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Low 5-HT$_{1B}$ receptor binding in the migraine brain: A PET study

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Abstract

Background: The pathophysiology of migraine may involve dysfunction of serotonergic signaling. In particular, the 5-HT$_{1B}$ receptor is considered a key player due to the efficacy of 5-HT$_{1B}$ receptor agonists for treatment of migraine attacks.
Aim: To examine the cerebral 5-HT$_{1B}$ receptor binding in interictal migraine patients without aura compared to controls.
Methods: Eighteen migraine patients, who had been migraine free for >48 hours, and 16 controls were scanned after injection of the 5-HT$_{1B}$ receptor specific radioligand [^{11}C]AZ10419369 for quantification of cerebral 5-HT$_{1B}$ receptor binding. Patients who reported migraine <48 hours after the PET examination were excluded from the final analysis. We defined seven brain regions involved in pain modulation as regions of interest and applied a latent variable model (LVM) to assess the group effect on binding across these regions.
Results: Our data support a model wherein group status predicts the latent variable ($p = 0.038$), with migraine patients having lower 5-HT$_{1B}$ receptor binding across regions compared to controls. Further, in a whole-brain voxel-based analysis, time since last migraine attack correlated positively with 5-HT$_{1B}$ receptor binding in the dorsal raphe and in the midbrain.
Conclusion: We report here for the first time that migraine patients have low 5-HT$_{1B}$ receptor binding in pain modulating regions, reflecting decreased receptor density. This is either a primary constitutive trait of the migraine brain or secondary to repeated exposure to migraine attacks. We also provide indirect support for the dorsal raphe 5-HT$_{1B}$ receptors being temporarily downregulated during the migraine attack, presumably in response to higher cerebral serotonin levels in the ictal phase.

Keywords

Headache, pain modulation, raphe, neuroimaging, serotonin 1B receptors, serotonin

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Introduction

One of the longest-standing theories on migraine pathophysiology holds that this highly prevalent condition is fundamentally a disorder of serotonergic transmission, involving chronic low brain serotonin levels (1). This 'serotonin theory' of migraine emerged from biochemical studies in the 1960s (2) and is supported by more recent electrophysiological and neuroimaging studies (3). In addition, the anti-migraine drugs, such as ergotamine and triptans, act on serotonergic targets (4) and are highly selective for pain modulation of migraine, for example, triptans have no effect in tension type headache. Triptans are unlikely to exert their action through binding to vascular serotonin 1B

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(5-HT_{1B}) receptors, but rather through binding to peripheral, neuronal 5-HT_{1D} (5) and central 5-$HT_{1B/1D/1F}$ receptors in the trigemino-cervical complex (6). The relative contributions in the anti-migraine effect of the 5-HT_1 subtypes (B, D, F) is still unclear, although the selective 5-HT_{1D} receptor agonist PNU-142633 is ineffective in migraine (7). Therefore, the 5-HT_{1B} receptor seems to play an important and specific role in migraine pathophysiology.

5-HT_{1B} receptors are present in all parts of the trigeminal pain-signaling pathway, including the dural vasculature, trigeminal ganglion, trigeminal nucleus caudalis, raphe nuclei, thalamus, hypothalamus and the cerebral cortex (8). In addition to being a heteroreceptor that modulates the release of gamma-amino-butyric acid (GABA), glutamate and acetylcholine, the 5-HT_{1B} receptor is also an autoreceptor located presynaptically on serotonergic neurons, inhibiting synthesis and release of serotonin in raphe (9) and in projection areas (8).

To our knowledge, no study has yet investigated *in vivo* cerebral 5-HT_{1B} receptor binding in migraine patients. The aim of this PET study was to examine cerebral 5-HT_{1B} receptor binding in interictal migraine patients without aura and compare it to healthy volunteers. We focused our analysis on brain regions of relevance for migraine, i.e., those involved in pain modulation or emotional and cognitive aspects of pain: The prefrontal cortex, sensorimotor cortex, anterior cingulate cortex, insula, and amygdala. We subsequently evaluated with an exploratory whole-brain voxel-based analysis whether differences in 5-HT_{1B} receptor binding could be detected in any other regions. Finally, we investigated whether 5-HT_{1B} receptor binding correlated with clinical characteristics of the patients' migraine attacks.

