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Safety, Feasibility, and Potential Clinical Efficacy of 40 Hz Invisible Spectral Flicker versus Placebo in Patients with Mild-to-Moderate Alzheimer's Disease: A Randomized, Placebo-Controlled, Double-Blinded, Pilot Study

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Abstract.

Background: Recent studies suggested induction of 40 Hz neural activity as a potential treatment for Alzheimer's disease (AD). However, prolonged exposure to flickering light raises adherence and safety concerns, encouraging investigation of tolerable light stimulation protocols.

Objective: To investigate the safety, feasibility, and exploratory measures of efficacy.

Methods: This two-stage randomized placebo-controlled double-blinded clinical trial, recruited first cognitive healthy participants ($n = 3/2$ active/placebo), and subsequently patients with mild-to-moderate AD ($n = 5/6$, active/placebo). Participants were randomized 1:1 to receive either active intervention with 40 Hz Invisible Spectral Flicker (ISF) or placebo intervention with color and intensity matched non-flickering white light.

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Results: Few and mild adverse events were observed. Adherence was above 86.1% of intended treatment days, with participants remaining in front of the device for >51.3 min (60 max) and directed gaze >34.9 min. Secondary outcomes of cognition indicate a tendency towards improvement in the active group compared to placebo (mean: –2.6/1.5, SD: 6.58/6.53, active/placebo) at week 6. Changes in hippocampal and ventricular volume also showed no tendency of improvement in the active group at week 6 compared to placebo. At week 12, a potential delayed effect of the intervention was seen on the volume of the hippocampus in the active group compared to placebo (mean: 0.34/–2.03, SD: 3.26/1.18, active/placebo), and the ventricular volume active group (mean: –0.36/2.50, SD: 1.89/2.05, active/placebo), compared to placebo.

Conclusion: Treatment with 40 Hz ISF offers no significant safety or adherence concerns. Potential impact on secondary outcomes must be tested in larger scale clinical trials.

Trial registration: This trial was registered at www.clinicaltrials.gov identifier: NCT04574921

Keywords: 40 Hz, Alzheimer's disease, gamma entrainment, invisible spectral flicker, light-based neurostimulation

INTRODUCTION

As the number of patients suffering from Alzheimer's disease (AD) increases, so does the need for novel effective disease-modifying treatments [1, 2]. Although research towards such disease-modifying treatments is vast [3], many have failed to show clinical improvement. Recently the monoclonal antibody Aducanumab failed to show clinical efficacy but obtained a US limited approval based on surrogate endpoints [4, 5]. Another monoclonal antibody, Lecanumab have just shown clinical efficacy in a large scale phase III clinical trial [6]. Although these antibodies are promising, the efficacy is still limited and there are concern of the safety and large scale implementation [7]; novel treatment option are thus still needed.

A prevailing target for treatment of AD is the protein deposits of amyloid-β (Aβ) and phosphorylated tau (p-tau) [3] which are among the hallmarks of AD pathology [2]. Targeting Aβ and p-tau has been tested in numerous trials with limited clinical success. One explanation may be that Aβ and p-tau are not the cause of the disease but rather a result or epiphenomenon of underlying pathological processes with maybe even a protective effect [8].

In addition to the molecular hallmarks of AD, a growing body of evidence is emerging that links disturbances of neural networks to AD, especially in the gamma frequency band [9, 10]. A novel approach of restoring these disturbances in the gamma frequency band using induction of 40 Hz neural oscillations has been suggested [11] using various entrainment methods, such as stroboscopic light [12], stroboscopic light and sound [13], and vibrotactile stimulation [14]. Induction of 40 Hz neural activity through non-invasive 40 Hz stroboscopic light not only reduced Aβ and p-tau in transgenic mice, but also improved visuospatial memory [13, 15, 16]. Stroboscopic light [12] and a combination of stroboscopic light and sound [17, 18] was also tested in patients with AD for a treatment duration of 60 min per day, without clear evidence of clinical efficacy. Another study using 40 Hz vibrotactile and sound stimulation found improvement in the St. Louis University Mental Status Test following 6 weeks of treatment.

