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Ref.: GMK

Date: October 25, 2023

To Whom It May Concern,

Regarding:
EudraCT identifier: 2018-003382-34
Title: “Prophylactic effects of psilocybin on chronic cluser headache an open-label clinical trial and neuroimaging study.”

The study was ended prematurely due to the covid pandemic, which substantially slowed down study progess. The study was terminated after 10 patients had completed the study (original aim: 20 patients). We anticipated comparing brain functional connectivity data of patients with data from healthy volunteers. However, the data from healthy volunteers was collected as part of other on-going studies at our lab, and after careful consideration of applied MRI sequences and the participant profiles, it was apparent that this comparison would be inappropriate due to inter-study differences.

A manuscript was prepared based on the available data and submitted for publication, and a pre-published version of this manuscript, containing the conducted analyses, is presented here.

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CCH attack frequency reduction after psilocybin correlates with hypothalamic functional connectivity

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Conflicts of Interest Statement

MKM has received an honorarium as a speaker for Lundbeck Pharma and the Lundbeck Foundation. DSS has received an honorarium as a speaker for the Lundbeck Foundation. GMK has received honoraria as a consultant for Sanos and as a speaker for Sage-Biogen. RHJ has given lectures for Pfizer, Eli-Lilly, Merck, TEVA, Novartis, Lundbeck and Allergan and is or has been primary investigator in clinical trials funded by Eli-Lilly, Novartis and Lundbeck. RHJ

has received research funding from University of Copenhagen, Rigshospitalet, Lundbeck Foundation, The Medical Society in Copenhagen, NovoNordisk Foundation and Tryg Foundation. All other authors report no conflicts of interest.

Key words: Psilocybin, psilocin, cluster headache, fMRI, functional connectivity, hypothalamus.

Funding

The study was sponsored by Augustinusfonden (grant number: 18-0317), Rigshospitalet's Research Council (grant numbers: R130-A5324 and R214-A9447), Innovation Fund Denmark (NeuroPharm, grant number: 4108-00004B), and Lundbeck Foundation through Danish Neurological Society (no grant number available).

Abbreviations: CH = cluster headache; ECH = episodic cluster headache; CCH = chronic cluster headache; CGRP = calcitonin-gene-related peptide; 5-HT2AR = serotonin 2A receptor; FC = functional connectivity; fMRI = functional magnetic resonance imaging; ICHD = International Classification of Headache Disorders; BL = baseline; FU = follow-up; ASC = Altered States of Consciousness; PPL = plasma psilocin level; FWER = family-wise error rate; Cmax = peak plasma concentration; AUC = area under curve; BOLD = blood-oxygen level dependent.

Acknowledgements

The assistance of Drummond McCulloch, Lone Freyr, Arafat Nasser, Annette Johansen, Dorthe Givard, Peter Jensen, Kristian Jensen, and Martin Snoer is gratefully acknowledged. COMPASS Pathways Ltd, UK, kindly provided the study drug.

Abstract

Objective. To evaluate feasibility and prophylactic effect of psilocybin as well as effects on hypothalamic functional connectivity (FC) in patients with chronic cluster headache (CCH).

Background. CCH is an excruciating and difficult-to-treat disorder of incompletely understood pathophysiology, although hypothalamic dysfunction has been implicated. Psilocybin may have beneficial prophylactic effects, but clinical evidence is limited.

Methods. In this small open-label clinical trial, ten patients with CCH were included and maintained headache diaries for ten weeks. Patients received three doses of peroral psilocybin (0.14 mg/kg) on the first day of week five, six and seven. The first four weeks served as baseline and the last four weeks as follow-up. Hypothalamic FC was determined using fMRI the day before the first psilocybin dose and one week after the last dose.

Results. The treatment was well-tolerated. Attack frequency was reduced by mean (SD) 31% (31) from baseline to follow-up (P_{FWER}=0.008). One patient experienced 21 weeks of complete remission. Changes in hypothalamic-diencephalic FC correlated negatively with percent change in attack frequency (P_{FWER}=0.03, R=-0.81), implicating this neural pathway in treatment response.

Conclusion. Our results indicate that psilocybin may have prophylactic potential and implicates hypothalamus in possible treatment response. Further clinical studies are warranted.

Trial registration

EU Clinical Trials Register (EudraCT) identifier: 2018-003382-34, first posted June 17, 2019. ClinicalTrials.gov identifier: NCT04280055, first posted February 21, 2020.

Introduction

Cluster headache (CH) is one of the most painful conditions known and affects 0.1% of the population, a prevalence similar to Parkinson's disease.[1] CH exists in two forms: episodic (ECH) or chronic (CCH).[2] Ten to fifteen percent of patients with CH have CCH, which means less than three months without attacks during a year.[2] CH is frequently associated with anxiety, depressive symptoms and suicidal ideation.[3] Medical treatment consists of acute abortive treatment and prophylactic treatment. Although standard prophylactic options (e.g., verapamil or lithium), novel anti calcitonin-gene-related peptide (CGRP) antibody medications, and transitional treatments (e.g. dihydroergotamine [DHE] pulse) can substantially reduce CH attack frequency in many patients, the treatment may have unacceptable side effects or insufficient treatment response, especially in CCH.[4–6]

Both observational reports [7,8] and a recent clinical trial [9] indicate that serotonin 2A receptor (5-HT2AR) agonists, including psilocybin, may have beneficial prophylactic effects. However, more data from prospective clinical trials are needed to evaluate safety, clinical efficacy and possible mechanisms related to response.

Here we evaluate the feasibility and prophylactic effects of psilocybin in patients with CCH in an open-label study. Since hypothalamic dysfunction may play a key role in CH pathophysiology, [10–13] we also assess the association of treatment response with hypothalamic functional connectivity (FC), measured using functional magnetic resonance imaging (fMRI).

We hypothesized that CH attack frequency would decrease after psilocybin and that attack frequency change would correlate with change in hypothalamic FC.

