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Migraine and aura triggered by normobaric hypoxia

Florian Frank[1], Martin Faulhaber[2], Karl Messlinger[3], Chiara Accinelli[1], Marina Peball[1], Alois Schiefecker[1], Katharina Kaltseis[1], Martin Burtscher[2] and Gregor Broessner[1]

Abstract

Background: For future experimental studies or the development of targeted pharmaceutical agents, a deeper insight into the pathophysiology of migraine is of utmost interest. Reliable methods to trigger migraine attacks including aura are desirable to study this complex disease *in vivo*.

Methods: To investigate hypoxia as a trigger for migraine and aura, we exposed volunteers diagnosed with migraine, with (n = 16) and without aura (n = 14), to hypoxia utilizing a hypoxic chamber adjusted to a FiO_2 of 12.6%. The occurrence of headache, migraine, aura, and accompanying symptoms were registered and vital signs were collected for 6 hours under hypoxia and 2 hours of follow-up. A binary logistic regression analysis examined the probability of triggering headaches, migraines, aura, photo- and phonophobia.

Findings: Of 30 participants, 24 (80.0%) developed headaches and 19 (63.3%) migraine, five (16.7%) reported aura. Two patients that developed aura never experienced aura symptoms before in their life. The increase of mean heart frequency was higher in patients developing headaches or migraine. Mean SpO_2 during hypoxia was 83.39%.

Conclusion: Hypoxia was able to trigger migraine attacks and aura independently of any pharmacological agent.

Keywords

Migraine, headache, hypoxia, experimental, aura

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Abbreviations

CSD: cortical spreading depression; HAH: high-altitude headache; ICHD-III: international classification of headache disorders, 3rd edition; NHC: normobaric hypoxic chamber; FiO_2: inspiratory fraction of oxygen; $FiCO_2$: inspiratory fraction of carbon dioxide; SD: standard deviation; SpO_2: peripheral oxygen saturation; TRP: transient receptor potential; MAP: mean arterial pressure.

Background

After decades of modern research, the pathophysiology of migraine is still elusive. Common theories regarding the induction of migraines propose an involvement of brainstem and hypothalamic neurons with a subsequent activation of meningeal nociceptors and sensitization of trigeminovascular neurons (1). In addition to the resulting debilitating headache, about one third of patients with migraine experience migraine aura that is believed to originate from transient neuronal depolarizations spreading across the cortex followed by a depression of neuronal activity (cortical spreading depression, CSD) (2). The pathophysiological role of migraine aura in the context of a migraine attack is still unclear (3).

[1]Department of Neurology, Headache Outpatient Clinic, Medical University of Innsbruck, Innsbruck, Austria
[2]Department of Sport Science, Leopold-Franzens University of Innsbruck, Innsbruck, Austria
[3]Institute of Physiology and Pathophysiology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

Corresponding author:
Florian Frank, Department of Neurology, Medical University of Innsbruck, Anichstraße 35, 6020 Innsbruck, Austria.
Email: f.frank@i-med.ac.at

A common link to trigger both phenomena might be hypoxia as proposed by Amery as early as 1985 (4). Human research with the aim of experimentally inducing migraine attacks is essential to further study this complex disease, possible remedies, and the interactions involved in the generation of migraine attacks. There are several factors that support hypoxia as a possible means to trigger migraines and aura.

First, data from observational studies of people travelling in or inhabiting elevated regions demonstrated three times more migraine attacks in this population compared to a population at sea level (5,6). Additionally, high-altitude headache (HAH) was found to be associated with a history of migraine in two large prospective studies (7,8). In our previous work, we could show that exposing 77 healthy volunteers (i.e. without any history of primary headache) to normobaric hypoxia induces headaches in up to 81.1% and migraine-like headaches in up to 15%, suggesting a role of hypoxia in migraine pathophysiology (9).

Second, in the crossover, double dummy study of Schoonmann et al., hypoxia triggered migraine headache in 42% (n = 6) compared to nitroglycerin with 21% (n = 3) (6). Another hypoxia study by Arngrim et al. (10) provoked migraine attacks in eight out of 15 migraineurs and one out of 14 controls by 3-hour inspiration of nitrogen and oxygen. Three patients additionally reported typical aura symptoms during the tests.

Third, oxidative stress has been discussed as a common denominator underlying migraine triggers (11), as it is able to disrupt the biochemical integrity of the central nervous system and might contribute to neuronal dysfunction in the migraine brain (12).

Fourth, hypoxia has been shown to induce spreading depression, and the waves of transient neuronal depolarization cause and correlate with tissue hypoxia (2,13).

These findings corroborate the use of hypoxia as an experimental trigger of migraines with an emphasis on migraine aura.

To elucidate potential oxidative mechanisms in migraine it is necessary to develop human experimental models, which allow an analysis of the clinical appearance of migraines (14). Hypoxia might be such a model to induce oxidative processes *in vivo* that naturally occur in the migraine brain.

Methods

The main goal of this prospective, interventional, open study was to investigate hypoxia in a normobaric hypoxic chamber as a potent trigger of migraine and aura in migraineurs. Our aim is to establish a reliable experimental human model for further research on migraine pathophysiology and possible pharmacological treatments.

Population

We recruited 35 participants from our tertiary headache outpatient clinic and via invitation directed at students and employees of the medical university. A full-time neurologist with extensive experience in migraine and headache research screened all volunteers to establish eligibility and confirm the diagnosis of episodic migraine with and/or without aura according to the International Classification of Headache Disorders 3rd edition (ICHD-III) (15). Adult female and male patients were required to have a history of migraine for over 12 months and a migraine frequency of at least 1 day per month over the last 3 months prior to screening. To minimize heterogeneity in our population we decided to exclude volunteers that have been acclimatized to an altitude $\geq$2500 m and/or were overusing abortive migraine medication (i.e. corticosteroids, opiates, triptans, ergotamines, non-steroidal anti-inflammatory drugs). Furthermore, we excluded patients that were currently or recently (within 12 months prior to screening) using preventative migraine medication. All volunteers were required to be able to distinguish migraine from other headaches. For detailed inclusion and exclusion criteria please refer to Supplemental Table 1.

Experimental design

Calendar. Screened patients completed a daily headache diary online for 10 days prior to the experiment to identify a possible state of increased headache susceptibility or medication overuse. On the day prior to the examination, participants received a phone call to verify absence of headache and possible acclimatization to high altitude. Female patients were excluded from the study if they were pregnant or breastfeeding, and the examination was postponed if menstruation was reported or expected on the day of the experiment. The experiment was also postponed if the participant used abortive migraine medication within 24 hours before exposure to hypoxia.

