The onset of the migraine attack

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Part II.

Imaging aspects

Chapter 7.

Hypothalamic functional MRI activity in the initiation phase of spontaneous and glyceryl trinitrate-induced migraine attacks

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Abstract

Objectives

The hypothalamus has been suggested to be important in the initiation cascade of migraine attacks based on clinical and biochemical observations. The few imaging studies performed so far were all small, clinically heterogeneous, lacked non-migraine control groups and did not disentangle the changes due to the attack from those due the trigger compound. With a novel approach, we assessed hypothalamic neuronal activity in the early premonitory phase of glyceryl trinitrate (GTN)-induced and spontaneous migraine attacks.

Methods

We measured the hypothalamic blood oxygen level-dependent (BOLD) response to oral glucose ingestion with 3T functional MRI in 33 women, 19 with migraine without aura and 14 controls group-matched for age and body mass index on one day without prior GTN-administration, and on a second day, 90 minutes after GTN-administration to coincide with the premonitory phase of an induced attack. Interestingly, subgroups of patients with and without GTN-triggered attacks could be compared. Additionally, five migraineurs were investigated in a spontaneous premonitory phase. Linear mixed models were used to study between- and within-group effects.

Results

Without prior GTN-infusion, the BOLD-response to glucose was similar in migraine participants and controls ($p = 0.41$). After prior GTN-infusion, recovery occurred steeper and faster in migraine participants (versus day 1; $p < 0.0001$) and in those who developed an attack versus those who did not ($p < 0.0001$). Prior GTN-infusion did not alter the glucose-induced response in controls (versus baseline; $p = 0.71$). Just before spontaneous attacks, the BOLD-response recovery was also faster ($p < 0.0001$).

Conclusion

In this study, we found new and direct evidence of altered hypothalamic neuronal function in the immediate preclinical phase of both GTN-provoked and spontaneous migraine attacks.

Introduction

Migraine is a common, multiphasic, paroxysmal neurovascular brain disorder with recurring disabling attacks of headache, associated autonomic features and, in one third of patients, aura [1,2]. In up to 80% of patients, attacks may be preceded the 2-48 hours before by premonitory symptoms such as yawning, craving for specific food, fluid retention, tiredness, and mood changes [3-9]. When challenged with glyceryl trinitrate (GTN), most migraineurs (but not non-migraineurs), develop premonitory symptoms approximately 90 minutes after infusion and a migraine-like attack a few hours later [10].

The pathophysiology of migraine is complex. Although the underlying mechanisms for the aura, headache and associated symptoms are relatively well understood, how attacks begin is largely unknown [11]. The observation that attacks are usually preceded by premonitory symptoms strongly suggest that attacks actually begin well before the aura and headache phase. The episodic nature of attacks [12, 13], the clinical characteristics of premonitory symptoms [4], the observation that attacks may be triggered by acute changes in sleep pattern[14-18], and the finding that serum [19-24] and cerebrospinal fluid [21,22] levels of hypothalamic hormones are abnormal during [19, 21, 23, 24] and in-between [20, 22, 24] migraine attacks, all suggest a role for the hypothalamus in the initiation cascade of migraine attacks [11].

Studies using blood oxygen level-dependent functional MRI (BOLD fMRI) [25] or PET [26, 27] have indeed found hypothalamic cerebral blood flow (CBF) changes in GTN-induced [27] and a few spontaneous [25, 26] migraine attacks. These studies, however, were all small, clinically heterogeneous, or lacked non-migraine control groups. Moreover, the fMRI studies mainly investigated connectivity rather than hypothalamic function and no attempt was made to disentangle changes caused by GTN from changes due to the attack [27].

In the present study, we sought to better determine the role of the hypothalamus in the initiation of migraine attacks. As spontaneous migraine attacks are erratic, complicating studying the initiation phase of spontaneous attacks, we took advantage of the GTN migraine attack-provocation model [10]. We assessed and compared hypothalamic neuronal activity in women with (n=19) or without (n=14) migraine, with and without prior GTN infusion. Measurements with prior GTN infusion were done at 90 minutes after infusion to optimally coincide with possible premonitory symptoms. Five migraine patients could also be investigated during the premonitory symptoms phase of a spontaneous attack, enabling clinical validation of the findings in GTN-provoked attacks. Hypothalamic neuronal activity was measured as the fMRI BOLD response to glucose ingestion, which specifically activates glucose-sensitive neurons within the hypothalamus [28, 29]. The normal persisting drop in BOLD signal in response to glucose ingestion reflects reduced neuronal metabolic activity and is considered a signal of glucose satisfaction [28, 30]. The design we used enabled disentangling the effects due to the attack from those due to GTN.