Light-based 40 Hz entrainment for the treatment of AD is usually done with a treatment duration of minimum 60 min per day [12, 17, 19]. This treatment duration is however arbitrary and other entrainment methods have used shorter duration for the treatment of AD [14]. In fact, there are various trials using 40 Hz entrainment in other diseases that are utilizing different entrainment method treatment duration that can vary from 23 min exposure once a week to 30 min exposure five times per week [20–23]. However, preclinical trials indicated that the amyloid lowering effect of 40 Hz entrainment in AD diminishes with time from last treatment and almost disappears after 24 h [16], indicating that daily, prolonged, likely lifelong treatment is likely needed. Regardless of what the optimal treatment duration is, daily treatment of 40 Hz entrainment can be quite uncomfortable and raise adherence concerns. Therefore, developing novel solutions that can not only stimulate neural activity in the brain but also potentially arrest disease progression while increasing comfort during treatment is imperative for real-world application and to improve the quality of life for patients.

This study utilized a novel invisible spectral flicker (ISF) [24] rather than stroboscopic light, which involves using color fusion to achieve induction of

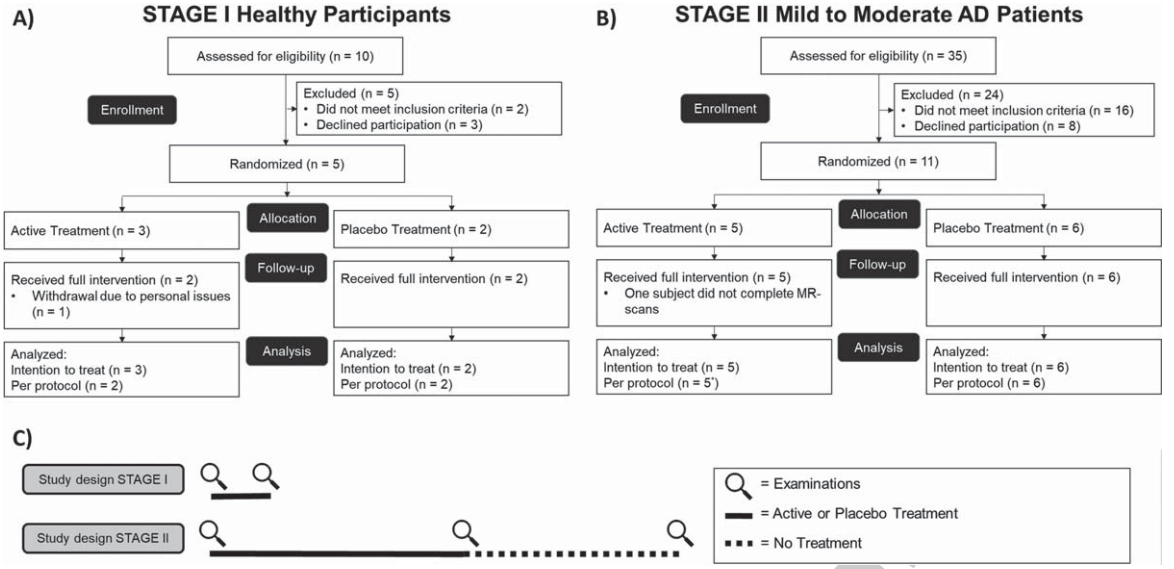

Fig. 1. Consort diagram and study design. A) Diagram of recruitment, and completion for STAGE I. B) As A but for STAGE II. C) Graphical illustration of the study design for STAGE I and STAGE II.

40 Hz neural activity without the perception of flickering light [25]. The use of ISF may help reduce the discomfort for the recipients, but more importantly, allows for trials with a high-quality placebo treatment, as the 40 Hz ISF is practically indistinguishable from non-flickering white light. Effects of induction of 40 Hz neural activity with ISF has previously only been tested in young healthy participants in an experimental setting with one session [25]. In contrast, this study investigated the safety and feasibility of 40 Hz ISF when given to healthy elderly participants and patients with AD for 1 h daily over a period of one to six weeks. To do this, a two-stage pilot study was designed in which STAGE I tested 1-h daily treatment with 40 Hz ISF in a group of healthy elderly subjects ($n = 5$) while STAGE II investigated the intervention in patients with mild to moderate AD ($n = 11$). The intervention lasted for 1 week in STAGE I and 6 weeks in STAGE II and in STAGE II all participants were re-examined at week 12 to assess any lasting or delayed effects (see Fig. 1C for illustration of study design). Following safety and feasibility assessment, clinically relevant outcomes were examined in an exploratory manner to determine if 40 Hz ISF may affect cognitive function. We further hypothesized that the amyloid lowering effect seen in the pre-clinical trials might affect disease progression by reducing atrophy rates based on brain volume measured structural MR images.