Normobaric hypoxic chamber (NHC). The experiment was conducted in the normobaric hypoxic chamber located on the campus of the University of Innsbruck's Department of Sport Science (590 m). So far, more than 500 volunteers have been examined in this chamber under strict experimental conditions with no severe side-effects. The chamber has a dimension of about 5×3 m and the hypoxic generator was set at high flow to keep the inspiratory fraction of oxygen (FiO_2) constant and to avoid an excessive increase of

inspiratory fraction of carbon dioxide ($FiCO_2$) throughout the entire study. The NHC system, FiO_2 and $FiCO_2$ were continuously controlled by an external sensor unit (Multiwarn, Draeger, Lübeck, Germany) and provided normobaric hypoxia (i.e. 1015.56 ± 4.72 hPa ambient barometric pressure) with consistent temperature, humidity, and oxygen partial pressure for each participant. The NHC was adjusted to a 12.6% FiO_2, resembling a sojourn at 4500 meters. We selected this level of hypoxia to match the parameters of our previously published study examining healthy subjects under hypoxia and under the scope of risk consideration (13). All participants received a medical routine examination, peripheral venous catheterization, and completed the first of eight questionnaire sets before entering the NHC. Peripheral oxygen saturation (SpO_2) was measured using a pulse oximeter (Pulox PO-300; Contec Medical Systems Co. Ltd, China) on the patients' index finger after ensuring no coloured nail varnish had been applied. Female participants were tested for pregnancy using a beta-hCG (human chorionic gonadotropin) urine test kit prior to the experiment and menstrual cycle details were collected. Peripheral venous blood was sampled, processed, and stored in a biobank for future analyses at hourly intervals. All volunteers entered the NHC at the same time of day and were free to consume food and beverages *ad libitum* (except for alcohol or caffeinated drinks). Recumbent position or sleeping was prohibited during the 6-hour examination to avoid potential headache relieving effects, other than that patients were allowed to move freely within the chamber. The first examination was done prior to hypoxic exposure, shortly before entering the chamber (T0 = baseline) and was repeated hourly (T1-Toff) under hypoxia. It comprised collection of vital signs, a questionnaire regarding their headache or aura presentation (based on the classification of the IHS), as well as evaluation of premonitory symptoms. Additionally to classifying the clinical phenotype of the patients' headaches during the examination according to the ICHD-III, we asked patients to self-assess their headache and report if it resembles their usual migraine headache. Intake of analgesic medication was not permitted during exposure to hypoxia (Figure 1). A full-time neurologist with experience in headache research conducted all examinations and closely monitored all subjects during the experiment.

Follow-up. After exposure to hypoxia, patients were followed up for 2 hours with completion of the questionnaires and examinations. For participants terminating prematurely the last observed time-point was termed "Toff" and they were asked to complete the 2-hour follow-up. Twenty-four hours after completing the experiment, all participants were contacted via telephone call to evaluate headaches, postdrome and efficacy of abortive migraine medication.

Statistics

All study data were collected and managed using REDCap electronic data capture tools hosted at the Medical University of Innsbruck (16). Frequencies of

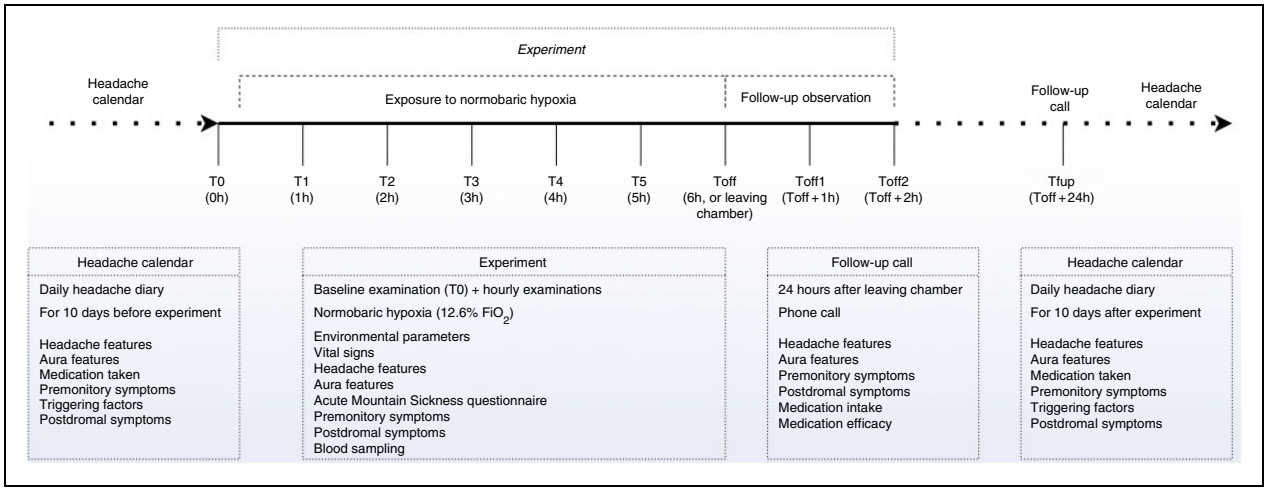

Figure 1. Study flowchart. Included patients were required to complete a headache calendar prior to and after the experiment to identify increased vulnerability for migraine generation. At baseline (T0), measurements and reports were acquired, before participants entered the normobaric hypoxic chamber (NHC). The examinations took place at hourly intervals until 6 hours (Toff) of exposition to hypoxia. For participants prematurely leaving the NHC, the last observation under hypoxia was also denoted as Toff. After-exposition follow-up was carried out for 2 hours after leaving the NHC. Additionally, all patients were followed up 24 h after the examination via telephone call.

headaches, migraines, and aura are presented as percentage of total patients for each time point. Total numeric values are presented as mean ± standard deviation (SD) or range where appropriate. The primary outcome was defined as the proportion of patients developing headache or migraines. The sample size was derived from our previous study examining healthy volunteers (n = 77) and other experimental studies utilizing hypoxia (6,10) assuming a 5% type-I error probability and a power of 90%. To test for any predicting factors group comparisons (patients developing symptoms of headache, migraine or aura vs. patients not developing symptoms) were performed for demographic characteristics at screening. For longitudinal analyses, values at T0 (i.e. baseline) were compared with subsequent time points (T1, T2, . . . , Toff2) with respective groups. Associations of dichotomous variables were calculated using a Fisher's exact test with Phi as measure of association. To compare mean group values, an unpaired two-sided t-test was performed with a 95% confidence interval. We performed binary logistic regression analyses with the parameters mean heart frequency, mean arterial blood pressure and body temperature as independent variables and outcome variables (i.e. headache vs. no headache, migraine vs. no migraine, aura vs. no aura . . .) as dependent variables. To assess possible influence of disease activity, participants were divided into three groups according to their migraine frequency (high: 8–12 days/month; moderate: 4–7 days/month; and low: <4 days/month). Pearson correlation coefficients were calculated for continuous variables. For differences between independent variables, Wilcoxon rank-sum test was applied. A *p*-value of 0.05 was considered significant. Missing data was addressed by excluding cases from further analysis. The analyses were performed with SPSS version 24.0 (IBM Corporation, Armonk, NY, US). Schematic images were created using "Biorender.com".