Material and methods

Participants

We included 20 women with migraine without aura according to the ICHD-2 criteria [31] and 16 age- and body mass index (BMI) group-matched control women without a personal and 1st degree family history of migraine or any other regularly occurring headaches. Migraineurs also fulfilled the new ICHD-3 criteria [2]. All participants were recruited from the Leiden University Medical Centre Migraine Neuro Analysis (LUMINA) programme including individuals with migraine and non-headache controls from the Dutch population who all agreed to participate in migraine-related scientific research[32]. Participants with migraine were to have 1-6 migraine attacks and no more than 10 days of non-migraine headache per month. In addition they had to be free of migraine for at least 3 days before and 2 days after each study day, as was checked by a telephone call 7 days after each study day. Exclusion criteria for all participants included diabetes, premenstrual syndrome, hypertension, any psychiatric or neurologic disease, fever in the week prior and use of vasoactive, neuroactive or antibiotic medication in the two weeks prior to the measurement days.

Standard protocol approvals, registration, and patient consents

The study was approved by the local medical ethics committee and all subjects provided written informed consent prior to participation. The study was conducted according to the Declaration of Helsinki [33].

fMRI BOLD response to glucose ingestion

fMRI BOLD provides an indirect and non-invasive method to assess changes in neuronal activity in the brain by measuring changes in the BOLD signal. These changes occur due to changes in local concentrations of oxygenated and deoxygenated haemoglobin, local perfusion (blood flow and volume) and haematocrit, that result from changes in neuronal activity [34, 35]. Glucose-sensitive neurons within the lateral hypothalamus respond to glucose triggering after fastening. Physiologically, the hypothalamic BOLD response to oral glucose administration follows a typical pattern with an initial, relatively rapid and steep decrease of the signal becoming noticeable after about four minutes, and reaching its nadir after another four minutes. This is then followed by a slow recovery towards baseline levels over the next ten to twelve minutes [28, 29]. For glucose ingestion, a standard solution as used for the glucose tolerance test was made by mixing 300 mL tap water with 75 g glucose (Natufood, Natudis, Harderwijk, the Netherlands)[28].

GTN migraine provocation model

After cannulating an antecubital vein, GTN (0.5 micrograms·kg^{-1}·min^{-1}) is administered over 20 minutes with the study participant in supine position [10]. Immediately after infusion, all study participants (migraineurs and non-migraineurs alike) develop a brief, non-specific mild headache without associated features. In approximately 80% of migraineurs, but in none of non-migraineurs, this is followed, 3-6 hours later, by a migraine-like attack [10, 36-39]. In many migraineurs, GTN-provoked migraine-like attacks are preceded by premonitory symptoms, which typically start at around 90 minutes after GTN infusion [10, 36-39].

Study design

All participants were scanned on two separate days after overnight fasting and abstention of coffee, tea and alcohol; water intake was allowed. Prior to scanning all participants underwent a detailed standardised interview and full neurological examination. On the first (baseline) day, at around 9:00 am, this was followed by an fMRI scan which lasted for 21 minutes. About 7 minutes after beginning the fMRI scan, all participants ingested a standard glucose solution via a perioral tube, while remaining in supine position within the continuously recording MR scanner. On the second (= provocation) day, a 20 minute GTN infusion was started at 08:30 am and the post-GTN fMRI scan (with ingestion of glucose at 7 minutes after onset) was performed 90 minutes (mean ± SD: 91 ± 20) after start of the GTN infusion,. This timepoint was chosen to afford the highest likelihood of capturing possible premonitory symptoms of an ensuing GTN-provoked attack[10, 36-39].

Spontaneous attacks

Participants with migraine were also asked to come to the MRI as soon as they noticed an impending spontaneous migraine attack. They were then scanned during the premonitory phase of a spontaneous attack, using the same fMRI and glucose ingestion protocols.

Clinical parameters

We assessed premonitory symptoms, headache and migraine characteristics (according to the ICHD-2 [31] (also fulfilling the new ICHD-3 criteria [2]), and pain severity (numeric rating scale [NRS], ranging from 0 [no headache] to 10 [most severe headache possible]) before, every 5 minutes during, and every 30 minutes after GTN-infusion. Sociodemographic and clinical variables including migraine subtype, attack frequency and medication use, were recorded during a structured interview before the baseline study day

Data acquisition

MRI was performed on a 3.0 Tesla Achieva clinical scanner (Philips Healthcare, Best, the Netherlands) using a 32-channel phased array head coil. The same scan protocol was used for all MR sessions. It comprised of a whole brain high resolution 3D T1 sequence for imaging anatomical structures (TR 9.7 ms; TE 4.6 ms; flip angle 8°; FOV = 220x174x156 mm; 130 slices with a thickness of 1.2 mm and a voxel size of 0.86*0.86 mm), a structural hypothalamus scan (single slice scan, TR 550 ms, TE 10 ms, FOV = 208x208 mm, voxel size = 0.52x0.52x14 mm, scan time 1.14 min) and mid brain single slice fMRI scan (TR 120 ms, TE 30 ms, FOV 208x208 mm, voxel size = 0.81x0.81x14 mm, scan time 21.2 min, 500 dynamics). Anatomic images were screened for accidental findings by a neuroradiologist (MCK).