Materials and methods

Subjects

All participants were recruited from a Danish website for recruitment of volunteers to health research (www.forsogsperson.dk), through online adverts on the intranet of the Capital Region of Denmark and from a local database. Patients were eligible for inclusion if they were 18–65 years old, had a verified diagnosis of migraine without aura according to the International Headache Society Criteria (10), had at least one migraine attack every other month but less than five migraine days per month, and reported having experienced successful treatment of migraine attacks with sumatriptan. Patients underwent a standardized interview and interview items included duration of disease (years), frequency (migraine days per month), maximum pain intensity of untreated headache as measured with the Numerical Rating Scale (NRS) (number 0–10), triptan use (days per month) and time since last migraine attack (days). Age and sex matched controls were eligible for inclusion if they did not have any history of migraine including probable migraine and had no first-degree relatives with migraine. Exclusion criteria were: A history of any other primary headache (except tension-type headache for less than five days per month), psychiatric disease, cerebro- or cardiovascular disease, contraindications for magnetic resonance imaging (MRI), pregnancy or nursing, and intake of daily medication. On the day of the scheduled PET scan, all subjects were headache free and had not had any intake of medications for the last 24 hours. Patients were also excluded if they had not been migraine free for at least 48 hours prior to the PET scan. Headache diaries were obtained from all patients for 48 hours after the scan. All included participants had a normal physical and neurological examination and brain MRI.

The Ethics Committee of the Region of Copenhagen approved the study (H-6-2014-057), which was conducted in accordance with the Helsinki II Declaration of 1964, with later revisions. All participants gave written consent after receiving detailed oral and written information prior to any study-specific procedures.

PET and MRI

[^{11}C]AZ10419369 was synthesized using an automated radiosynthesis system as previously described (11). The subjects were placed in a supine position on the scanner bed with their head in a specialized head holder to minimize movement. [^{11}C]AZ10419369 was given intravenously as a bolus over 20 seconds followed by a 90 min PET dynamic data acquisition. PET scanning was performed with the high-resolution research tomography (HRRT) PET scanner (CTI/Siemens, Knoxville, TN, USA) with an in-plane resolution of approximately 1.4 mm (12). Reconstruction was done using ordinary Poisson 3-dimensional ordered-subset expectation maximization with point spread function modeling (16 subsets, 10 iterations) (13,14) with attenuation map improvements as previously described (15).

MRI was conducted using a Siemens Prisma 3T scanner (Siemens, Erlangen, Germany) with a 64-channel head coil. Structural T1 and T2 weighted images were recorded for each subject. MR images were used to rule out structural pathology, for co-registration with PET and delineation of regions of interest (ROI), and for segmentation in SPM8 into grey matter, white matter and cerebrospinal fluid.

Region of interest analysis

PET images were co-registered and aligned to the corresponding T1-weighted MRI image using SPM8 (16).

Confirmation of accurate co-registration was done by visual inspection for each subject across all planes. Correction for intra-scan movement was performed on all PET images using the AIR 5.2 software. All frames were aligned to the first five-minute frame. Delineation of ROIs was done automatically on each subject's MRI using PVElab software (www.nru.dk) as previously described (16), and time activity curves (TAC) and grey matter volumes for each ROI were extracted. Grey matter volumes were quantified summing the grey matter voxels from the SPM8 segmentation.