Materials and Methods

Patients

Ten patients (mean age (SD); median[25th,75th] = 49.4 (12.9); 54.5[39.4, 58,1], 5 females) with a verified diagnosis of CCH were recruited from Danish Headache Center, Glostrup Hospital, Denmark (**Table 1**) and completed the present open-label study.

All patients were thoroughly informed about the study, including possible effects and side effects of psilocybin including physiological changes (e.g., blood pressure increases) and psychedelic effects. After giving written informed consent, all patients underwent a medical examination, including neurological and somatic examination, blood screening panel for common biomarkers, ECG, and a screening for present and past psychiatric disorders using the Mini-International Neuropsychiatric Interview, Danish Translation, version 6.0.0.[14] Eleven patients were included in the study (**Fig 1**).

Inclusion criteria were: 1) age 18-65 years, 2) a diagnosis of CCH according to ICHD-III,[15] 3) ability to separate cluster headache attacks from other types of headache, 4) a history of at least four attacks per week in the last four weeks before inclusion. Exclusion criteria were: 1) a history of using a serotonergic hallucinogen for CH, 2) participation in any clinical trials within 30 days preceding study enrollment, 3) use of other prophylactic CH medication within the last two weeks, 4) current use of drugs suspected to interfere with treatment (e.g. antipsychotic medication) or to be hazardous in combination with psilocybin, 5) presence of other trigeminal autonomic cephalalgias than CH, 6) known hypersensitivity or allergy to multiple drugs

(including psilocybin), 7) a history of or current medical or psychiatric condition that might render participation unsuitable, 8) present or previous manic or psychotic disorder or critical psychiatric disorder, 9) current substance use disorder, 10) MRI contraindications, 11) pregnancy or breastfeeding, 12) not using safe contraception (if fertile woman), 13) stroke (<1 year from inclusion), 14) myocardial infarction (<1 year from inclusion), 15) hypertension (> 140/90 mmHg at inclusion), 16) clinically significant cardiac arrhythmia (<1 year from inclusion).

The study was approved by the ethics committee for the capital region of Copenhagen (journal identifier: H-18040896, H-amendments: 64708, 65371, 65944, 71621, 72461) and Danish Medicines Agency (EudraCT identifier: 2018-003382-34, amendments: 20191209), ClinicalTrials.gov identifier: NCT04280055. The study abided by the Helsinki declaration. The study was approved by the GCP-monitoring unit September 25, 2019; the first patient was recruited January 20, 2020; the first psilocybin intervention was conducted on February 20, 2020; the study was concluded November 9, 2022.

Procedures

After study inclusion, patients completed an online headache logbook daily for ten weeks. The information entered in the headache logbook included the number of CH attacks and estimated average pain intensity (i.e., one summary estimate for all attacks prior to acute treatment on that day (0-10, numeric rank scale)), the number of times of acute treatment use (either oxygen or sumatriptan s.c.), and the estimated average duration of all attacks experienced each day (options: A) 15-30 min; B) 30-60 min; C) 60-120 min; D) 120-180 min; E) > 180 min). A four-week baseline (BL) period was followed by three psilocybin treatments spaced by one week, and then followed by a four-week follow-up period (FU). For study design, see **Fig 1**. The day prior to the first psilocybin treatment and one week after the last treatment, patients underwent an MRI

scan session. Patients were contacted by phone after three and six months to obtain information of potential remission duration and perception of the psilocybin treatment (**Table 2**).

Psilocybin Treatments

On the day before the first psilocybin treatment, every participant met with two trained session facilitators, following an established protocol at our lab previously employed in healthy participants.[16–18] The overall aim of preparation was to build a safe alliance between the facilitators and the patients, and prepare the patient for the intervention. During preparation, facilitators explored the patient's expectations and motivations for undergoing the treatment and inquired about the patient's personal history including major life events and any previous experience with altered states of consciousness.

Psilocybin dose was determined according to body weight at inclusion (0.14 mg psilocybin per kg bodyweight), using 1 mg and 5 mg capsules/tablets (COMP360, COMPASS Pathways' proprietary pharmaceutical-grade synthetic psilocybin formulation that has been optimized for stability and purity). The 0.14 mg/kg dose and pulse treatment regimen were determined based on both patient accounts that low to moderate psilocybin doses spaced by 4-7 days is effective[19] and our previous 5-HT2AR PET occupancy study, which indicated that a dose of 0.14 mg/kg produces high psilocin occupancy at cerebral 5-HT2ARs while still having limited psychoactive effects.[16] Patients were asked to limit to a minimum the intake of food and caffeine on the morning of all treatment days and were asked to standardize their food intake across all three treatment days. Sessions were conducted at the hospital in a hotel-room like environment. During dosing sessions, facilitators provided interpersonal support. A curated standardized music program of primarily classical music was played during dosing sessions to further assist and support the patients and create a pleasant setting. If a patient disliked the music, the facilitator

would change the track. At the end of every treatment day, when psychoactive effects had waned sufficiently, patients completed the 11-dimension (11-D) Altered States of Consciousness (ASC) questionnaire, which quantifies eleven dimensions of subjective experience associated with psychedelic use.[20] After each psilocybin treatment, patients met the day after with the session facilitators to share and integrate their experience. During integration, the facilitators explored the time elapsed since the preceding psilocybin treatment, including first sharing of the experience with individuals in the patient's life outside the research group, behaviors, thoughts and feelings that the patient may have had after returning to home/the overnight facilities, and inquiry about sleep, dreams, appetite and residual drug effect. Facilitators elicited a complete narrative of the experience using deep listening skills (i.e., listening to learn, listening for understanding and not agreement or analytical interpretation) and asking questions that evoke presence, curiosity, innovative ideas, and meaning-making. Psychotherapy was not part of the study set-up.

Blood Pressure Measurements

Blood pressure was measured immediately prior to psilocybin administration and at 40, 80, 120, 180, and 240 minutes after drug administration, using an electronic blood pressure measurement device (Omron M3 Comfort, Kyoto, Japan).