MATERIALS AND METHODS

Study design

A two-stage setup was utilized, with a 1:1 randomization to either an active intervention with 40 Hz ISF or a placebo intervention with color and intensity matched non-flickering white light. As treatment with intensity matched non-flickering white light is attempted for the first time by anyone, the need to examine the usage of ISF for safety purposes is needed. Therefore, STAGE I of the pilot study recruited five healthy elderly individuals to examine ISF exposure on the safety and adherence of the device prior to examining the effect of ISF light in AD participants. Three participants received active ISF intervention, whereas, the other two participants received placebo light that was intensity (200–900 lux at 100-33 cm) and color matched (color temperature 3191 K, CIE 1931 chromatic color coordinates (x,y) = (0.410, 0.367). Upon completion of STAGE I, mild to moderate AD participants ($n = 11$?) were recruited for a subsequent STAGE II trial. Five participants received active ISF intervention, whereas six participants received placebo. For completeness, all assessments were performed on all participants (STAGE I and II).

Participants were required to use the Light Therapy System (LTS) (Optoceutics ApS, Copenhagen Den-

mark) intervention within arm's reach (50–100 cm) daily for 1 h before noon, to avoid potential influence on sleep patterns. Additionally, participants were encouraged to direct their gaze towards the LTS as much as possible. Identical LTSs were randomized to deliver either active or placebo intervention prior to initiation of the study. The LTS is similar to the one used in a previous study that examined ISF effect on brain entrainment [25]. The intervention in the first stage (STAGE I) lasted for 1-2 weeks and for 6 weeks ± 7 days in the second stage (STAGE II). Stage II only initiated after the completion of stage I. Participants in STAGE II were followed for an additional 6 weeks ± 7 days with no intervention to examine any delayed or potential lasting effects. Thus, participants in STAGE I ($n = 3/2$, active/placebo) were examined at baseline and at week 1, whereas participants in STAGE II ($n = 5/6$, active/placebo) were examined at baseline, week 6, and week 12 (Fig. 1C).

Study population

STAGE I and II examined different study populations. Participants in STAGE I were cognitively healthy elderly ($n = 3/2$, active/placebo), whereas, in STAGE II participants were patients with mild to moderate AD ($n = 5/6$, active/placebo) [26]. STAGE I recruited only 5 participants with sole purpose of identifying major problems with novel treatments such as the ISF device and give confidence to continuation into STAGE II with AD participants. To significantly determine whether ISF may induce adverse events and can be adhered to, 11 participants (recruitment was continued until 10 participants were completed, therefore, an additional participant was included in the STAGE II) were recruited in STAGE II.

Participants in each study were prescreened to ensure reasonable likelihood that they fit the inclusion/exclusion criteria which was confirmed at inclusion (Inclusion: age 55–80 years, >8 years of education, Ishihara colorblindness test >17, Wi-Fi internet access at home, probable mild to moderate AD*, Mini-Mental State Examination (MMSE) score 10–27*, Montreal Cognitive Assessment score 6–21*, Cognitive healthy caregiver capable of daily assistance with treatment sessions*. Exclusion: visual acuity <0.5 or anopsia, history of neurological or psychiatric disorder, use of antiepileptic or sedative medication, history of substance abuse, planned hospital admission for other disease within the duration of the study at inclusion, Dysregulated diabetes, use of >1 Alzheimer's medication, contraindications to MR-scans) criteria were applied for the recruitment). *Only applied in STAGE II.

Participants in STAGE I were recruited amongst close relatives of patients with AD, usually spouses. This ensured that the participants of STAGE I had similar technical skills as the AD patients would have had prior to their illness, thus, giving the most realistic measures of feasibility and adherence in STAGE I.

Primary outcomes

Adverse events

The primary outcomes variables were safety and feasibility. Safety was measured by the number of adverse events (AEs), which were categorized by severity (severe, moderate, or mild) and based on their relationship to the intervention (probable, possible, or unlikely). The evaluations and recording of the AE were performed by a medical doctor according to the European MDCG (the Medical Device Coordination Group) guidelines on AE reporting. Participants were asked to report any adverse symptom or reaction that might be correlated with the intervention and the electronic patient journal was monitored to detect contact with the healthcare system (primary sector not included). In addition, medical doctors conducted a single visit per participant in STAGE I and three visits per participants in STAGE II to inquire and record whether there were incidences of AEs. During these visits, the medical doctor did not specifically suggest any possible symptoms to ensure that the participants were not biased.