The kinetic modeling was performed in Matlab using the simplified reference tissue model (SRTM), with the cerebellum (excluding vermis) as a reference region. The cerebellum is suitable as a reference region since it is almost devoid of 5-HT_{1B} receptors (17). We used the SRTM to calculate the non-displaceable binding potential (BP_{ND}), which has been validated for quantification of [^{11}C]AZ10419369 in humans (18). The person performing the kinetic modeling was blinded to group status (migraine patient or control).

Statistical analysis

Group differences in demographics, ROI specific grey matter volumes (corrected for total grey matter volume) and PET variables were compared using two-sample t-tests. We used a latent variable model (LVM) to evaluate group effects on BP_{ND} across the predefined ROIs, using the Lava package in R (19). LVM is a linear regression statistical framework used to model associations with shared information across observations, e.g., receptor binding across different brain regions. With this approach, it is possible to avoid multiple comparisons. In addition, the concept of an underlying common regulation of 5-HT_{1B} receptor binding is also captured by the latent variable model. Here, we estimated a single latent variable, modeling the shared correlation between 5-HT_{1B} receptor binding within regions involved in pain-processing including cognitive and affective aspects of pain: Anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), ventrolateral prefrontal cortex (VLPFC), orbitofrontal cortex (OFC), sensorimotor cortex (SenMot), insula (Ins), and amygdala (Amy). An identifiable model was chosen such that covariate effects can be interpreted relative to effects on VLPFC 5-HT_{1B} receptor binding. Individual additional model paths were considered based on score tests. A score test is similar to a likelihood ratio test, and it tests whether including additional paths benefits the overall model fit. All estimates and significance values were determined simultaneously, and p-values < 0.05 (two-tailed) were considered statistically significant.

As an exploratory post hoc analysis, the group effect (patients vs. controls) in each region included in the LVM was evaluated using univariate linear regression analyses. Age was included as a covariate in all models, since cerebral 5-HT_{1B} receptor binding declines significantly with age (20). Cerebral 5-HT_{1B} receptor binding does not differ between males and females (21) and, accordingly, sex was not included as a covariate.

For all analyses the significance threshold was set at $p < 0.05$ (two-tailed) and p-values are reported without correction for multiple comparisons. Statistical tests were carried out using Prism Version 6 and R Studio 3.2.3.

Voxel-based analysis

Parametric images of 5-HT_{1B} receptor binding were generated using FreeSurfer (http://surfer.nmr.mgh.harvard.edu, version 5.3) as previously described (22–24) (Figure 1). In summary, each single-subject structural T1 was normalized to Montreal Neurological Institute

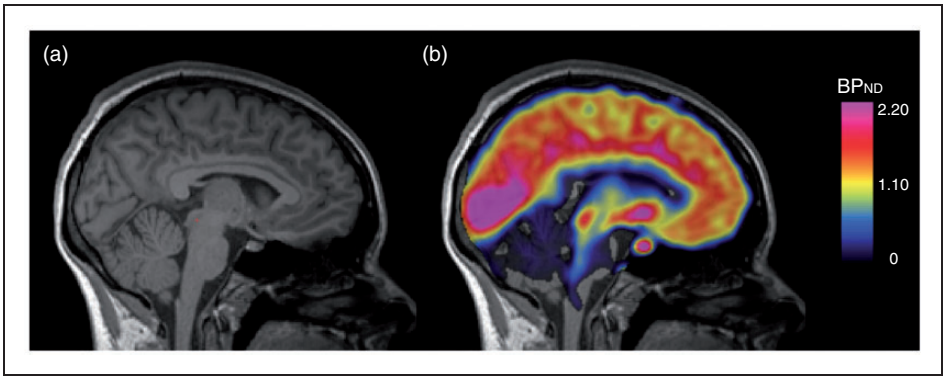

Figure 1. Parametric image of 5-HT_{1B} receptor binding. (a) Structural T1-weighted MRI image. The 5-HT_{1B} binding is not identifiable. (b) [^{11}C]AZ10419369 PET image superimposed on the corresponding structural image, highlighting the 5-HT_{1B} system.