Standard protocol approvals, registrations, and patient consents

The study was approved by the Ethics Committee of the Medical University of Innsbruck (AN2016-0126 363/4.14). All participants provided written informed consent in accordance with the Declaration of Helsinki.

Data availability

All study data, including anonymised participant data and a data dictionary defining each field in the set, will be made available to other researchers after ethics approval and receipt of a signed material transfer agreement. Only de-identified individual data that underlie the results reported in this manuscript will be made available. Proposals should be directed to the corresponding author. Data will only be shared via individual secured network connections.

Findings

At screening, 16 patients (11 females) fulfilled the ICHD-III criteria for episodic migraine with aura, while 14 patients (11 females) were diagnosed with episodic migraine without aura. These 30 participants (22 females, eight males) were exposed to normobaric hypoxia and included in the analysis. The examinations were carried out for 23 patients per protocol, enduring 6 hours under hypoxia. Three participants left the NHC prematurely due to severe migraine headache and one examination was discontinued for safety reasons due to a pronounced decrease in systolic pressure in one asymptomatic patient. All participants were followed up 24 hours after leaving the NHC. Mean age was 27.56 years (20.10–47.00 years), mean body mass index 21.74 kg/m^2 (16.41–27.72 kg/m^2). Prior to the hypoxic challenge, peripheral oxygen saturation at baseline was between 95 and 99% with a mean SpO_2 of 98.37% (CI: 97.97–98.77; SD ±1.07). During the hypoxic challenge mean SpO_2 decreased to 83.39% (CI: 82.52–84.28; SD ±5.83).

Mean monthly migraine attack frequency, as reported by the patients, was 3.25 attacks (SD ±3.05). Mean monthly intake of abortive migraine medication was 3.39 days (SD ±5.88). We classified participants based on their baseline migraine day frequency, yielding a high-frequency (8–12 days/month; n = 6, 20.0%), medium-frequency (4–7 days/month; n = 10, 33.3%), and low-frequency group (< 4 days/month; n = 14, 46.7%). Complete demographic details as well as headache characteristics and features at screening are presented in Supplemental Table 2.

Headaches and migraine incidence under hypoxia

A total of 24 patients (80.0%) reported headaches during the examination. Nineteen (63.3%) developed migraine headache and five (16.7%) developed migraine aura (Table 1). One patient experienced headache during the hypoxic experiment, corresponding to his usual migraine attack that did not fulfil the ICHD criteria for migraine headache due to a lack of nausea or photophobia and phonophobia. Incidence of total headache and migraine was increasing throughout the experiment and peaked at Toff, which entailed volunteers completing 6 hours of exposition to hypoxia as well as those terminating prematurely (Figure 3). Migraine aura was observed first after 1 hour and the frequency gradually increased up to 4 hours of hypoxia, followed by a decline towards the end of the