Data processing

Pre-processing and analysis of fMRI data was done using FSL version 5.03 [40]. Data was pre-processed as described in earlier studies [41]. Data were averaged for each set of 4 subsequent volumes, reducing the 500 dynamic scans to 125. The hypothalamus was segmented manually on the middle volume of the single slice MRI scan according to anatomical landmarks as previously described [41]. To correct for scanner drift, all hypothalamic BOLD values were corrected for the BOLD signal obtained in an internal reference ROI, drawn in grey matter, superior of the genu of the corpus callosum. To establish the post-ingestion

hypothalamic BOLD response to intervention, the mean pre-glucose signal (first 7 minutes of the 21 minute fMRI scan) was used for contrast. All data points (n=125) were divided by the mean baseline value and converted to percentages, yielding the percentage signal change relative to baseline, this percentage signal change was then averaged per minute.

Statistical analysis

General characteristics were compared using Mann-Whitney U tests for continuous variables, and Fisher exact tests for categorical data. Continuous data are presented as mean ± standard deviation, or as median with minimum-maximum. All fMRI results are reported as percentage BOLD change relative to the mean pre-glucose (reference) BOLD signal (0-7 minute pre-drinking). Data between minute 8 and 11 were omitted from the statistical analysis for artefacts in BOLD signal due to swallowing of the glucose solution. Data from minute 11 and up (11-21) were considered the post-glucose drinking BOLD response and were used for statistical analysis. Statistical analysis for comparison between groups (migraine; control), GTN (baseline; provocation) and/or migraine attack was performed by mixed model analysis as described earlier [41, 42]: group and GTN status were used as a fixed effect, time point as a variate and subject as a random factor. For within-group comparisons this model was applied to paired datasets.

Primary analysis was the difference in fMRI BOLD response to glucose at 90 minutes after GTN infusion versus the fMRI BOLD response to glucose without prior GTN infusion (i.e. baseline measurement) in participants with migraine, while focussing on those who developed an GTN-induced migraine-like attack. Secondary analyses included: i) baseline day differences between participants with migraine and controls; ii) effects of GTN on the fMRI BOLD response to glucose in participants and controls; iii) differences in fMRI BOLD response to glucose in GTN-induced versus spontaneous attacks; and iv) differences in post-GTN fMRI BOLD signal response to glucose in migraine participants in whom GTN did not provoke an attack versus controls . For further exploratory analyses, the fMRI BOLD response after glucose (average derived from 11 to 21 minutes post-glucose) was correlated with clinical migraine and demographic parameters using Pearson correlation coefficients. Uncorrected *p*-values of <0.05 were deemed significant for all tests. All statistical analyses were performed with the Statistical Package of Social Sciences (SPSS, version 23.0; SPSS, Chicago, III).

Data availability Statement

All data, methods and materials used to conduct this research are mentioned in this article.

Results

Participants

The study flow is depicted in Figure 1 (reasons for exclusion are given in the legends). Of the 20 migraine participants with a baseline scan two were excluded, leaving 18 participants eligible for the GTN scan. In two, post-GTN scans could not be performed. One post-GTN scan had to be excluded, leaving 15 post-GTN scans in migraine participants with also a baseline scan. Of the 16 migraine participants with a post-GTN scan, 13 (81%) developed

a migraine-like attack including the one of whom the scan had to be excluded due to movement artefacts. Paired data with both baseline and post-GTN scans were available for 15 migraine participants, 12 with and 3 without a provoked attack. In five migraine participants, scans could also be performed in the premonitory phase of a spontaneous attack. Of the 16 controls with a baseline scan, two were excluded and in four no post-GTN scan could be performed, leaving 11 controls with a post-GTN scan. Paired data with both baseline and post-GTN scan were available for 10 controls.