(MNI) space using the combined volumetric and surface registration algorithm (CVS), as this has been shown to be particularly sensitive to both cortical and subcortical alignment (25). Subsequently, this was applied to the co-registered PET images. Finally, the PET images were volume-smoothed with a 6 mm full-width half-maximum 3D Gaussian kernel, and voxel-level BP_{ND}'s were estimated using the Multilinear Reference Tissue Model 2 (MRTM2), with the cerebellum as the reference region and the neocortex as the high-binding region for estimation of k_2'.

Group differences were evaluated at voxel level using multiple linear regressions. Lastly, whole-brain voxel-wise multiple regressions were performed with measures of clinical severity, including time since last migraine attack. All analyses were adjusted for age. Correction for multiple comparisons was performed using 3dClustSim, a program within AFNI (National Institute of Mental Health, Bethesda, MD; http://afni.nimh.nih.gov/afni) that uses a Monte Carlo simulation method to determine a cluster extent threshold unlikely to have occurred by chance ($\alpha < 0.05$). The cluster extent threshold for a whole-brain search volume given a voxel-level of $p < 0.005$ uncorrected, was $k > 2248$.

Results

Eighteen migraine patients and 16 age- and sex-matched controls completed the study. Three patients had a migraine attack within 48 hours after the scan and were excluded from the final analysis. All other patients were migraine free for at least 48 hours following the scan. One patient was excluded due to excessive head movement in the scanner. Hence, 14 patients and 16 controls were included in the final analysis. The clinical data of the migraine patients are presented in Table 1 and demographic data and details of the injected radioligand for both groups are shown in Table 2. The regional distribution of the tracer was in concordance with previous studies showing high binding in the occipital region and low binding in the cerebellar cortex (Figure 1) (18,26). There were no differences in grey matter volumes between patients and controls in any of the included ROIs.

Latent variable model

The basic model predicted a high correlation between regions captured by the latent variable (all factor loadings: $p < 1.6 \times 10^{-5}$). To this model we added group status (patient or control) as a predictor of the latent variable and age as a predictor of binding. Score tests revealed an additional shared correlation between $5\text{-}HT_{1B}$ receptor binding in (a) the orbitofrontal cortex and dorsolateral prefrontal cortex ($p = 0.004$) and (b) the orbitofrontal cortex and sensorimotor cortex ($p = 0.009$). No subsequent model paths were supported following an additional score test, consistent

Table 1. Migraine characteristics of included subjects.

Subject	Years with migraine	Frequency (days/month)	Side R/L (%)	Triptan intake (days/month)	Severity of migraine attack (NRS)	Days since last migraine attack
1	8	1	50/50	1	6	17
2	21	1	Bilateral	0	9	22
3	25	3	50/50	3	5	11
4	19	1	70/30	1	8	19
5	15	2	Bilateral	0	9	31
6	20	1	50/50	1	7	4
7	8	2	50/50	1	8	50
8	9	1	50/50	1	9	8
9	17	1	Bilateral	1	7	15
10	6	2	Bilateral	2	8	10
11	7	3	10/90	1	8	29
12	36	4	50/50	3	7	5
13	2	4	100/0	4	7	4
14	16	1	Bilateral	1	9	7
Median (range)	15.5 (2–36)	1.5 (1–4)		1 (0–4)	8 (5–9)	13 (4–50)

NRS = Numerical Rating Scale.

Table 2. Summary of demographics and PET variables in the two groups.