Plasma Psilocin Level

Blood samples were collected on the first treatment day only prior to psilocybin intake and 20, 40, 60, 80, 100, 120, 140, and 240 minutes after drug. Blood samples were always obtained after blood pressure measurement. PPL was determined using ultra high performance liquid chromatography and tandem mass spectrometry, as previously described[16] with minor changes. The transition of the internal standard psilocin-d10 was m/*z* 215 -> 65.8 with collision energy of

14 eV corresponding to the quantitative transition of psilocin. The reconstitution mixture after precipitation and evaporation were added 1 mM ascorbic acid.

Magnetic Resonance Imaging

Structural and functional MRI data was acquired using a 32-channel head coil on a 3T Siemens Prisma scanner (Siemens, Erlangen, Germany). Blood oxygen level dependent (BOLD) fMRI data was obtained using a T2*-weighted gradient echo-planar imaging (EPI) sequence (TR = 800 ms, TE = 37 ms, flip angle = 52°, in-plane resolution = 2x2x2 mm, 72 slices (thickness = 2.0 mm, no gap), multiband acceleration factor=8). The first eight acquired volumes were discarded. For the first five participants' BOLD fMRI data acquisition, 375 volumes were acquired (5 min). An optimization of the scan protocol allowed for additional BOLD data acquisition for the last five patients for whom 750 volumes were acquired (10 min) for at BL and FU. Participant were instructed to keep their eyes closed, let their mind wander freely and not fall asleep. A high-resolution, T1-weighted 3D structural MP-RAGE image was acquired each scan session (inversion time = 920 ms, TE = 2.41 ms, TR = 1810 ms, flip angle = 9°, resolution = 0.8x0.8x0.8 mm, 224 slices; slice thickness = 0.8 mm, no gap). Nine individuals completed both BL and FU scan sessions; a Covid-19-related lock-down prevented acquisition of follow-up MRI data in one patient.

Image Preprocessing

BOLD image preprocessing was performed in SPM12 (http://www.fil.ion.ucl.ac.uk/spm), including unwarping using B0 field-maps, realignment, coregistration, segmentation of structural image and normalization to Montreal Neurological Institute (MNI) space, and smoothing with an 8 mm Gaussian kernel, as described previously.[17]

Functional Connectivity Analysis

Denoising was performed using CONN (v17c) as previously described[17] and included band-pass filtering (0.008 Hz to 0.09 Hz), motion and spike regression and principal-component based noise-reduction, as described previously,[17,21] and FC estimation (Fisher-transformed R-to-z values) between an 8 mm radius sphere seed placed in the posterior hypothalamus (MNI coordinates: 3, -9, -10)[22] and the rest of the brain, conducted across all images using both BL and FU BOLD images (statistical significance threshold: voxel-level p-value threshold = 0.001, cluster-level significance level $p_{FWER} = 0.05$). FC estimates for clusters exhibiting significant FC with the seed region across all scans (BL and FU) were extracted for subsequent data analysis.

Exploratory Functional Networks fMRI Analysis

An exploratory networks analysis was conducted using FC estimates within and between seven networks (default mode, ventral attention, dorsal attention, fronto-parietal control, visual, limbic, and somatomotor), which were delineated using Schaefer's 400-region parcellation of the Yeo atlas.[23,24] The test-retest reliability of FC fMRI analysis generally low but depends on outcome measure and analysis choices.[25,26]

Statistical Analysis

Clinical Efficacy Evaluation

The primary outcome was change in CH attack frequency per week (BL vs. FU). In the event of multiple headache diary data entries per date, the higher CH attack entry was used. The secondary outcome was change in average pain intensity (BL vs. FU). Statistical analyses for BL vs. FU were conducted using the non-parametric Wilcoxon-signed-rank test, two-tailed. The family-wise error rate (FWER) was controlled for all tests in the present study using the Bonferroni-Holm method (here, two tests, alpha: $P_{FWER}<0.05$).[27] Data analysis was performed using R Studio version 1.2.5042. No available data existed on which to base a formal sample size

calculation, and the original sample size goal of n=20 was deemed as appropriate. We calculate weekly headache frequency mean, median, standard deviation, and percent change.

Neuroimaging Analysis

The primary analyses were to evaluate the change in hypothalamic FC from BL to FU (Wilcoxon-signed-rank test, two-tailed) and to analyze the correlation between percent change in attack frequency from BL to FU with change in hypothalamic FC (simple linear regression). The FWER was controlled (four tests, alpha: $P_{FWER}<0.05$). Assumptions for statistical tests were evaluated using histograms and QQ plots.

Exploratory post hoc analyses of psilocybin effects on FC within and between networks were performed, evaluating change from BL to FU and the correlation between percent change in attack frequency and change in neuroimaging outcome. Effect sizes (Cohen's d and Pearson's R) are reported as outcome measures, along with P-values and FWER-corrected P-values (Bonferroni-Holm).

Plasma Psilocin Analysis

We also explored a possible dose-response relation of maximum measured PPL (C_{max}) and psilocin area under curve (AUC) with percent change in attack frequency and pain intensity, using linear regression (n=9, given incomplete psilocin data for Patient 2). Plasma psilocin AUC was calculated using the trapezoidal rule.

Results

Safety and Feasibility

Nine patients completed all three psilocybin treatments; one patient was due to logistical issues unable to attend the third treatment but completed the rest of the study. The dosing regime was

well tolerated by all patients, and we observed no serious adverse reactions (**Table 1 & 2)**. One patient (Patient 5) reported beneficial long-lasting psychological effects, lasting at least six months (**Table 2**). Psilocybin induced variable acute subjective psychoactive effects (**Fig 2**) and not associated with changes in blood pressure (**Fig 3**). Interestingly, one patient (Patient 6) reported complete absence of psychoactive effects of psilocybin. Objective effects of psilocybin (e.g., dilated pupils) were also imperceptible to the staff. This patient displayed the lowest C_{max} PPL of all patients (5.9 μg/L).