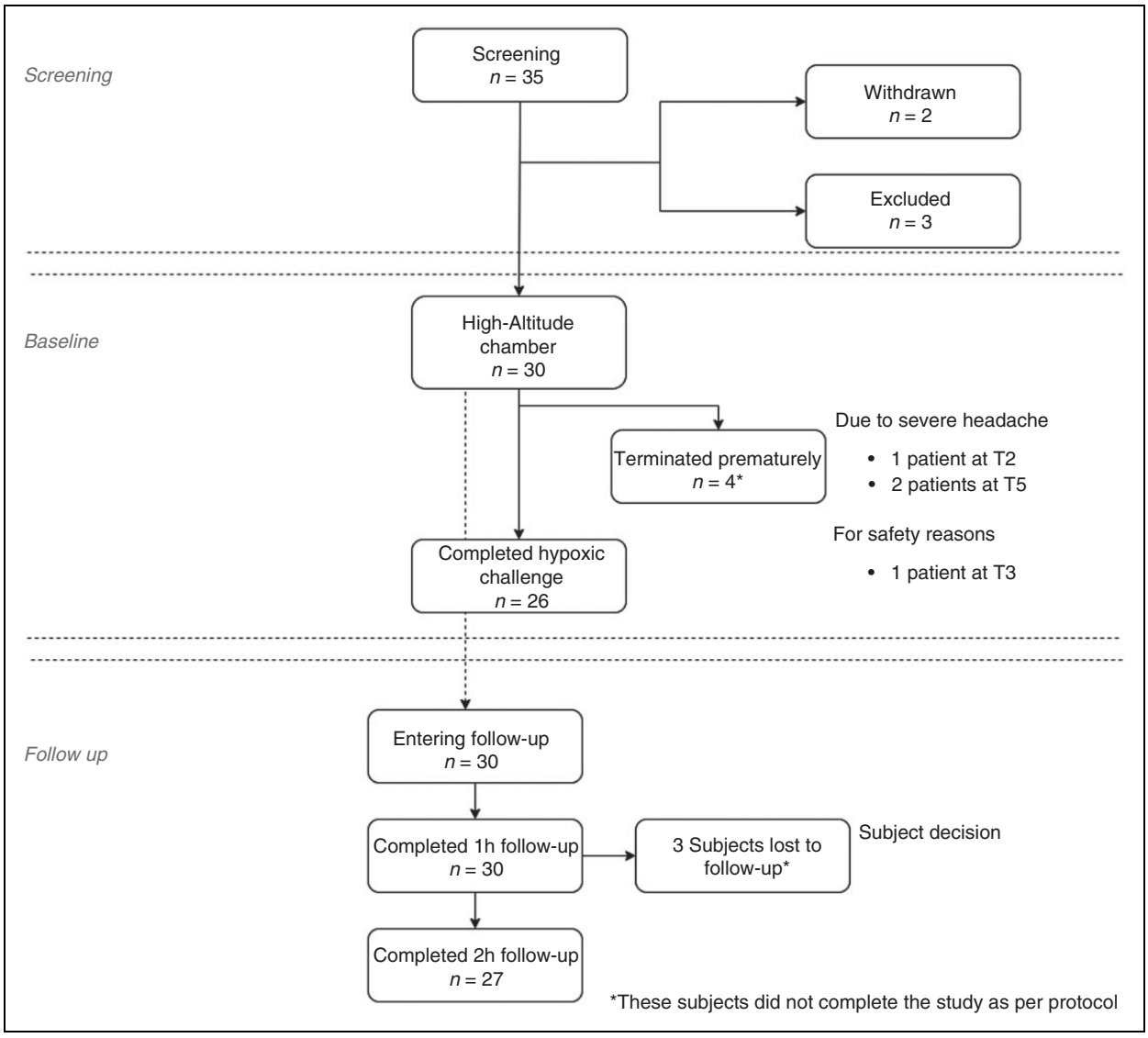

Figure 2. Patient disposition chart. Thirty patients were exposed to normobaric hypoxia (22 females, eight males; mean age was 27.56 years, SD ±7.55 years). Three patients were excluded for not meeting inclusion criteria. Sixteen patients were diagnosed with migraine with aura (11 female, five male). Three patients terminated prematurely due to severe headache, one patient was dismissed by the investigator for safety reasons.

experiment. Following up on the patients 24 hours after the examination, no aura but migraine was reported in 10.0% and headaches in 33.3%. There was no statistically significant difference between patient self-rated migraines and migraines according to the ICHD-criteria, as evaluated by a neurologist.

Aura incidence

Overall, five patients (16.70%) reported experiencing aura symptoms according to the ICHD-III. In all cases, aura was not preceded by headaches. Two subjects developed paraesthesia of the face or tongue that did not fulfil the ICHD-III criteria. Two other participants reported symptoms that were evaluated as migraine aura with positive unilateral visual symptoms (fortification spectrum, scotoma, zig-zag figures, and bright spots) that developed gradually over 5 minutes, persisted for 30 minutes, and were accompanied by migraine headache. One of these two patients additionally developed dysarthria. Both never experienced migraine aura before in their life and were initially classified as patients with migraine without aura at screening. For these subjects, we extended the initially agreed upon follow-up period to 6 months. Thorough follow-up did not reveal any aura symptom ever since in those patients. A full description of reported aura symptoms is listed in Table 2.

Table 1. Overview of headache descriptions.

ID	Headache characteristics	Photophobia	Phonophobia	Nausea	Mimics usual migraine	Onset
2	Unilateral / 5 / throbbing / +	1	1	0	Yes	T4
7	Unilateral / 3 / throbbing / +	1	1	0	Yes	T4
8	Bilateral / 5 / throbbing / +	1	0	1	Yes	T3
9	Bilateral / 10 / throbbing / +	1	0	1	Yes	T3
11	Unilateral / 5 / throbbing / +	1	1	0	Yes	Toff
13	Unilateral / 8 / throbbing / +	1	1	1	Yes	T5
14	Unilateral / 8 / throbbing / +	1	0	1	Yes	T5
17	Bilateral / 4 / throbbing / +	1	1	1	Yes	Toff1
20	Unilateral / 6 / throbbing / +	0	0	1	Yes	Toff
21	Unilateral / 6 / throbbing / +	1	0	1	Yes	T3
22	Bilateral / 7 / throbbing / +	0	0	1	Yes	T1
23	Unilateral / 7 / throbbing / +	1	1	1	Yes	T4
24	Unilateral / 7 / throbbing / +	0	1	1	Yes	T4
25	Bilateral / 7 / pressing / +	0	0	1	Yes	T3
28	Unilateral / 7 /throbbing / +	1	0	1	Yes	T4
29	Unilateral / 4 / dull / +	1	1	1	Yes	T5
30	Bilateral / 6 / throbbing / –	1	0	1	Yes	Toff
31	Bilateral / 4 / throbbing / +	1	0	1	No[a]	Toff1
32	Unilateral / 6 / throbbing / –	1	1	1	Yes	T5

Note: Overview of migraine description by all patients during the experiment. Headache characteristics include laterality/pain intensity (VAS 1-10)/pain quality/+ (aggravation or exertion by routine physical activity). Onset = first observation when migraine criteria was fulfilled. In most cases, headache intensity increased and accompanying symptoms developed gradually.

[a]This patient reported that his usual migraine attacks are unilateral, the other characteristics reported corresponded to this patients' usual migraine headache.