	Patients	Controls	*p*-value
Number of subjects (male/female)	14 (1/13)	16 (3/13)	
Age (years)	30.4 ± 10.0	28.9 ± 9.9	0.69
BMI (kg/m^2)	22.5 ± 1.4	24.1 ± 4.6	0.25
Injected radioactivity (MBq)	588 ± 13	580 ± 51	0.62
Specific radioactivity (GBq/μmol)	629 ± 304	492 ± 134	0.13
[^{11}C]AZ injected mass per kg (μg/kg)	0.012 ± 0.014	0.009 ± 0.003	0.35
[^{11}C]AZ cerebellum AUC/Injected dose (counts/MBq)	10.53 ± 1.91	9.71 ± 2.23	0.29

Values are given as mean ± SD. *p* values represent differences between groups evaluated using two-sample t-test. [^{11}C]AZ = [^{11}C]AZ10419369; AUC = Area under the time activity curve for cerebellum, representing the non-displaceable binding.

with sufficient model fitting ($\chi^2 = 23.54$ on 18 degrees of freedom giving a *p*-value of 0.17). In addition, the effect size of group on the latent variable was consistent throughout these model modifications (range −0.105 to −0.113). Within this final model, group significantly predicted the latent variable (parameter estimate and 95% confidence interval: −0.113 [−0.219; −0.006]), $p = 0.038$, with patients having lower binding across regions (Figure 2).

Linear regression analysis

Post hoc ROI analyses showed a significantly lower binding in the anterior cingulate cortex of migraine patients compared to controls (1.64 ± 0.15 vs. 1.78 ± 0.20, $p = 0.041$) and within the sensorimotor cortex (1.24 ± 0.13 vs. 1.34 ± 0.14, $p = 0.048$). There was a trend towards a lower 5-HT$_{1B}$ receptor binding in patients compared to controls within insula (1.54 ± 0.15 vs. 1.65 ± 0.17, $p = 0.071$) and ventrolateral prefrontal cortex (1.63 ± 0.14 vs. 1.74 ± 0.18, $p = 0.070$).