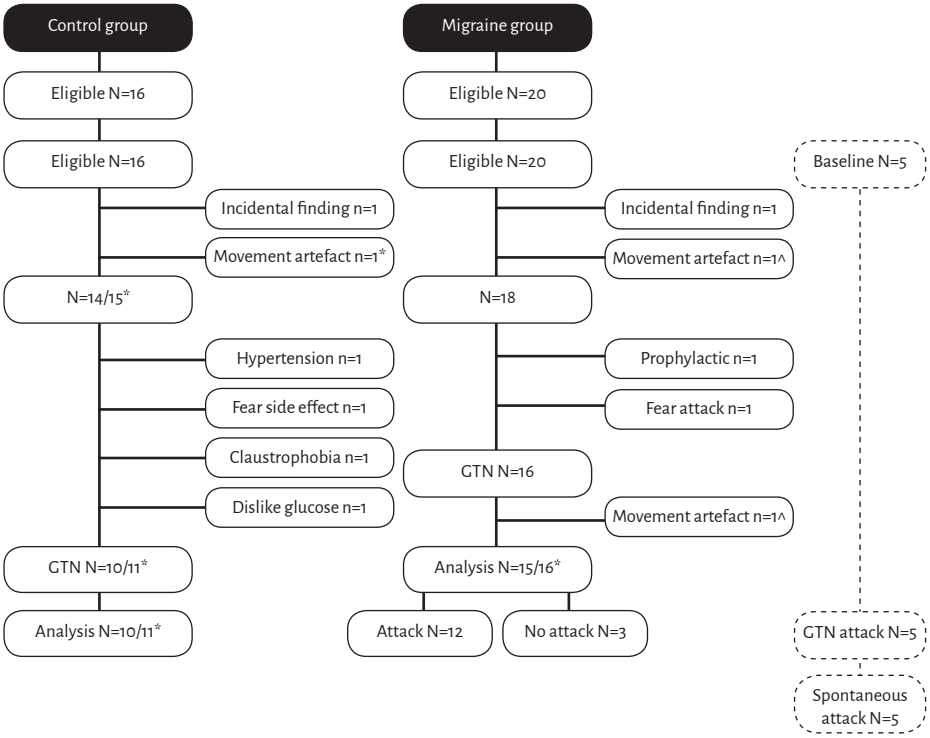

Fig. 1. Flowchart of study participants

Flowcharts depicting eligibility and exclusion of study participants at different stages of the study.
* = control participant in whom the baseline scan was excluded due to movement artefact, but GTN scan could be included in the unpaired analysis. ^ = migraine participant in whom both baseline and GTN scan were excluded due to movement artefacts.

Clinical and demographic characteristics

Clinical and demographic characteristics from all participants whose data were used in the analyses are summarized in Table 2. Demographic characteristics did not differ between the two groups. Participants with migraine had been free of migraine for 12.8 ± 8.7 (range 4-30) days before and >3 days after the attack-free measurement, and 10.3 ± 6.8 (range 4-30) days before and >3 days after the provocation day. There were no major baseline differences between the 13 migraine participants who developed a migraine attack after GTN and the 3 who did not.

Table 1. Description of clinical characteristics at baseline and after GTN-provocation in study participants.

Group	Age	Baseline	GTN-90	GTN effect	Headache onset delay (h:min)	Headache characteristics[a]	Headache intensity[c]	Associated symptoms[b]	Mimics usual migraine	Premonitory Symptoms
M	44	No HA	No HA	+	4:30	Left/throb/+	6	-/-/+/+	+	Yawning; nose feeling warm
M	40	No HA								
M	44	No HA								
M	46	No HA	No HA	-	n.a.	n.a.	n.a.	n.a.	n.a.	None
M	35	No HA	No HA	+	4:30	Right/throb/+	7	+/+/+/-	+	Yawning; nausea
M	50	No HA	No HA	+	5:20	Right/throb/+	9	+/+/+/+	+	Stiff neck; fatigue; problems concentrating
M	25	No HA	No HA	-	n.a.	n.a.	n.a.	n.a.	n.a.	None
M	50	No HA								
M	44	No HA	No HA	-	n.a.	n.a.	n.a.	n.a.	n.a.	None
M	48	No HA	No HA	+	3:35	Bil/pres/+	5	-/-/+/+	+	Stiff neck; osmophobia
M	50	No HA	Premonitory	+	2:50	Left/pres/+	8	+/+/+/+	+	Yawning; fatigue; feeling cold; polyuria
M	49	No HA	No HA	+	3:20	Right/pres/+	10	+/+/+/0	+	Fatigue; dry mouth
M	40	No HA	No HA	+	9:00	Right/throb/+	3	-/-/+/+	+	Feeling warm; fatigue; nausea
M	35	No HA	No HA	+	5:40	Left/pres/+	8	+/-/+/+	+	Craving; osmophobia; yawning; restless
M	51	No HA	No HA	+	6:20	Left/throb/-	5	+/-/+/-	+	Nausea; yawning;
M	28	No HA	Premonitory	+	3:40	Bil/pres/+	6	+/+/-/-	+	Fatigue; dry mouth; problems concentrating
M	32	No HA	No HA	+	4:10	Right/pres/+	6	+/+/+/+	+	Yawning; fatigue; heavy eyes; no appetite
M	24	No HA	No HA	+	7:40	Left/pres/+	6	+/-/+/-	+	Stiff neck; feeling cold
M	28	No HA	No HA	+	2:40	Bil/pres/+	9	+/-/+/+	+	Pressure feeling; light dizziness

table continues

Group	Age	Baseline	GTN-90	GTN effect	Headache onset delay (h:min)	Headache characteristics[a]	Headache intensity[c]	Associated symptoms[b]	Mimics usual migraine	Premonitory Symptoms
C	27	No HA	No HA	-						
C	25	No HA	No HA	-						
C	40	No HA	No HA	-						
C	46	No HA	No HA	-						
C	50	No HA	No HA	-						
C	44	No HA	No HA	-						
C	39	No HA	No HA	-						
C	44	No HA	No HA	-						
C	30	No HA	No HA	-						
C	45	No HA	No HA	-						
C	43	No HA	No HA	-						
C	46	No HA	No HA	-						
C	28	No HA	No HA	-						
C	29	No HA	No HA	-						
C	25	No HA	No HA	-						