Feasibility

The feasibility of the intervention was also examined in both STAGE I ($n = 5$) and II ($n = 11$). Feasibility is defined as adherence to the intervention. Adherence was measured by a built-in camera in the LTS device. The camera recorded images at a sampling rate of 1 Hz. These images were used to estimate gaze direction and detect presence of a face before the image was discarded to ensure privacy of the participants. Three measures of feasibility were used: 1) Percentage of days with use of the device, defined by switching on the device; 2) Mean minutes per day present in front of the device, determined by detection of a face; 3) Mean minutes per day of direct gaze towards the LTS device. Gaze directions are calculated based on the angles of the pupils using the assumption that the eyes are perpendicular to the device and constantly at 60 cm distance.

Secondary outcomes

The secondary/exploratory outcomes were defined prior to study initiation. Cognitive function assessment using The Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-Cog) plus executive functioning (EF) and functional ability (FA) (ADAS-Cog + EF&FA) [27] and brain morphology by structural MRI (T1 MPRAGE MR scan, Slice thickness: 0.9 mm, Field of view: 230 mm, Repetition time: 2000 ms, Echo time: 2.41 ms, Flip Angle: 9 deg and Acceleration Mode: GRAPPA, with a 64-channel receive coil, 3T Siemens Magnetom Vida) were examined to assess possible intervention effects.

Data analysis

Differences in primary outcomes for active and placebo intervention were examined using students *t*-test in RStudio (R.4.0.2). Secondary and exploratory outcomes are examined by graphical representation using matplotlib (3.5.2) running in Python3 and as summary statistics in parenthesis as active/placebo.

An algorithm embedded in the LTS device was used to calculate gaze direction. Firstly, it used the face detection algorithm available in the dlib library (dlib ver. 19.21.1), which is built using Histogram Oriented Gradients for feature extraction and a linear Support Vector Machine (SVM) for face and landmark classification [28]. From these landmarks the position of the eyes was extracted and the location of the pupil relative to the eyeball was used to compute the angle of gaze direction.

ADAS-Cog + EF& FA were translated to Danish and validated by experienced bilingual professionals. The individual tasks were summarized to a total score (0 to 135, a higher score reflects greater cognitive impairment) using a pre-defined scoring sheet (Supplementary Table 1) adapted from [27] to incorporate all scores.

MR scans were used to analyze changes in the volume of the hippocampi and whole brain atrophy, using the total volume of the ventricles as a marker of central/whole brain atrophy. Longitudinal pipeline in FreeSurfer v7.2.0 [29] with the -3T handle, automatically analyzed the images to estimate volumes. To quantify the quality a T1 images, an Image Quality Rating (IQR) was calculated in CAT12 [30] running in the SPM12 extension to MATLAB (MathWorks, Inc., Natick, MA USA). Generally, IQR >60 % is considered acceptable, but the IQR is a composite of three measures (resolution, noise, bias). Thus, high-resolution images, as in this study, are at risk of having acceptable >60 % IQR while still having significant noise or bias. Consequently, this study will exclude images with an IQR >2 SDs from the mean IQR.

All included participants ($n = 5$ STAGE I, $n = 11$ STAGE II) were included in the analysis of primary outcomes with the intention to assess the population effects. Analysis of secondary/exploratory outcomes used the per-protocol population which included only participants ($n = 4$ STAGE I, $n = 11$ STAGE II) with available data. Missing observations were adjusted for using a last observation carried forward approach, or in case of a missing baseline value the mean of later values were carried backwards.

Ethics approval and consent to participate

All participants provided informed consent after receiving careful verbal and written information. Participation did not affect their treatment outside the study. The scientific ethics committee of Region Zealand (SJ-806) and the Danish Medical Agency (CIV-19-12-031124) approved the project. The Unit for Good Clinical practice at Frederiksberg Hospital monitored the project.

RESULTS

Recruitment and baseline characteristics

Recruitment for STAGE I required screening of ten potential participants of which five were included. One participant in STAGE I withdrew participation after three days of intervention due to personal reasons. Recruitment for STAGE II required screening of 35 potential participants of which 11 were included. All 11 participants completed STAGE II, although one participant did not complete the MR scans due to inability to lie sufficiently still within the scanner (Fig. 1).