Prophylactic Effects of Psilocybin

The primary analysis was prophylactic effects of psilocybin and was evaluated by comparing the four-week BL to the four-week FU. We observed a statistically significant reduction in headache frequency from mean (SD); median[25^{th},75^{th}] = 13.3 (7.3); 11.4[8.9, 16.1] attacks/week at BL to mean (SD); median[25^{th},75^{th}] = 9.8 (7.4); 9.8[4.4, 13.3] attacks/week at FU (P_{FWER} = 0.008, mean change (SD) = -3.57 (3.87) attacks/week, percent change (SD): -31 (31)% (**Fig 4 and 5**)). One subject had more than 50% reduction in attacks per week. Notably, this patient (Patient 4) experienced complete remission for 21 weeks, which began one day after the first psilocybin session. Effects of psilocybin treatment on average self-rated pain intensity showed a statistically significant reduction from BL to FU (P_{FWER} = 0.03, mean change (SD) = -0.89 (0.94) pain intensity, percent change (SD): -13 (15) % (**Fig 5)**). Although numerically reduced, the use of oxygen for acute attacks was not significantly changed from BL (average number of weekly use (SD); median[25^{th},75^{th}] = 48.1 (56.1)); 33.3[0, 74.4] to FU (average number of times oxygen used per week = 37.8 (51.8); 21.8[0, 39.4], $P_{uncorrected}$ = 0.3). Similarly, the use of subcutaneous triptan was not statistically significant from BL (average number of weekly administrations (SD); median[25^{th},75^{th}] = 20.5 (30.6); 21.9[0, 39.4]) to FU (average number of weekly

administrations (SD); median[25^{th},75^{th}] = 13.3 (29.0); 0[0; 39.4], $P_{uncorrected} = 0.07$). Self-reported average daily CH attack duration is visualized in **Fig 6**.

Hypothalamic Functional Connectivity and Treatment Effect

After analyzing prophylactic effects, we investigated effects on hypothalamic FC. Three clusters showed significant positive FC with the hypothalamic seed across BL and FU scans: 1) a large diencephalic cluster encompassing the hypothalamus, thalamus, caudate and brain, 2) a cluster in the left lingual gyrus and 3) a cluster in the right cerebellum (**Table 3**). One cluster showed negative FC: a cluster in occipito-temporal white matter. Linear regression analysis showed a significant negative correlation between percent change in CH attack frequency and change in hypothalamic FC with the diencephalic cluster ($p_{FWER} = 0.03$, R = -0.81, **Fig 7**). We found no significant associations for the remaining three FC estimates (P-values > 0.7) and no significant change in hypothalamic FC from BL to FU (P-values > 0.6).

Exploratory Post Hoc Networks Functional Connectivity Analysis

The exploratory post hoc networks FC analysis did not show statistically significant changes (BL vs FU). Also, we observed no significant associations between percent change in headache frequency and changes in network FC (**Fig 8**).

Plasma psilocin level and treatment effect

We observed substantial interindividual variability in PPL (**Fig 9**): maximum PPL concentration ($C_{max)}$) (mean (SD) =11.8 (4.3) μg/L, median[range]=10.4[5.9;17.9] μg/L and area under curve (AUC) (mean (SD) 1711 (682) μg*min/L, median[range]=1483[968;2615] μg*min/L. PPL from one patient (Patient 2) was not included due to missing data (IV access failure). Exploratory linear regression analyses did not show significant associations between PPL C_{max} or AUC with change in attack frequency or pain intensity (all P-values > 0.9).

Discussion

Here, we evaluated prophylactic psilocybin effects in CCH and possible relations to hypothalamic FC. The treatment was safe, well tolerated and was associated with a substantial but variable reduction in CH attack frequency, supporting that psilocybin therapy may constitute a valuable therapeutic option in some patients with CCH. Our data suggest a transitional effect of psilocybin as the reduction in CH attack frequency persisted beyond the pulse of drug administration, with one patient displaying a 21-week complete remission from CH attacks. Such an effect, which lasts longer than the acute pharmacological effect, is not dissimilar from other transitional headache treatments, such as DHE or steroid pulses or occipital nerve blocks. [29]

We observed a negative correlation between percent reduction in attack frequency and FC changes between the hypothalamic seed and a large diencephalic cluster (**Fig 7**). Hypothalamic dysfunction is a prime candidate for pathophysiological involvement in CH, as supported by several neuroimaging studies.[10–13,30] Increased hypothalamic-diencephalic FC in patients with CCH has previously been observed, and the authors speculated that this signified an imbalance between the orexin/hypocretin system in the hypothalamus and dopaminergic pathways in diencephalon.[31] Although we are unable to measure direct alterations in dopamine or orexin neurotransmission, our data support modulation of hypothalamic-diencephalic neuronal pathways in psilocybin-induced treatment response to psilocybin. Although our results are intriguing, the present sample size was small and more studies are needed to confirm this finding.

Psilocybin acutely alters the brain's normal functional architecture, including reduced functional integrity of individual functional networks (i.e. reduced FC), increased FC between networks, and increased thalamic FC.[17] Accumulating evidence suggest increased neuroplasticity

after psilocybin, including increased dendritic spines and synaptic density as observed in preclinical studies.[32–34] We speculate that a recalibration of aberrant hypothalamic-diencephalic neuronal pathways, supported by increased neuroplasticity, may underlie part of psilocybin's prophylactic potential in CH.

We conducted a post hoc exploratory networks analysis, evaluating FC changes within and between seven networks (BL vs FU). We also assessed associations of percent headache frequency change with network FC changes. Previous studies found higher FC in the fronto-parietal control network in out-of-bout ECH patients relative to healthy controls[35] and wide-spread FC alterations within networks comparing patients with ECH to healthy controls.[36] Acutely, psilocybin reduces FC within and increases FC between networks in healthy individuals.[17,37] Our study design does not allow for a direct comparison with previous studies, however we do not observe evidence for substantial protracted effects of psilocybin on network FC, indicating that alterations in cortical networks may not be important for therapeutic effects of psilocybin in CCH.