For assessing the effect migraine attack phases, measurements were individually labelled as *no headache*, premonitory and *headache*. 'Premonitory symptoms' indicate symptoms that were reported by migraine patients >30min after GTN infusion, and that were recognised as their usual premonitory symptoms. M = Migraine patient; C = healthy control; GTN-90 = 90min after GTN infusion; + = provoked migraine attack after GTN infusion; - = no migraine attack provoked; HA = headache

[a] = lateralisation (left / right / bil = bilateral) / quality (throb = throbbing; pres = pressing) / aggravation

[b] = nausea / vomiting / photophobia / phonophobia

[c] = Numeric rating scale (NRS) score for maximum headache severity from 0 (no pain at all) to 10 (worst pain ever)

Table 2. Baseline characteristics of study population.

Variable	Migraine without aura n=19			Controls n=15		*p*
Socio-demographic						
Age, y [a]	44		24-51	40	25-50	0.34
BMI, kg/m² [a]	23.8		20.1-26.2	23.1	20.5-27.2	0.85
Righthandedness, n (%) [b]	17		(89.5%)	14	(93.3%)	0.69
Migraine specific						
Attack frequency / month ^	2	±	1-6			
Age at onset, y "	18.5	±	7.9			
Disease duration, y "	21.3		11.2			
Attack severity, NRS ^	8		5-10			

[a] = Mann-Whitney U test, depicted as median and minimum-maximum; [b] = Fisher exact test; ^ = depicted as median and minimum-maximum; " = depicted as mean ± standard deviation; Y = years; BMI = Body Mass Index; NRS = numeric rating scale

Clinical effects of GTN-infusion

GTN-infusion caused an immediate transient mild non-specific headache in all participants with migraine (mean numeric rating scale score = 3.8 ± 2.4) and controls (2.8 ± 2.0; $p = 0.25$). A delayed migraine-like attack developed in 13/16 (81%) participants with migraine at a mean of 4:54 ± 2:06 hours after start of the GTN infusion versus in 0/12 controls (0%; $p = 0.007$). All participants who developed a migraine-like attack reported one or more premonitory symptoms from 60 ± 54 minutes (median 180; range 30-240 minutes) after onset of the GTN-infusion and from 135 ± 116 minutes (median 112; range 30-460 minutes) before the headache started (Table 1). In contrast, none of those who did not develop a migraine-like attack, reported any premonitory symptom.

BOLD response to glucose: with versus without prior GTN-infusion

Without prior GTN infusion, the BOLD response to glucose was prototypical (steep decrease followed by a slow recovery) as reported previously [28, 29] and similar in the migraine and control group (Fig 2; $p = 0.41$). After prior GTN infusion, the BOLD response remained the same in the control group ($p = 0.71$; Fig 3A), but was clearly changed in the migraine group, with a much faster and steeper recovery phase after prior GTN infusion compared to the response without prior GTN infusion (total migraine group; intra-individual comparison: $p < 0.0001$; Fig 3B). A post-hoc analysis revealed that this response (after GTN-infusion) differed between patients with and without provoked migraine attack (between-group comparison; $p < 0.0001$; Fig 4B). The fast recovery had only occurred in the 13 migraine participants who later developed a migraine attack (intra-individual comparison versus own baseline: $p < 0.0001$). In those three who did not get an attack the recovery, in contrast, was slower (intra-individual comparison versus own baseline; $p < 0.004$).

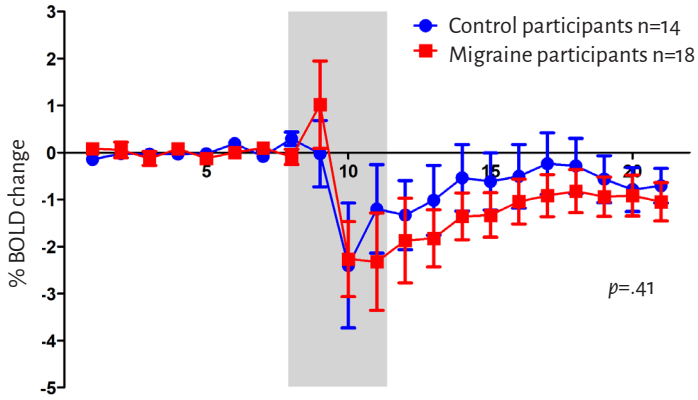

Fig. 2. Comparison of BOLD responses to glucose ingestion at baseline day in migraine and control participants

The BOLD response to glucose at a baseline day did not differ between migraine and control participants. Grey box indicates drinking period, of which data were omitted from the analysis since they are considered drinking artefacts. Error bars indicate 1 standard error.