At baseline, the active and placebo groups in either STAGE I or II were not significantly different (see Table 1). The variation at baseline characteristics for STAGE I (see Table 1) was small despite the small sample size. For STAGE II, there were more variations, especially for the measures of cognitive performance as expected with the relatively broad inclusion criteria of mild to moderate AD, the variations are similar in the active and placebo groups.

Table 1
Baseline characteristics for STAGE I and STAGE II

Characteristic	STAGE I			STAGE II		
	Active ($n=3$)	Placebo ($n=2$)	p	Active ($n=5$)	Placebo ($n=6$)	p
Sex (Female/Male)	3/0	1/1	0.819	3/2	5/1	0.853
Age (y)	65.7 (9.07)	63 (11.31)	0.808	72.2 (5.16)	68.5 (9.77)	0.446
	56, 74	55, 71		65, 78	55, 78	
ADAS-Cog	4 (2.00)	5 (1.41)	0.561	25.9 (14.92)	19.8 (8.28)	0.500
	2, 6	4, 6		6, 46	10, 33	
ADAS-Cog + EF&FA	18.67 (5.03)	18 (0)	0.839	70.2 (19.38)	63.3 (18.35)	0.565
	14, 21	18,18		50, 95	41, 90	
Months Since Diagnosis	N/A	N/A	N/A	23.6 (17.07)	13.33 (6.22)	0.259
				12, 53	3, 21	
Years of Education	14.33 (3.21)	17 (1.41)	0.299	15 (2.74)	14.5 (3.02)	0.780
	12, 18	16, 18		11, 18	10, 17	
MMSE	28.67 (1.53)	30 (0)	0.269	21 (2.55)	20.17 (5.23)	0.740
	27, 30	30, 30		18, 24	13, 26	
MoCA	29,33 (1.15)	28 (1.41)	0.388	13.8 (5.85)	15.5 (5.65)	0.638
	28, 30	27,29		8, 20	6, 21	

Data presented as mean (±SD) and range (min, max). No statistically significant differences were seen between either groups in STAGE I or STAGE II. Months since Diagnosis are not available (N/A) for STAGE I as these participants were without a diagnosis of AD. Student's t-test were used to compare groups at baseline.

Table 2
Primary outcome for STAGE I and STAGE II. Upper panel: Adverse Events (AEs)

Adverse Events	STAGE I			STAGE II		
	Active ($n=3$)	Placebo ($n=2$)		Active ($n=5$)	Placebo ($n=6$)	
Total AEs	4	2		2	1	
Participants with ≥1 AEs	2	2		2	1	
Relation to Treatment Probable/Possible/Unlikely	0/1/3	1/0/1		0/1/1	0/0/1	
Intervention days (mean, range)	19 (6.33 4–9)	23 (11.5, 10–13)		213 (42.6, 40–47)	290 (48.3, 42–54)	
Feasibility	Active ($n=3$)	Placebo ($n=2$)	p	Active ($n=5$)	Placebo ($n=5^*$)	p
Days, % (±SD)	86.1 (12.7)	95.5 (6.4)	0.42	97.9 (4.7)	90.1 (9.8)	0.15
Time Present min (±SD)	51.3 (10.1)	59.7 (0.6)	0.34	57.1 (6.6)	55.9 (6.2)	0.77
Direct exposure min (±SD)	34.9 (15.7)	41.4 (2.5)	0.61	42.1 (9.6)	45.8 (6.3)	0.49

No serious AEs were observed in this study. STAGE I: 6 total AEs (4 in the active group), 1 AE had a probable relation to the intervention (troubles falling asleep after improper use of the intervention late at night), 1 AE with possible relation to the intervention (headache). STAGE II: 3 total AEs (2 in the active group), 1 AEs had a possible relation to the intervention (eyestrain). The AEs with possible relation to the intervention are categorized as possible as all participants habitually had these events prior to enrollment in the trial. Lower panel: Adherence to intervention measured by: 1) Percentages of days with device turned on of the total number of days. 2) mean time in minutes of being present in front of the device per day, max of 60 min. 3) Mean time in minutes with direct exposure as defined by gaze directed within the diffuser of the device max of 60 min. *One device had a defective data recording resulting in no data, thus excluded from the adherence analysis.