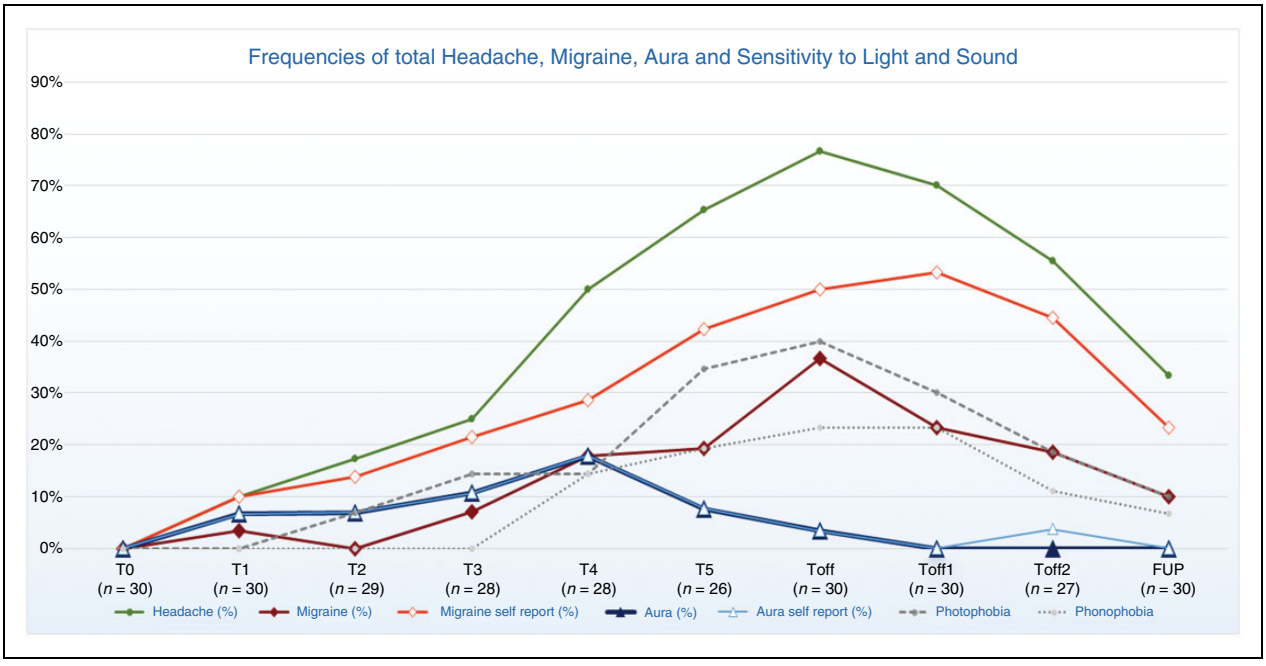

Figure 3. Distribution of reported headaches and symptoms. Shown are the absolute percentages of reported symptoms for each time-point with standard errors. In 18 of 19 cases of patients reporting migraine, ICHD-III criteria for migraine headaches were fulfilled. One patient reported having migraine aura at 1 hour after leaving the normobaric hypoxic chamber; however, the symptoms did not match the ICHD-III criteria. The percent values include onset of, as well as ongoing symptoms at each time point. Patients were asked if their symptoms resembled their usual migraine or migraine aura, this is displayed as "self-report". The graphs for "self-report" and migraine or aura diverge, as some patients recognize the emergence of migraine (and aura) prior to fulfilling the complete ICHD-III criteria. The number in brackets () signifies patients completing the respective timepoint.

Table 2. Aura presentation. Description of reported aura symptoms of five patients in accordance with the International Classification of Headache Disorders 3rd edition criteria.

Patient	1	2	3[a]	4[a]	5
Aura symptom	Visual	Sensory + dysphasic	Visual + dysphasic	Visual	Visual + dysphasic
Spreading gradually over ≥5 minutes	Y	Y	Y	Y	Y
Two or more symptoms in succession	N	Y	Y	N	Y
Each symptom lasts 5–60 minutes	Y	Y	Y	Y	Y
At least one symptom unilateral	N	Y	Y	Y	Y
At least one symptom positive	Y	N	Y	Y	Y
Aura is accompanied or followed by headache within 60 minutes	Y (M)	Y (HA)[b]	Y (M)	Y (M)	Y (M)

[a]These patients were classified as migraine patients without aura at screening. They reported having developed migraine aura for the first time in their life. We performed an unscheduled follow-up on these patients 6 months after the experiment, at which they reported not having experienced migraine aura since. Both patients fulfilled the ICHD-III criteria for migraine aura.
[b]In this patient, headache was already present at the time migraine aura was reported. The headache did not fulfil the ICHD-III criteria for migraine headache. The headache subsided 30 minutes after onset of migraine aura.
M: migraine headache; HA: non-migraineous headache.

Premonitory symptoms

Most common reported premonitory symptoms were yawning (23.3% after 1 hour, max: 40.0% after 3 hours) and fatigue (10.0% after 2 hours, max: 26.7% after 4 hours). Other symptoms including craving, irritability, muscle stiffness, gastrointestinal symptoms, and blurred vision were observed in less than 10.0% in total.

Group comparisons

Patients reporting headaches had a higher mean heart frequency than asymptomatic patients (+11.43 bpm, $p = 0.022$) as well as lower mean body weight (−9.25 kg, $p = 0.025$). No other differences regarding age, sex, baseline migraine attack frequency, blood pressure, SpO_2, FiO_2, or body temperature were found. A binary logistic regression modelled with the variables mean heart frequency and mean weight revealed a correlation of higher heart frequency and lower weight with the induction of headaches (Chi^2 9.521, $p = 0.009$, R^2 0.43) (Table 3). A comparison of the patients reporting migraines and those not reporting migraines revealed a moderately higher, but statistically not significant, mean baseline migraine attack frequency in the symptomatic group (+2.22/month, $p = 0.053$). A difference in mean heart frequency was found with higher values in symptomatic patients compared to those not developing migraine (+10.28 bpm, $p = 0.004$). The regression analysis, including mean heart frequency, yielded a medium effect size (Chi^2 7.921, $p = 0.005$, R^2 0.317). Patients using prior migraine prophylaxis (> 12 months prior to screening) also had a higher likelihood to develop migraines, than those without any prior preventive medication (Chi^2 5.286, $p = 0.029$). Analysis of aura-positive and aura-negative patients did not provide any significant differences. The respective groups of migraine attack frequency (high, medium, low) did not differ in regard to development of migraine ($p = 0.317$), headache ($p = 0.520$), migraine aura ($p = 0.454$), photophobia ($p = 0.358$), or phonophobia ($p = 0.269$). There was no difference regarding the FiO_2 between headache positive ($p = 0.188$), migraine positive ($p = 0.564$), and aura positive ($p = 0.292$) compared to asymptomatic patients, indicating stable testing conditions throughout the experiment. The pain intensity did not correlate with mean heart frequency (Bravais-Pearson $r = 0.056$; $p = 0.793$), implying independency between sympathetic response and pain. Twelve patients (40.0%) reported triptans as their usual abortive migraine medication. There was no statistical difference between triptan users and non-triptan users in regard to vital parameters and no association with triggered headaches, migraines, and aura ($p = 0.358$, $p = 0.442$, $p = 0.622$, respectively). Sixteen patients (53.3% of total) self-administered analgetic medication after leaving the NHC; six of them (37.5%) used triptans. Six participants still reported migraine headache at Toff2 after administering acute medication. (Figure 4(a),(b))

Discussion

Migraine research has advanced remarkably in the last decades, providing clinical tools and targeted therapies (17–19). These accomplishments relied considerably on data generated from basic animal and human research. Generating concepts about the underlying mechanisms in a distinct disease entity is of the utmost importance to enable further clinical research. To date, there still exists a substantial uncertainty about the mechanisms initiating, sustaining and even terminating migraine

Table 3. Group comparisons of mean vital signs and mean FiO_2.