Although the BOLD response to glucose after prior GTN infusion was visually different (faster and steeper recovery) in the total migraine group compared to controls, this difference did not reach statistical significance (between-group comparison: $p = 0.11$; Fig 3C), also not when only the 13 migraine participants who got an attack were included ($p = 0.12$).

Similar BOLD response in spontaneous and GTN-provoked attacks

Five migraine participants could also be studied in the premonitory phase of a spontaneous attack. In these patients the BOLD responses to glucose in spontaneous and GTN-provoked attacks were similar (intra-individual comparison; $p = 0.42$; Fig. 4C). Both responses differed visually from the attack-free measurement (Fig. 4D), but the response pattern in spontaneous attacks reached statistical significance at between-group level (versus baseline; $p < 0.0001$) but not at the intra-individual level likely due to small sample sizes ($p = 0.30$).

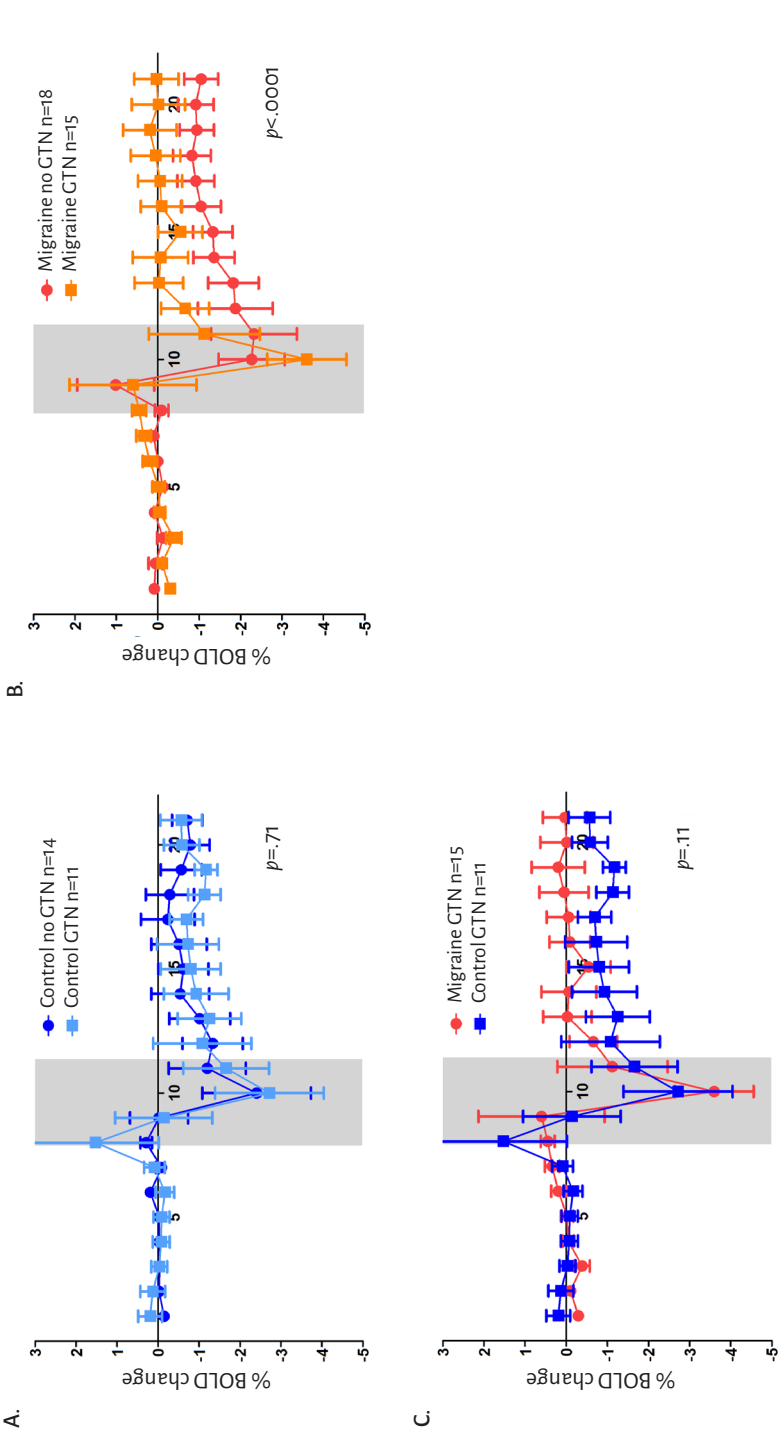

Fig. 3. Comparison of BOLD responses to glucose ingestion after GTN-provocation in migraine and control participants
The BOLD response to glucose after GTN did not differ from the BOLD response at baseline (without prior GTN-infusion) in controls (3A), but was higher in migraine participants (3B). The BOLD response to glucose after GTN is higher in migraine participants then in control participants, although not significant (3C). Grey box indicates drinking period, of which data were omitted from the analysis since they are considered drinking artefacts. Error bars indicate 1 standard error.