Primary outcomes

The primary outcome of safety and feasibility was analyzed using the intention to treat populations (Table 2). In STAGE I, the active group had four AEs (in two participants) and the placebo group had two (in two participants) during 39 days of intervention (total days: 19/23, mean days: 6.33/11.5, active/placebo), all of which were categorized as mild. One of the AEs in the placebo group had a possible relation to the intervention (trouble falling asleep following improper use late at night) the other AE (headache) were unlikely related to the intervention as it was a preexisting symptom with no worsening. In the active group, one of the AEs in the active group had possible relation to the intervention (fatigue) while the remaining AEs (dizziness, early wakening, and irritability) were unlikely related to the intervention as they occurred during a particular stressful event in the private life of the participant.

In STAGE II, the active group had two AEs (in two participants), and the placebo group had one AE

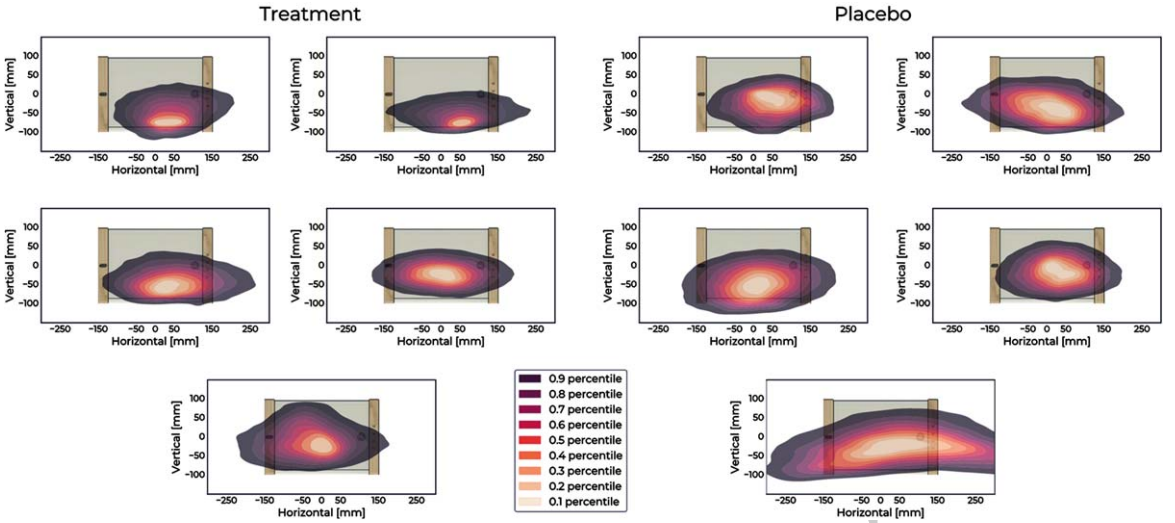

Fig. 2. Heatmaps of gaze direction per participant in STAGE II. The gaze tracking camera was defective in one of the devices resulting in no available data. All participants were looking at the device or in close proximity for most of the time. Most of the participants tend to have their gaze directed at the lower part of the device, this is likely because all participants incorporated the daily session into their breakfast routine, during which they are likely to place food and drinks between themselves and the device. This was allowed to make the treatment more acceptable. Heatmaps for STAGE I see Supplementary Figure 1.

during 509 days of intervention (total days: 212/290, mean days: 42.6/48.3, active/placebo), all of which were mild. One of the AEs in the active group had a possible relation to the intervention (eyestrain), the other AE (erythasma) was unlikely related to the intervention. In the placebo group, one AE was recorded (uticaria) which was unlikely to be related to the intervention as a likely dietary trigger was identified by the non-affiliated treating healthcare provider.

The feasibility of 1-h daily intervention was assessed using three measures of adherence (Table 2). In STAGE I, participants used the intervention for >86 % (mean: 86.1/95.5, $p = 0.42$, active/placebo) of the intended days. During the days with device use, the participants were present for >51.3 min (51.3/59.7, $p = 0.34$, active /placebo) of the 60-min intervention, and had gaze directed at the LTS for >34.9 min (34.9/41.1, $p = 0.61$, active/placebo). Differences in adherence between the active and placebo groups were not statistically significant. In STAGE II, the participants used the intervention for >90.1 % (97.9/90.1, $p = 0.15$, active/placebo) of the intended days, during which they were present for >55.9 min (57.1/55.9, $p = 0.77$, active/placebo) of the 60-min intervention. Their gaze was directed at the LTS for >42.1 min (42.1/45.8, $p = 0.49$, active/placebo). Differences between the active and the placebo group were not statistically significant. Participants tended to direct their gaze at the lower parts of the LTS (Fig. 2 and Supplementary Figure 1).