A recently published small exploratory placebo-controlled trial, showed a large effect size (d=1.25) in patients with CCH (n=8).[9] The reduction in CCH attacks were 2.8 per week, which is comparable to the average reduction of 3.6 attacks per week we observe. Together these findings suggest that larger trials are warranted to evaluate prophylactic effects.

Interestingly, one patient in our study reported no subjective psychoactive effects and did not show expected signs of psilocybin (e.g. dilated pupils). We did not identify potential medications likely to alter the response to psilocybin (e.g. antidepressants, antipsychotics etc.) in this person or the other patients. Although this patient displayed the lowest level of PPL, consistent with limited effects, we have previously observed psychoactive effects at this PPL in

healthy individuals.[16,17] Interestingly, the patient displayed a reduction in headache frequency of 22% from BL to the FU, indicating a possible prophylactic effect even in the absence of psychoactive effects. This is also supported by a previous study on the non-psychedelic LSD analogue 2-bromo-LSD (also known as BOL-148), which showed promising clinical effects in a small open-label study[38] and case-reports in patients with depression.[39,40] More generally, this supports the idea that it may be possible to induce clinically beneficial effects, using non-psychedelic 5-HT2AR agonists, which some researchers are actively pursuing.[41]

Importantly, one patient (Patient 5) reported substantially enhanced quality of life despite long-term unchanged headache burden (**Table 2**). This is consistent with long-lasting subjective psychological benefits in both patients and healthy individuals.[42] It is possible that psilocybin treatment could modulate both the disease burden itself and, in the proper context, the psychological response to the disease burden. Thus, psilocybin therapy may constitute a valuable intervention in patients with CCH or other chronic pain conditions.

Although psilocybin overall has a favorable safety profile,[43] it is important to emphasize that psilocybin has potent psychedelic properties, is illegal in most countries, and that serious negative health-related outcomes have been observed in non-clinical settings due to poorly regulated behavior.[44] Thus, we caution that our results are preliminary and advise that patients abstain from self-medicating with psychedelics.

Our study is not without limitations. Our original aim of including 20 patients was hampered by Covid-19-related societal and institutional restrictions and data from ten patients only were available for data analysis (nine for fMRI analysis). Although previous CH neuroimaging studies employ also small sample sizes (presumably due to the inherent difficulties with conducting CH studies), we recognize that our small sample size limits the ability to draw

strong statistical inference, especially concerning the fMRI analysis, and to observe rare side effects. We increased the fMRI scan acquisition time from five to ten minutes after the first five patients, and future studies should consider evaluating effects of acquisition time on FC measures for a this or a similar sequence. We chose to evaluate percent change of headache frequency changes with FC changes, and future studies should consider employing a larger sample size and complementary analytical strategies (e.g., using absolute changes instead of percent change). Three patients used intranasal triptan during the study, and this use was not recorded in the daily headache logbook, and the present study is thus unable to evaluate effects of psilocybin on intranasal triptan use in CCH. Patients provided for each day one overall estimate for attack duration and pain intensity, and future studies may improve the data quality by collecting duration and pain intensity data for each individual CH attack. Further, we did not employ a placebo-control condition. The included patients were relatively treatment refractory to standard prophylactic treatment and recruited from a tertiary headache center; this may limit the generalizability to other CH-patients. Intake of prophylactic medication was not allowed in our trial, and it is also possible that our results do not generalize to patients on regular prophylactic agents. We used a fixed dose, which was relative to bodyweight, however it is possible that further treatment effect occurs at a higher dose.

In conclusion, the administration of three moderate doses of psilocybin, conducted in a controlled clinical setting, was feasible and the well-tolerated by all patients. Both attack frequency and pain intensity decreased after the treatments. Changes in hypothalamic-diencephalic FC correlated negatively with percent change in attack frequency, implicating a neural pathway in treatment response. Although encouraging, future placebo-controlled studies

in carefully controlled settings are needed to confirm the clinical utility of psilocybin in CH prophylaxis.

Author Contributions

Conceptualization: MKM, GMK, RHJ, ASP, DSS.

Grant applications: MKM, GMK, RHJ. Data collection: MKM, ASP, DSS, IMS, HS, TF, CHN, SMUL, MG, TM, TLK, OOH, KBB. Psilocin analysis: KL, SSJ. Data analysis: MKM, PMF, GMK. Writing original draft: MKM, GMK.

All authors have engaged in interpretation of the data, critical review of the present manuscript, and have approved of and are responsible for the final version.

Data Availability

Data can be made available upon reasonable request, as permitted by applicable law.

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Tables

Patient	Sex	Age	Years since onset	Years since chronic	Previous prophylactic medication	Current abortive treatment	Neuro-stimulation device	Previous transitional treatment	Adverse event
1	F	50s	51	13	Verapamil, candesartan, lithium, gabapentin, indomethacin, amitriptylin	Oxygen, triptans s.c. & nasal and SPG stimulation	SPG	Prednisone, GONB	CH attack (dose 1 and 2)
2	F	50s	6	6	None	Oxygen	No	None	Nausea & facial muscle spasms (dose 1)
3	M	30s	11	11	Verapamil	Oxygen	No	GONB	Nausea (dose 1 & 2), irritability (dose 2)
4	M	40s	5	5	Verapamil	Oxygen & oral triptan	No	GONB	No adverse events
5	F	50s	19	11	Verapamil, lithium, topiramate, gabapentin	Triptan s.c. and SPG stimulation	SPG	Prednisone, GONB	No adverse events
6	F	50s	19	19	Verapamil, topiramate, gabapentin, indomethacin, candesartan, pregabalin	Oxygen	No	Prednisone, GONB	No adverse events
7	F	20s	9	7	Verapamil, lithium, indomethacin, candesartan	Oxygen & triptan s.c.	SPG	Prednisone, GONB	Lethargy (dose 1)
8	M	60s	10	10	Verapamil, lithium, topiramate, gabapentin, indomethacin, candesartan	Oxygen, triptans s.c. & nasal	No	GONB	CH attack & nausea (dose 1 and 2)
9	M	40s	29	2	None	Oxygen, triptans s.c. & nasal	No	None	CH attack (dose 1)
10	Male	60s	31	11	Verapamil, lithium, topiramate, gabapentin, indomethacin	None or SPG stimulation	SPG	Prednisone, GONB	CH attack (dose 1, 2 & 3), fatigue after dose 3

Table 1. Characteristics of study patients and reported adverse events. F: female, M: male. SPG: sphenopalatine ganglion stimulation device, GONB: greater occipital nerve block, s.c.: subcutaneous.