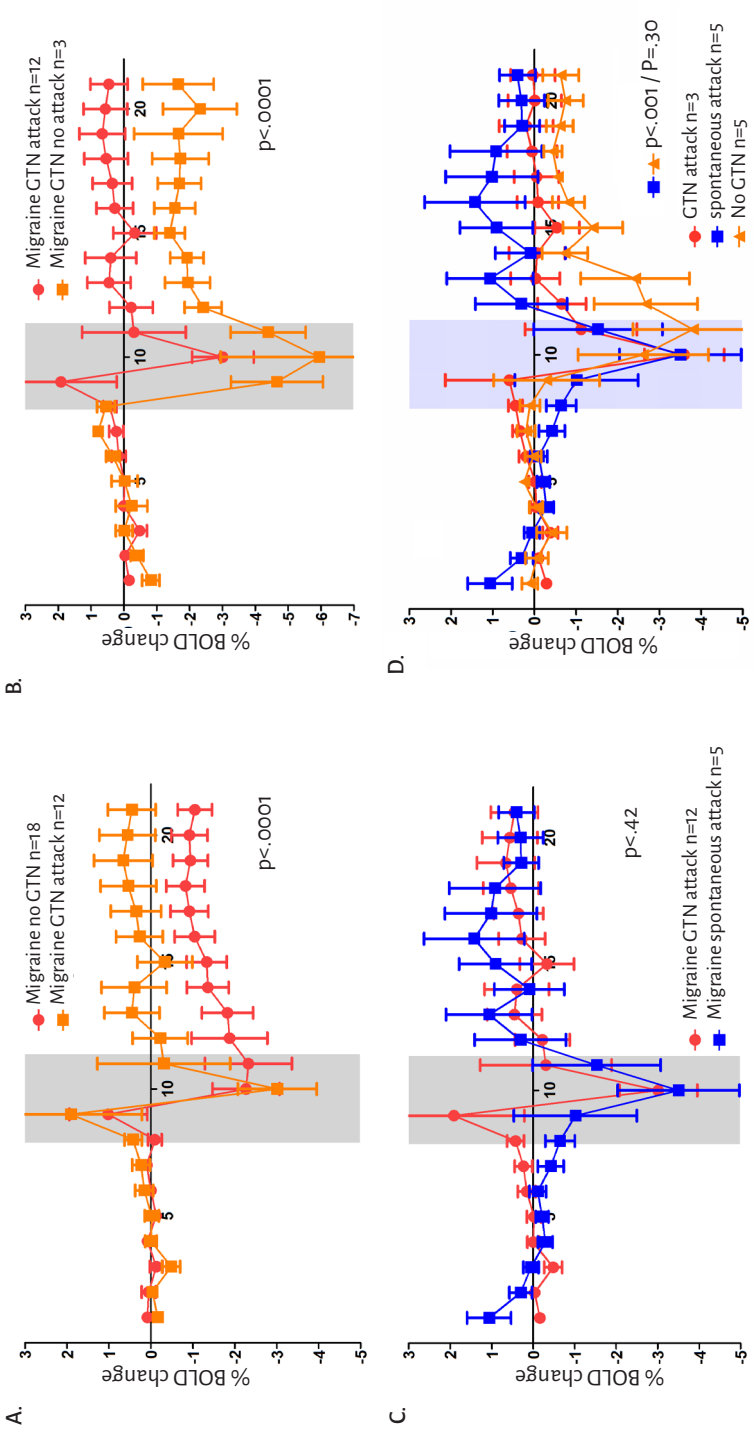

Fig 4. Comparison of BOLD responses to glucose ingestion in migraine participants in provoked and spontaneous attacks

In the pre-ictal phase of a GTN-induced migraine attack, the BOLD response to glucose is higher compared to the interictal response at baseline (paired analysis; 4A). The response pattern in spontaneous attack is similar to the pattern in GTN-induced attacks (4B). After GTN-infusion, this abnormal response >

(Continuation of fig 4 legend) pattern was only seen in the subgroup of migraine patients who developed a migraine-like attack, i.e. who were in the premonitory early phase of the attack. In the five migraine participants scanned during a spontaneous attack, the response pattern in the spontaneous attack was similar to that in a GTN-provoked attack (Fig 4C). Visually, the BOLD responses in both spontaneous attacks and GTN-provoked attacks differ from the response at baseline without GTN. The response pattern in spontaneous attacks reached statistical significance on between-group level ($p < 0.0001$) but not on intra-individual level due to small sample sizes ($p = 0.30$) (Fig. 4D). Grey box indicates drinking period, of which data were omitted from the analysis since they are considered drinking artefacts. Error bars indicate 1 standard error.