	Headache[a]			Migraine[b]			Aura[c]			Total
	Headache n = 24 (80.0%)	No headache n = 6 (20.0%)	*p*-value	Migraine n = 19 (63.3%)	No migraine n = 11 (36.7%)	*p*-value	Aura n = 5 (16.7%)	No aura n = 25 (83.3%)	*p*-value	n = 30
Mean systolic blood pressure, mmHg ± SD	118.1 ± 11.1	124.9 ± 21.9	0.491	120.2 ± 10.4	118.3 ± 18.7	0.722	126.5 ± 8.1	118.1 ± 14.3	0.218	119.5 ± 13.7
Mean diastolic blood pressure, mmHg ± SD	74.5 ± 8.1	78.8 ± 15.0	0.340	75.2 ± 8.2	75.7 ± 12.2	0.900	78.7 ± 7.4	74.7 ± 10.1	0.414	75.4 ± 9.7
Mean SpO_2, % ± SD	88.9 ± 2.7	87.1 ± 4.3	0.375	88.5 ± 2.5	88.5 ± 4.0	0.995	89.2 ± 1.7	88.4 ± 3.2	0.568	88.5 ± 3.0
Mean heart frequency, bpm ± SD	83.0 ± 11.0	71.6 ± 6.2	0.022	84.5 ± 11.8	74.2 ± 5.9	0.004	78.2 ± 5.9	81.2 ± 11.9	0.589	80.7 ± 11.1
Mean body temperature, °C ± SD	36.7 ± 0.3	36.5 ± 0.6	0.455	36.6 ± 0.3	36.6 ± 0.5	0.588	36.6 ± 0.3	36.6 ± 0.4	0.662	36.6 ± 0.4
Mean FiO_2, % ± SD	12.6 ± 1.1	12.2 ± 0.5	0.188	12.4 ± 1.0	12.7 ± 1.0	0.564	13.3 ± 1.8	12.4 ± 0.8	0.292	12.5 ± 1.0

Note: A binary logistic regression analysis was performed to analyse possible correlations of continuous variables with the triggering of headaches, migraine or aura. Statistical significance is assumed at a *p*-value of ≤ 0.05.

[a]Headache is defined as any subject developing any headache during the experiment. No-headache subjects are those that did not report any headache.

[b]Migraine subjects are defined as all patients reporting headaches that fulfil the ICHD-III criteria for a migraine attack. No-migraine subjects could still be subjects experiencing any other headache not classified as migraine attack headache.

[c]Aura subjects are all patients reporting migraine aura during the experiment. No-aura subjects are all other subjects independent of headache or migraine development.

attacks – oxidative dysregulation might be a crucial factor embedded in the origin of this disease (11). To reinforce the pivotal role of oxidative processes in migraine, we employed controlled hypoxia as a trigger for migraine headache, premonitory symptoms, aura and acompanying symptoms.

Hypoxia induced headaches and migraine

In this experiment, headache was induced in 24 (80.0%) patients with history of migraine; migraine was triggered in 19 (63.3%) patients. It is important to point out that the FiO_2 (percentage of available inspiratory oxygen) was constant throughout the experiments, confirming stable and equal experimental conditions for all participants. The frequencies of triggered headaches and migraines are comparable to other studies investigating hypoxia as a potential migraine trigger (6,9,10) (Table 4). In addition to a high rate of successful migraine induction, we also showed a unimodal headache response. This is in contrast to headaches triggered by nitroglycerin that expose an early headache response followed by a delayed (up to 6 hours) migraine headache (25,27). Compared to our previous study (9), we elicited headaches in about the same percentage of the study population (~80%) but eight times more migraines (63.3%) than migraine-like headaches (7.8%) in healthy volunteers, indicating reproducibility and a higher susceptibility in patients with positive migraine history. Comparing the screening characteristics of patients, we found that all participants previously using migraine-preventive medication developed migraine during the experiment (n = 7, 23.3%, $p = 0.029$), which might indicate a higher susceptibility for migraine development in this subgroup. However, patients were required to not be using preventative medication for at least 12 months prior to screening. Since baseline attack frequency and monthly migraine days did not differ significantly between this group and patients never using preventive medication, a comparable disease activity at enrollment can be assumed. Gender was not a significant independent risk factor for the development of headache, migraines, or aura.

Hypoxia-induced aura

Migraine aura was elicited in five (16.6%) participants, further underlying a possible involvement of oxidative mechanisms in the induction of CSD, as proposed by previous studies (16,20). Several groups have examined pharmacologic or common migraine triggers in regard of their potential to induce migraine aura (28–30). Experimentally provoking migraine aura without pharmacological agents in humans is seldom reported (10). Performing hourly examinations allowed us to depict not only the absolute occurrence of headaches and

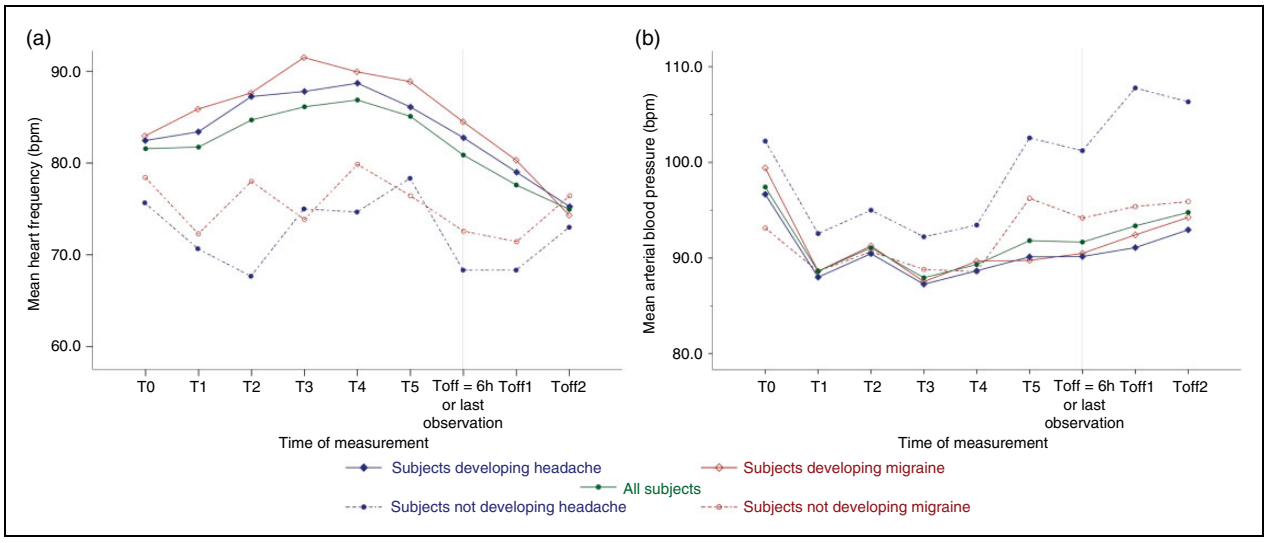

Figure 4. Comparison of mean heart frequency (a) and mean arterial blood pressure (b) between groups. Asymptomatic patients (no headache or no migraine) showed a statistically significant lower overall mean heart frequency (−11.4 bpm and −10.3 bpm respectively) than symptomatic patients. Group differences in mean arterial blood pressure (MAP) were moderate, with higher MAP in asymptomatic patients.

migraines but also to plot the progression of symptoms. Consistent with a naturally occuring migraine, we observed gradually increasing aura events that were accompanied or followed by migraine headaches and ceased prior to the end of the hypoxic exposure. Surprisingly, in contrast to earlier experiments, migraine aura according to the ICHD-III criteria was even reported by two patients who have never experienced migraine aura before. These patients were carefully followed up over months and reported that no migraine aura had occurred ever since. This finding is of utmost interest to the scientific community due to the current lack of a feasible experimental human model for migraine aura (31). Any interpretation of this result, however, should be taken with care, since only a limited fraction of our population developed aura for the first time. It is highly speculative that a hypoxic challenge is capable of inducing CSD and subsequently a "genuine" migraine. Further research confirming our findings might add to the hypothesis that each migraine is preceded by a subclinical, conceivably even undetectable, CSD (32).