Patient	Verbatim Comment
1	Good experiences all three. Each time I had been there, it seemed the effect waned. Not something I feel like going to do for regular treatment. I didn't like the loss of control and therefore don't want to do it again. I didn't feel bad after, no bad after effects. I can relive the experience by listening to music, see colors for my inner eye. Wouldn't take it at home, but would like to take it under controlled conditions.
2	I had two good experiences and one experience that was sort of scary. But I felt well taken care of. But the experiences wouldn't deter me from doing it again. I guess that it is a treatment that would have to be repeated. If it were possible, I would try it again.
3	I had good experiences. I would be willing to trying it again.
4	I had no problems and felt completely safe. However, I can imagine that for some people it would not be something to do, like if you had had a psychosis.
5	I felt very safe. Nothing negative to say. I would recommend anyone with CH to do it because of the psychological benefits. Right now I'm going for a walk in the woods and enjoying it even though I just had 2 days of living hell due to cluster headache attacks. This is a substantial change for me, it has improved my life quality.

6	I felt safe, well taken care of. I had no subjective effects. I would like to participate again with a higher dose.
7	NA
8	It was a huge experience, my body was relaxed. I am more relaxed now during attacks than before. It was a safe experience all the way through. I think this could be taken further as a treatment.
9	Good experience, it's probably important not to be afraid of taking the drug. I think I had a partial effect on my cluster headache. Probably a bit anxiety-provoking if you haven't tried it before. I feel I learned some things about myself. The three times I took psilocybin, the experiences were very different. The last one I was very much out of my body.
10	I found it safe and very pleasant. I would like to try it every week.

Table 2. Patient comments about drug administration and therapeutic effects.

Cluster	Peak voxel MNI coordinate	Size (voxels)	Size (mm^3)	Peak voxel T-score
1. Diencephalon: hypothalamus, thalamus, caudate, brain stem	+2,-10,-10	3294	26352	40[a]
2. Left lingual gyrus	-18, -44, 0	339	2712	7.5
3. Right cerebellum	14, -68, -12	221	1768	6.14
4 Occipito-temporal white matter	-30, -36, 26	158	1264	-6.8

Table 3. Clusters exhibiting significant functional connectivity with hypothalamus seed.
MNI: Montreal Neurological Institute. [a]Cluster includes seed region.

Figures & legends

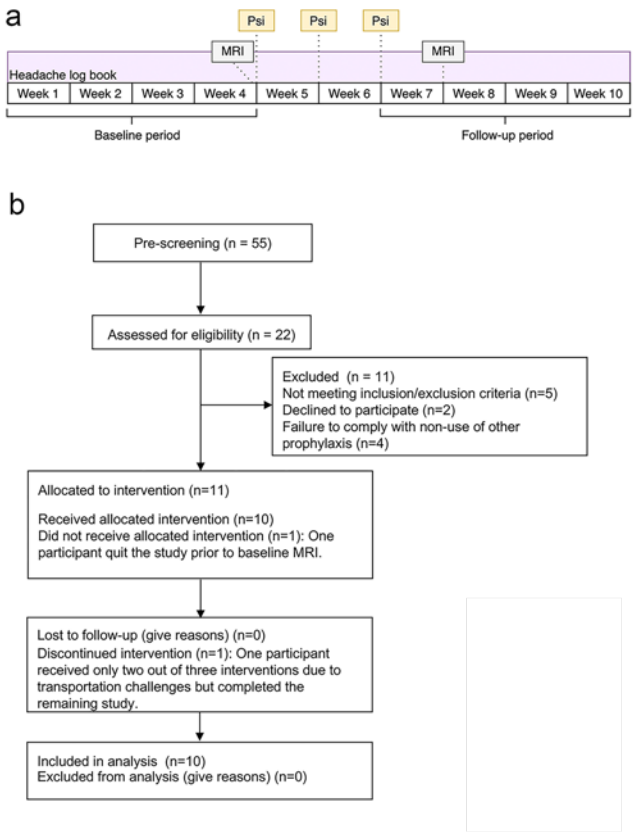

Fig 1. a) Study design. MRI: magnetic resonance imaging. Psi: psilocybin treatment. MRI data acquisition was done the day before the first psilocybin treatment and one week after the last treatment. Plasma psilocin level (PPL) was measured during the first treatment only; psychoactive effects and blood pressure were measured on all treatment days. **b)** Flow-chart of study.