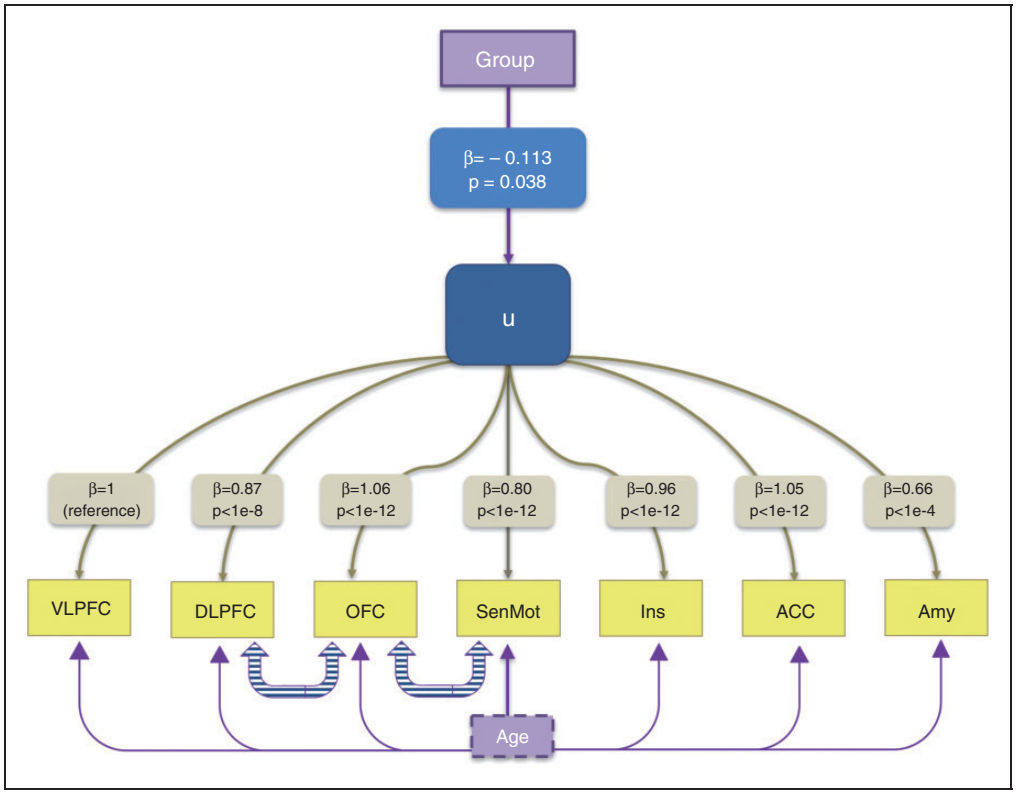

Figure 2. Latent variable model of group effects on [^{11}C]AZ10419369 BP$_{ND}$. Purple boxes represent observed predictors. The blue box (u) represents the latent variable, whereas yellow boxes represent regional [^{11}C]AZ10419369 binding potential. Striped arrows indicate additional shared correlations. The parameter estimate, β, is noted for each model path as well as significance, *p*, of parameter estimates.

Voxel-based analysis

Whole-brain voxel-wise multiple regression analysis revealed no significant group differences in 5-HT_{1B} receptor binding. The whole-brain voxel-wise multiple regression analyses with measures of clinical severity revealed a positive correlation between 5-HT_{1B} receptor binding and days since last migraine attack in a cluster spanning the midline. By visual inspection using xjView, a viewing program for SPM (http://www.alivelearn.net/xjview8/), this cluster was determined to cover bilateral red nucleus, left substantia nigra, and the dorsal raphe ($k = 3589$, $t(11) = 6.41$, $p < 0.05$ corrected, $x = -15$, $y = -22$, $z = -8$, Figure 3). No other correlations were detected.

Discussion

We found that patients with migraine without aura have lower 5-HT_{1B} receptor binding than controls across brain regions involved in pain modulation. The association between the latent variable and the large, 5-HT_{1B} receptor high-density control region (occipital cortex), was the weakest of all regions (beta = 0.65), indicating that the current finding is specific for the pain modulating regions. Hence, our data indicate that migraine patients (at least when interictal) have low 5-HT_{1B} receptor binding in brain regions involved in pain-processing including emotional and cognitive aspects of pain. In contrast to previous studies (27), we found no group difference in grey matter volume between patients and controls within any of the ROIs and thus, the lower 5-HT_{1B} receptor binding is not driven by differences in brain structure. Given that all subjects were scanned at the same time of the day, and that all migraine patients were truly interictal, we do not expect diurnal or ictal variations in brain serotonin levels to explain differences in 5-HT_{1B} receptor binding. Instead, we believe that the low binding reflects a decreased density of the 5-HT_{1B} receptor in migraine patients.

5-HT_{1B} receptor binding in pain modulating regions

There may be several alternative or possibly co-existing reasons why the 5-HT_{1B} receptor density is low in pain modulating brain regions in migraine patients.