Group comparisons

Notably, the most robust difference between the symptomatic (reporting headache or migraine) and asymptomatic groups was found in mean heart frequency (headache +11.4 bpm, migraine +10.3 bpm) (Table 3). Since the highly standardized conditions applied to all participants, a similar reflectory cardiopulmonary response to counteract hypoxia could be expected. This is somewhat contrary to the findings of another group utilizing hypoxia to trigger migraines (10), as they found no significant difference in mean heart rate of patients and controls during hypoxia. They showed an almost immediate (20 min) response to hypoxia with an increase of mean heart rate of around 15 to 25 min^{-1}. Similarly, we could reproduce a lower overall increase in mean heart rate of up to 10 min^{-1} but with a more pronounced distinction between symptomatic and asymptomatic patients. A possible explanation might be the different approach in the exposition to hypoxia, as this group constantly adapted the inspiratory FiO_2 to maintain a peripheral pulsoxymetric oxygen saturation (SpO_2) of 70–75% (10), whereas we provided a constant inspiratory oxygen fraction (FiO_2) of around 12.6%. Therefore, our approach provided less interference with the patients' hemodynamic counter-regulation to hypoxia. Analyzing mean arterial blood pressure (MAP) in our groups, we found no significant differences under hypoxia. Patients developing headaches or migraines, however, showed a tendency to lower MAP values, possibly indicating a hemodynamic dysregulation under hypoxia. We have found no statistically significant difference in migraine baseline, although there is a clear trend ($p = 0.054$). This might indicate that baseline migraine days could be a surrogate marker for susceptibility.

Strengths and limitations

In this study, we employed normobaric hypoxia as a trigger for headaches and migraines in a large

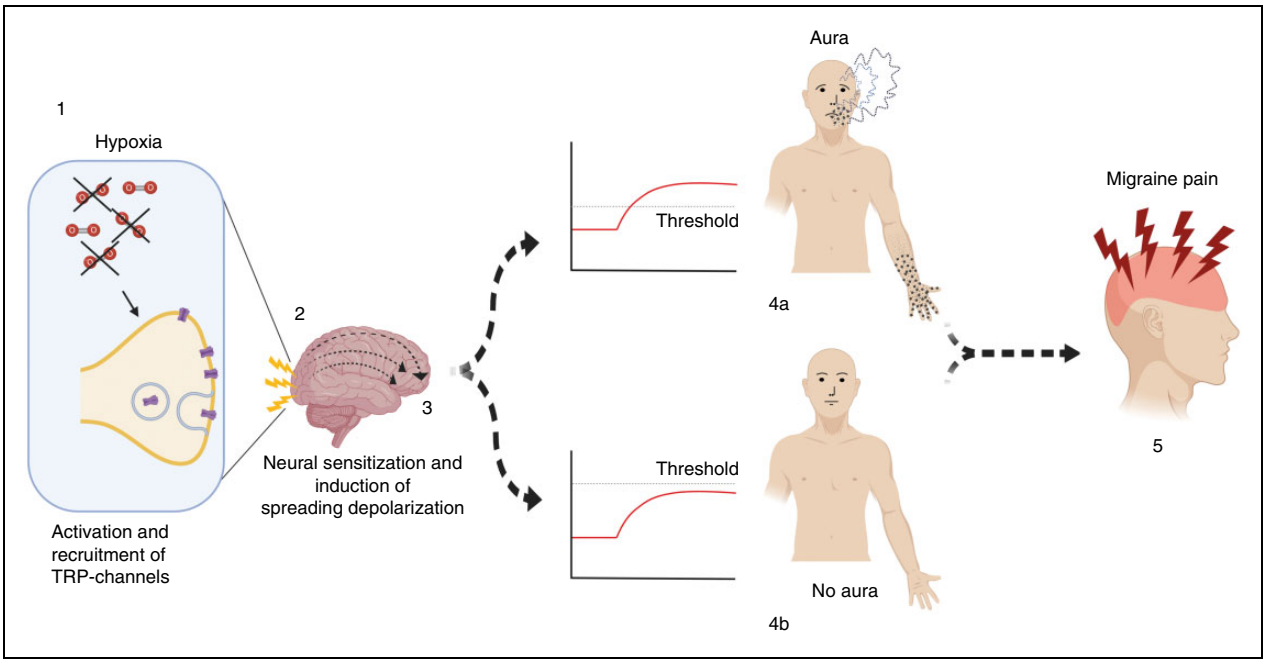

Figure 5. Hypothetical role of hypoxia-induced cortical spreading depression (CSD) in migraine. 1: Upon exposure to normobaric hypoxia, reactive oxygen species (ROS) and reactive nitrogen species (RNS) activate redox-sensitive transient receptor potential (TRP) channels and enable plasma membrane insertion of TRP channels (32). 2: Meningeal nociceptors become sensitized and facilitate excitatory signals within central trigeminal projections. 3: This induces CSD in patients with higher susceptibility for cortical excitation. 4(a): By exceeding an individual threshold, the patient experiences migraine aura. 4(b): In patients with higher thresholds than the neural activation, CSD proceeds subclinically. 5: Both scenarios promote sensitization of central pain processing networks, resulting in migraine headache.

Table 4. Comparison of studies experimentally inducing headaches. This is an incomplete overview of important studies that evaluated different triggers for migraines in human.