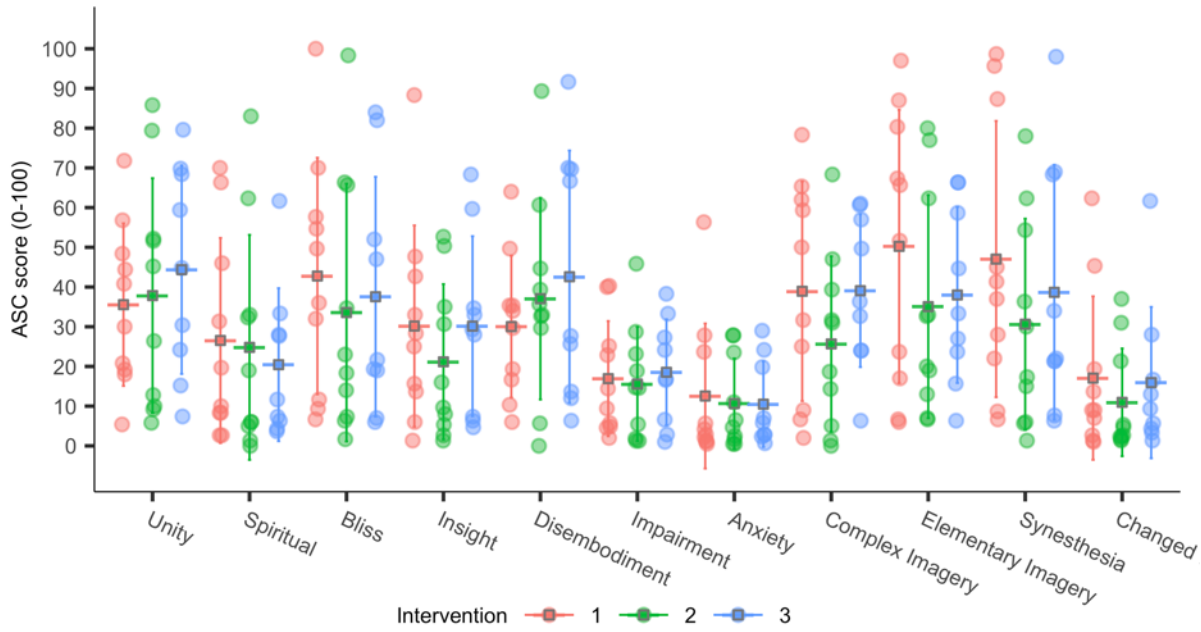

Fig 2. Psychometric evaluation of psychoactive effects. Scores on the 11-dimension Altered States of Consciousness (ASC) questionnaire rated at the end of each treatment day. Error bars show mean and standard deviation.

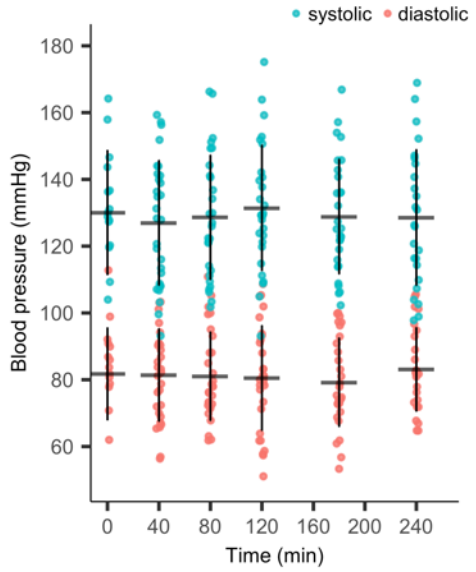

Fig 3. Blood pressure. Measurements pooled across all three treatments. Time is after psilocybin administration.

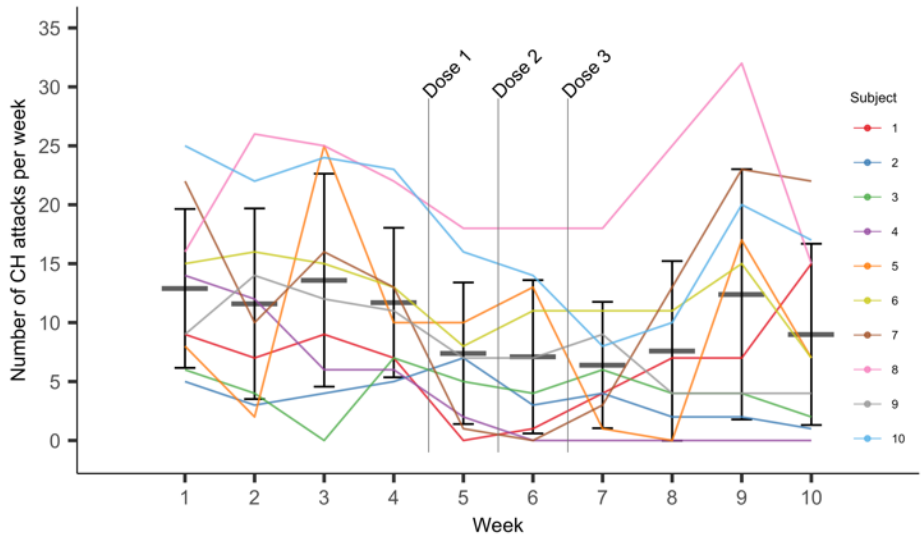

Fig 4. Average weekly cluster headache attacks. Patient 8 did not undergo the third treatment.

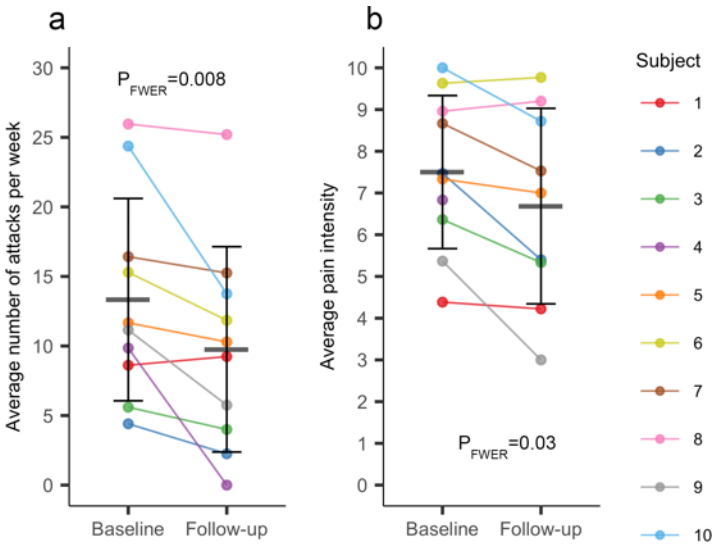

Fig 5. Clinical effects of psilocybin. a) Effects of psilocybin on weekly CH attack frequency. **b)** Effects of psilocybin on average pain intensity. Statistical test: Wilcoxon signed-rank test. Baseline: four weeks preceding the first psilocybin dose. Follow-up: four weeks after the last psilocybin dose.

