same as a provoked aura. However, all aura and migraine attacks are provoked by some factors (stress, light, etc).[41] Furthermore, the auras fulfilled the ICHD classification[14] of classical aura and according to the patients the aura mimicked their usual aura. In the present study, aura was induced by either hypoxia (Patients 1, 3, 5), sham hypoxia (Patient 2), or exercise combined with photo stimulation (Patient 4). Theoretically, the BOLD signal could be influenced by hypoxia. During hypoxia, the BOLD response to visual stimulation is reduced.[42] However, the patients were scanned after hypoxia, and published data from migraine with aura patients and gender- and aged-matched healthy controls, who were exposed to the same hypoxic challenge without developing aura, have shown that after determination of hypoxia the cerebral blood flow and the BOLD fMRI response to visual stimulation is normalized.[43] Furthermore, similar changes were observed in the patients who experienced scotomas after sham procedure/hypoxia with concurrent flickering after exercise and photostimulation/hypoxia.

Conclusions

We here demonstrate that the clinical heterogeneity of visual migraine aura symptoms corresponds to specific, equally heterogeneous physiological responses in the visual cortex, which are detectable by BOLD fMRI. Scotomas (ie, "negative" symptoms) and flickering (ie, "positive" symptoms) affect the BOLD response differently. Furthermore, we demonstrate bihemispheric BOLD changes during bilateral visual aura symptoms. These data indicate that the physiological changes that occur during CSD episodes vary markedly between individual patients. We suggest this may be caused by different effects of CSD on the brain activity, metabolism, and hemodynamics. Characterization of these different pathophysiological responses could potentially have prognostic and therapeutic implications. Future studies should investigate the variability of physiological changes within patients by provocation of several attacks, once such provocation becomes possible. Furthermore, it would be interesting to specifically study regional cerebral blood flow changes along with BOLD fMRI responses during aura using PET MRI.

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Author Contributions

All authors contributed to the conception and design of the study; N.A., A.H., K.A., M.B.V., and F.M.A. contributed to the acquisition and analysis of data; N.A., A.H., K.A., M.B.V., M.B.H., and M.A. contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

Nothing to report.

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