Agent	Population (n)	Headache	Migraine	Aura
Present study				
Hypoxia	M± (30)	80.0%	63.3%	16.6%
Hypoxia (9)	Healthy (77)	81.8%	0%[c]	0%
Hypoxia (6)	M± (16)	50.0%	43.0%	NR
Hypoxia (10)	M+[a] (15)	73.3%	53.0%	20.0%
CGRP (21)	M− (12)	100%	27.3%	NR
CGRP (22)	M+ (14)	86.0%	57.0%	28.0%
PACAP38 (23)	M− (12)	100%	63.6%	NR
Sildenafil (24)	M− (12)	91.7%	83.3%	NR
GTN (25)	M± (197)	NR	82.1%	13.6%
GTN (26)[b]	M± (53)	100%	83.0%	13.0%

PACAP38: pituitary adenylate cyclase activating peptide-38; NR: not reported; M+: migraine with aura; M−: migraine without aura; M±: migraine with and without aura.

[a]This population consisted of patients who usually experienced migraine aura with each migraine attack.

[b]This study applied a randomized, crossover design to evaluate re-triggering rates of nitroglycerine infusion. Besides reporting a relatively fast onset of migraineous headaches (median 107 min) they also provide data on triggering of premonitory symptoms.

[c]In this study, migraine-like headaches were triggered in 7.8% in a headache-native population (as percentage of whole study population).

population of 30 otherwise healthy patients with and without migraine. The results demonstrate that genuine migraine attacks can be elicited under hypoxia without chemical stimuli. However, an interaction of oxidative agents with molecular or cellular mechanisms not inherent to authentic migraines cannot be completely excluded. As there are virtually no strict contraindications against exposure to normobaric hypoxia, it can be

applied even to those excluded from receiving common triggering substances. The reported visual aura symptoms were in no way different to the aura symptoms usually experienced by the migraineurs with aura and not accompanied by any other neurological symptoms. Therefore, we conclude that these symptoms signal proper aura events and not ischemic vascular events during the long normobaric hypoxia, even in the two subjects who never experienced aura before. Our procedures and experimental conditions were highly standardized to rule out external influences such as temperature, sunlight, exertion and dehydration. By utilizing an NHC, we also prevented disruptive factors that might occur when applying hypoxia through a breathing mask (odours, pericranial pressure, atmospheric pressure differences, breathing resistance). As was pointed out in a previous review of experimental migraine models (14), experimentally induced migraines cannot fulfill the complete ICHD-III criteria. In this study we tried to approximate the experimentally induced migraines to authentic migraines by patient self-evaluation. A potential drawback in the design of our study is the lack of a control group or sham exposition. However, after conducting hypoxic research for almost 2 decades and thorough sicentific consideration, we refrained from utilizing a sham approach. Most volunteers subjected to an FiO_2 of 12.6% almost immediately perceive the lack of oxygen due to shortness of breath followed by an increased respiratory drive, rendering an accurate blinding unlikely. As this study is based on an earlier experiment published by our group to evaluate migraine-like headache in healthy non-migraineurs under hypoxia (9), we abstained from including another group of healthy volunteers.

Every year, millions of people travel to high-altitude regions such as the Alps, the Andes or the Himalayas. These environments provide various challenges for the human organism including hypoxia. Epidemiological studies and studies analysing mountaineers in those regions provided insight into the remarkable capability of humans to adapt to such high altitudes (33–35). Adaption to hypoxia starts as early as 1 hour after exposure, with the stabilization of the transcription factor HIF-1α leading to an increased expression and/or translation of enzymes and proteins ensuring sufficient oxygen supply to the tissues (36,37). Regarding our study, other early mechanisms such as increased ventilation and cerebral perfusion counteract the acute exposure to hypoxia within minutes, regulating the partial pressure of carbon dioxide ($PaCO_2$) (38). These mechanisms presuppose an unimpaired cardiovascular and pulmonary system, which was ensured in our study by conservatively screening our subjects and their medical history. It is important to point out that the level of hypoxia is a crucial factor in the planning of hypoxic studies. Moderate hypoxia, as utilized in our study, has been applied by several groups without the occurrence of serious adverse events and is generally regarded as safe in an otherwise healthy adult population (10,39–44). Taken all together, we want to point out that such experiments should be planned carefully and carried out by investigational groups that have long-term experience in applying hypoxia. Relevant inclusion and exclusion criteria are mandatory, as well as close monitoring of patients under hypoxia. However, if all these precautions are considered, moderate hypoxia, as utilized in our experiment, can be deemed safe and feasible.

Conclusion

Normobaric hypoxia is an effective, safe, and feasible experimental human model to trigger migraines (63.3%) and to some extent aura (16.6%). Utilizing hypoxia as a trigger could help to investigate pathomechanisms of migraine, including aura, and support future drug development. We found no significant baseline differences between groups and provided stable testing conditions for all participants. Higher mean heart rate in symptomatic patients might indicate an impaired hemodynamic response to hypoxia, encouraging further research on oxidative cellular reactions in migraine. The suprising finding of two patients developing migraine aura for the first time in their life may support the pivotal role of CSD as a migraine facilitator. To further investigate the compelling oxidative mechanisms in migraine, head-up tilt table testing, sonography or other medical imaging modalities such as magnetic-resonance spectroscopy could be employed under hypoxia.

Key findings

- Normobaric hypoxia is able to safely trigger migraines and migraine aura.
- The course of symptom onset and progression indicates that hypoxia-triggered migraines resemble naturally occurring migraine attacks.
- Migraine aura was elicited in a subset of subjects who never experienced aura before in their life.

Authors and contributors

Name	Location	Contribution
Florian Frank, MD	Medical University of Innsbruck, Austria	Designed and conceptualized study; acquired patients and data; analysed the data; designed and prepared all figures; drafted the manuscript for intellectual content.
Martin Faulhaber, MD	University of Innsbruck, Austria	Designed study; acquired data; revised manuscript for intellectual content.
Karl Messlinger, MD	University Erlangen-Nürnberg, Germany	Interpreted the data; revised the manuscript for intellectual content.
Chiara Accinelli	Medical University of Innsbruck, Austria	Major role in the acquisition of data.
Marina Peball, MD	Medical University of Innsbruck, Austria	Interpreted the data; revised the manuscript for intellectual content.
Alois Schiefecker, PhD	Medical University of Innsbruck, Austria	Acquisition of patients; revised the manuscript for intellectual content.
Katharina Kaltseis, MD	Medical University of Innsbruck, Austria	Revised manuscript for intellectual content.
Martin Burtscher, PhD	University of Innsbruck, Austria	Designed study; interpreted the data; revised the manuscript for intellectual content.
Gregor Broessner, MD	Medical University of Innsbruck, Austria	Designed and conceptualized study; acquired patients; analysed and interpreted the data; revised manuscript for intellectual content.

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ORCID iDs

Florian Frank https://orcid.org/0000-0002-7236-5557
Karl Messlinger https://orcid.org/0000-0002-8099-3860

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