Discussion

To assess the role of the hypothalamus in the initiation of migraine attacks, we intra-individually compared hypothalamic neuronal activity in the premonitory symptom phase of 13 GTN-induced and 5 spontaneous migraine attacks with the activity outside an attack. Activity was also compared inter-individually with measurements with and without prior GTN infusion in 3 migraine participants and 11 controls who did not develop premonitory symptoms or an attack after GTN infusion. Hypothalamic neuronal activity was measured as the hypothalamic fMRI BOLD response to glucose ingestion, reflecting hypothalamic glucose-sensitive neuronal activity. Without prior GTN infusion, the hypothalamic BOLD response was similar in migraine participants and controls. However, while the response did not change after GTN infusion in controls, in the 13 migraine participants who developed a migraine attack, but not in the 3 who did not so, hypothalamic activity was different, with a faster and more abrupt recovery phase. Importantly, all hypothalamic responses and changes from baseline were similar in GTN-induced and spontaneous attacks, validating the findings in GTN-induced attacks as representative for what is occurring in spontaneous attacks.

In the early phases of provoked and spontaneous migraine attacks, migraine patients did not respond to glucose ingestion with a normal, persisting drop in the BOLD signal. Such a drop is considered to reflect reduced neuronal metabolic activity in the lateral hypothalamic area where glucosensitive neurons are located. This normal response is known across species[28] and is seen as a signal of glucose satisfaction, i.e. a normal 'satisfied' state after glucose ingestion[28,30]. The migraine patients rather showed an unresponsiveness to the glucose trigger during the early attack phase, implying a migraine-attack related disturbance of normal hypothalamic functioning; a disinhibited hypothalamic satisfaction. This abnormal response seems attack-specific, as it was not found in the control group, nor in the migraine group when there was no forthcoming attack. Although it is tempting to link this to the common premonitory symptom of craving, it would be oversimplifying to do so as the hypothalamic control of different homeostatic mechanisms is rather complex.

GTN influences cardiovascular parameters[43] and the changes we observed could theoretically have been due to GTN rather than related to the initiation cascade of an attack. However, one would then have expected similar changes to occur in the control

group. Moreover, we measured 90 minutes after GTN-infusion which significantly exceeds GTN $\tau_{1/2}$ (2.5-4 minutes).
Only two studies have previously investigated the role of the hypothalamus in the initiation phase of migraine attacks. The study by Maniyar et al showed activations in the posterolateral hypothalamus in 8 migraine with aura patients with premonitory symptoms after GTN infusion, using $H_2{}^{15}O$ PET cerebral blood flow as a marker for neuronal activity [27]. Although this suggests that the hypothalamus is pivotal in the early, premonitory phase of the migraine attack, a possible GTN effect cannot be excluded as there was no contemporaneous control group. Schulte et al [25] daily assessed the hypothalamic fMRI blood flow response to a trigeminal nociceptive stimulus (nasal administration of gaseous ammonia) for 30 consecutive days in a single migraine patient. They prospectively captured three migraine attacks and found an increased hypothalamic response in the 24 hours prior to onset of the migraine headache[25]. Although they did not include a control group to correct for possible diurnal, weekly or menstrual effects, the findings nonetheless suggest an important role for the hypothalamus in the early phases of the migraine attack. A third study found hypothalamic activation in migraine headache, but did not perform measurements during the premonitory phase. Collectively, these and our data suggest a pivotal role of the hypothalamus in the early phases of migraine attack initiation.

There is a growing interest in the hypothalamus as the site of initiation of a migraine attack initiator based on clinical and biochemical arguments [44-46]. In this study we have shown an increased hypothalamic BOLD response patterns to glucose after fasting in migraine patients. The ingestion of oral glucose induces a normal, transient silencing of the activity of glucosensitive neurons in the lateral hypothalamus. However, in the preictal phase of both spontaneous and GTN-triggered attacks this was followed by a much faster and steeper response than normally. The suppression of this hypothalamic state of hyperactivity was apparently temporarily and shorter than in non-migraine individuals and was suggestive of an impending migraine attack. We might draw an analogy between this 'overdrive' of 'craving' state of these neurons and the clinical symptom of craving experienced the hours before the migraine headache starts.

Our study has several strengths. The paired design in patients and controls enabled to disentangle the effects of GTN and the attack. Using a validated model to provoke premonitory symptoms and migraine-like attacks enabled us to capture the preclinical initiation phase of attacks [47] which is hardly possible for spontaneous attacks due to their erratic nature. Finally, we did manage to capture the presymptomatic phase of migraine attacks in 5 patients which allowed for a clinical validation of the findings in GTN provoked attacks. We included only female migraine without aura patients, limiting the overall generalizability. The result, however, was a homogeneous study group. Technically, the small region of interest made the data acquisition susceptible to e.g. drinking movement artefacts, leading to exclusion of three subjects from the analyses. We were not able to distinguish between the different hypothalamic nuclei. Possibly, different nuclei could be involved in different migraine attack phases, being hyperactive in one phase and hypoactive in the other. More sophisticated imaging techniques could perhaps be used in the future to disentangle these possible differential effects.

To conclude, this is the first study showing a disturbed function or reactivity of the hypothalamus during the earliest phases of both GTN-provoked and spontaneous migraine attacks. This emphasizes the role of the hypothalamus in the early phase of migraine attacks.

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