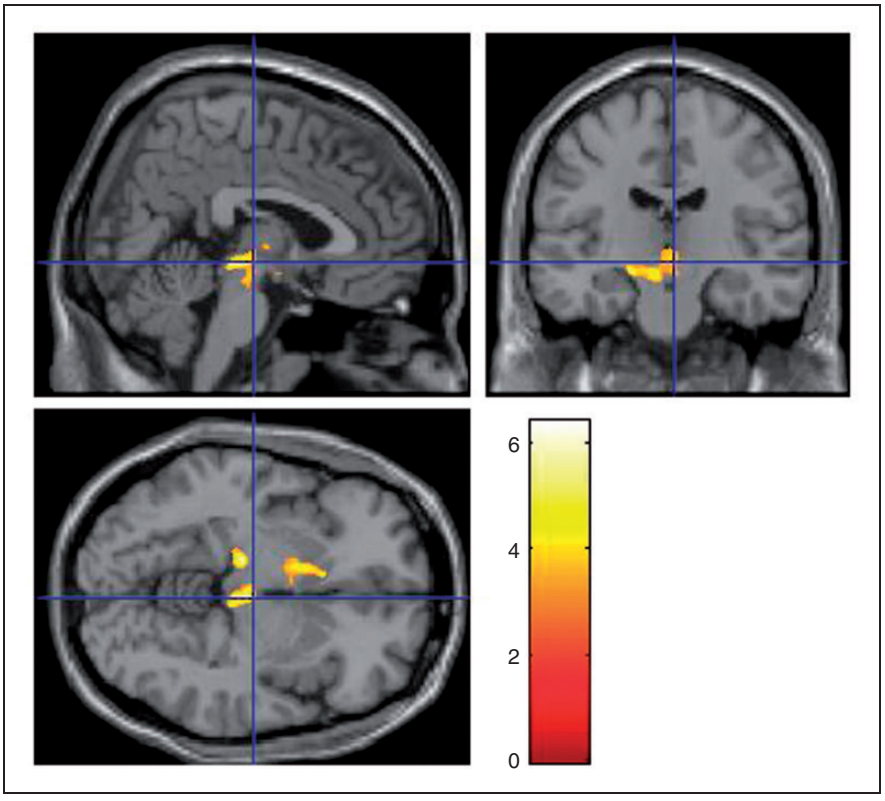

Figure 3. Voxel based analysis. Whole-brain voxel-based analysis in the migraine patients showed a positive correlation between 5-HT_{1B} receptor binding and days since the last attack in a cluster in the brainstem and midbrain (($k = 3589$, $t(11) = 6.41$, $p < 0.05$ corrected, $x = -15$, $y = -22$, $z = -8$). Color bar indicates t-score. Image shown at $z = -5.84$.

Firstly, the finding could represent a primary abnormality, e.g., a genetically determined trait marker for migraine susceptibility with an ensuing altered pain sensation and processing. This hypothesis is supported by the lack of correlation with time since last attack or duration of disease within the predefined ROIs. On the other hand, we did not observe any correlation between pain severity or headache frequency and 5-HT_{1B} receptor binding, which could have been expected if the latter is a trait marker. The 5-HT_{1B} receptor serves both as an auto- and a heteroreceptor and PET neuroimaging does not allow for a distinction between the two receptor types. Decreased 5-HT_{1B} autoreceptor density diminishes the ability to regulate synaptic serotonin levels (28). Low levels of 5-HT_{1B} autoreceptors in certain pain-related areas could thus increase the susceptibility to pain stimuli and trigger migraine attacks due to a decreased capacity to normalize serotonin levels. In the rat cingulate cortex, activation of 5-HT_{1B} heteroreceptors leads to inhibition of glutamate release (29). Low 5-HT_{1B} heteroreceptor neurotransmission could therefore lead to increased excitatory neurotransmission in ACC. In support thereof, changes in glutamatergic neurotransmission have been described in both insula and ACC interictally in migraine patients (30). In summary, our findings could reflect a functional modification of pain processing in migraine patients, caused by low 5-HT_{1B} autoreceptor and/or heteroreceptor neurotransmission.

Secondly, the low 5-HT_{1B} receptor density in the pain modulating brain regions could be a result of repeated migraine episodes, with a migraine-induced modulation of 5-HT_{1B} receptor density in brain areas involved in the pain matrix. Several studies provide support for the idea that the continuous activation of the pain related areas in migraine leads to neurochemical, metabolic or even structural modifications (30–32). In the insula and anterior cingulate cortex, the glucose metabolism is negatively correlated to duration and lifetime headache frequency (31), and similarly, a negative association between these regions' grey matter volume and headache duration and lifetime headache frequency has been found (32).

Theoretically, our observation of lower 5-HT_{1B} receptor binding could also result from higher regional serotonin levels in the pain modulating regions. From studies with acute pharmacological interventions aiming at increasing cerebral serotonin levels, there is some evidence that [^{11}C]AZ10419369 binding is reduced with acutely increased endogenous serotonin levels. This was, however, only shown in non-human primates (33), but not in humans (34). It is currently unknown if chronically elevated serotonin levels alter the 5-HT_{1B} receptor binding.