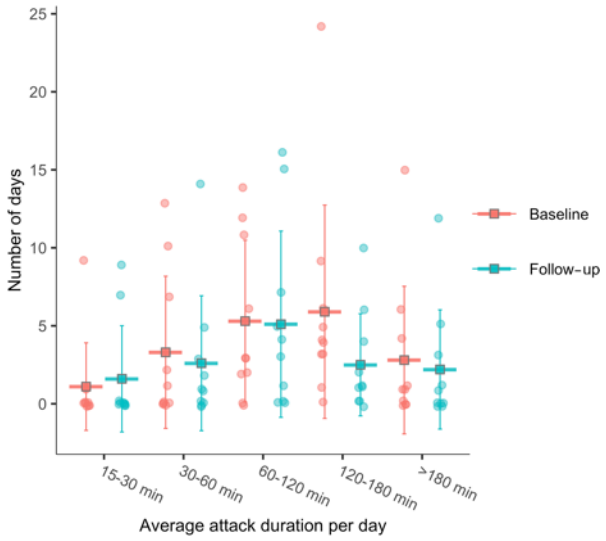

Fig 6. Average cluster headache attack duration at baseline and follow-up. Patients rated the average duration of all CH attacks experienced per day.

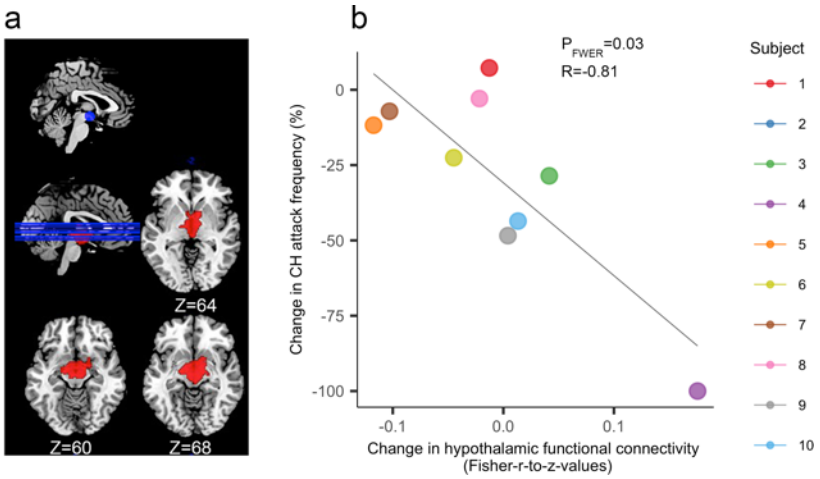

Fig 7. Hypothalamic functional connectivity and clinical response to psilocybin. **a)** Hypothalamic 8 mm radius sphere seed (blue) centered on posterior hypothalamus and diencephalic cluster (red) exhibiting significant functional connectivity with the seed region across baseline and follow-up scans. **b)** Negative correlation between percent change in attack frequency and change in functional connectivity between seed region and diencephalic cluster. Statistical test: simple linear regression.

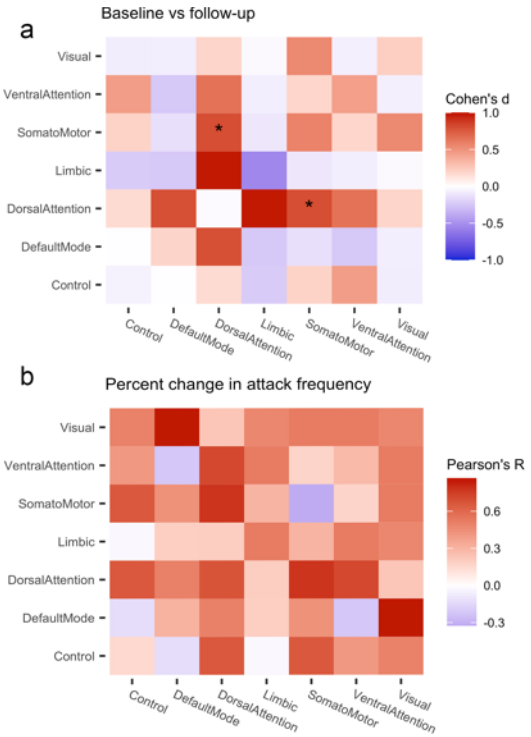

Fig 8. Exploratory functional networks analysis. a) Heat map shows Cohen's d effect size estimates of change in functional connectivity from baseline to follow-up within and between functional networks. b) Heatmap showing correlation between percent change in attack frequency and change in functional connectivity from baseline to follow-up. *P<0.05, **P_{FWER}<0.05.

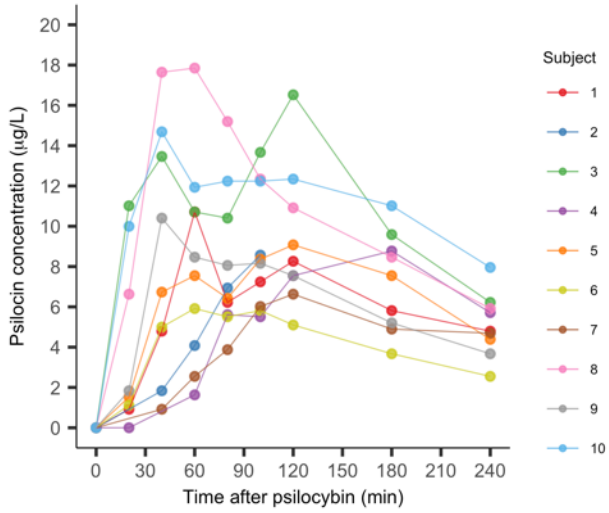

Fig 9. Plasma psilocin concentration. Individual subject PPL (plasma psilocin level) measured at the first treatment. It was not possible to obtain blood samples after the 100 min measurement for Patient 2 due to IV access failure.