Raphe nuclei

In our supplementary voxel-based correlation analysis, we did not identify any clusters showing significant group differences. However, in migraine patients we found that time since last migraine attack correlated positively with 5-HT_{1B} receptor binding within a cluster encompassing the dorsal raphe and other parts of the midbrain. The presence of this correlation was also confirmed in a ROI based approach where we analyzed dorsal raphe separately (uncorrected $p = 0.001$, data not shown). This suggests that 5-HT_{1B} receptors in dorsal raphe, critically involved in the regulation of serotonin synthesis and serotonergic neurotransmission, are regulated in synchrony with the migraine cycle.

Some authors have suggested that brain serotonin levels are temporarily increased in the ictal phase of a migraine attack (35). Such a stimulation of the raphe nuclei could lead to a temporary downregulation of 5-HT_{1B} autoreceptors that only gradually recovered. This explanation finds support in animal studies that show downregulation of presynaptic 5-HT_{1B} autoreceptors in the raphe nuclei following an increase in synaptic serotonin (36,37). Thus, it is possible that our results reflect spontaneous increases in synaptic serotonin levels in migraine patients compared to controls.

Interestingly, the raphe nuclei, including the dorsal raphe, have previously been implicated in migraine pathophysiology. In particular, [^{15}O]H_2O PET studies reported an increase in regional cerebral blood flow during spontaneous attacks, which persisted after effective treatment with 5-$HT_{1B/1D}$ receptor agonist sumatriptan (38,39). More recently, a functional MRI study reported strong functional connectivity between the hypothalamus and the dorsal pons during a migraine attack (40). Collectively, these findings point toward an involvement of the dorsal raphe during migraine attacks. In our analysis, we only included patients that were migraine free for 48 hours before and after the scan, but in the light of our findings it would be relevant to investigate the dynamic changes in raphe related to the migraine cycle in the period from 48 hours before attack onset to 48 hours post-ictally.

Limitations

A few shortcomings of our study need to be mentioned. Since we only examined patients in their inter-ictal phase we cannot exclude that we would have had a different outcome had we studied them immediately before, after, or during a migraine attack. Further, since most of the migraine participants were occasional triptan users, we cannot exclude that the 5-HT_{1B} receptors were desensitized in response to the periodic intake of 5-HT_{1B} receptor agonists. However, the absence of a

global downregulation of 5-HT_{1B} receptors and the lack of correlation between duration of disease, frequency or triptan use and 5-HT_{1B} receptor binding speaks against this explanation.

Conclusion

We report novel evidence that patients with migraine without aura have low 5-HT_{1B} receptor binding in pain modulating regions of the brain, and we explain it as either a primary constitutive trait of the migraine brain or secondary to the repeated exposure to migraine attacks. We also provide some indirect support for the dorsal raphe 5-HT_{1B} receptors being temporarily downregulated during the migraine attack, presumably in response to higher cerebral serotonin levels in the ictal phase. Further studies are warranted to clarify the mechanisms underlying our observations.

Key findings

- We found a lower binding of 5-HT_{1B} receptors in migraine patients compared to controls in pain modulating regions of the brain.
- The lower binding is either a trait marker of the migraine or a consequence of repeated activation of the pain modulating areas.
- We found that 5-HT_{1B} receptor binding increased in the midbrain with days since the last attack, supporting the involvement of the dorsal raphe in migraine pathophysiology.

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Declaration of conflicting interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr. Knudsen has received honoraria as a consultant/speaker for H Lundbeck and Pfizer, and as a board member of Brain Prize and the Elsass Foundation. She is also on the advisory board for the Kristian G Jebsen Foundation and a field editor for *Int J Neuropsychopharm*. Messoud Ashina is a consultant and/or scientific adviser/speaker for the ATI, Allergan, Amgen, Alder and Eli Lilly. All other authors declare no conflicts of interest and report no financial disclosures.

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