Exploratory outcomes

The remainder of this section will focus on STAGE II, results from STAGE I are available in the Supplementary Material.

In the active group, 4 out of 5 participants had a lower ADAS-Cog + EF&FA score at week 6 than at baseline compared to 2 out of 6 in the placebo group. At week 12, 3 out of 5 had a lower score than at baseline in the active group compared to 1 out of 6 in the placebo group indicating less cognitive impairment (Fig. 3A). On a group level, the active group had an indication of slight improvement (Fig. 3B) in ADAS-Cog + EF&FA compared to the placebo group at week 6 (mean: –2.6/1.5, median: –1.00/3.50, SD: 6.58/6.53), and at week 12 (mean: –2.2/–0.17, median: –5.0/3.0, SD: 8.78/9.95). Results from the original ADAS-Cog are available for comparative analysis in the Supplementary Material.

One participant, allocated to active treatment, was unable to complete MR scans, and is thus excluded from the analysis of MRI. Quantification of scan quality revealed that all remaining scans had a sufficient quality of >60 IQR (mean IQR = 76.41 std = 4.67); however, two scans that had an IQR >2 std from

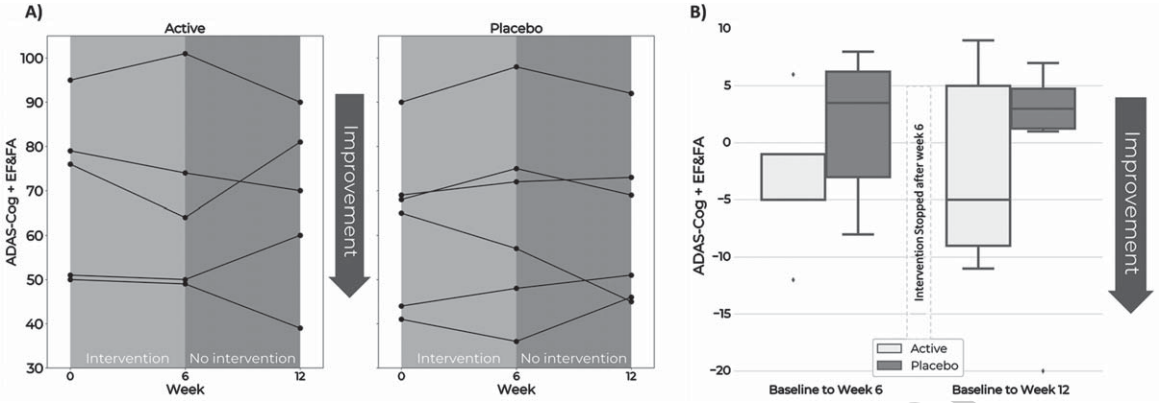

Fig. 3. ADAS-Cog +EF&FA for STAGE II: A) Spaghetti plot of individual participants, 4/5 participants has a lower score at week 6 than at baseline, compared to 2/6 in the placebo group. At week 12 3/5 in the active group has a lower score than at baseline, compared to 1/6 in the placebo group. B) Boxplots of change in ADAS-Cog + EF&FA from baseline to week 6 (Active: mean: –2.6 SD: 6.58, Placebo: mean: 1.5 SD: 6.53) and 12 (Active: mean: –2.2 SD: 8.79, Placebo: mean: –0.67 SD: 9.95). The distributions of the active and the placebo groups are overlapping at week 6 and 12, but at both week 6 and 12 the median for the active group are slight below zero while the median in the placebo group are slightly above zero.

the mean were excluded, and a last observation carried forward approach was used. See Supplementary Figure 2 for full dataset analysis, regardless of data quality, whereas Fig. 4 shows the two scans with bad quality discarded. The measures of volumetric changes in the hippocampus and the lateral ventricle showed high inter-individual variation, which makes tracing of individual change difficult as individual changes are minor compared to the inter-individual variation. The inter-individual variation is accounted for by evaluating the change from baseline rather than absolute values (Fig. 4B, C). At week 6, no difference was observed between the active and placebo group in hippocampal volume (mean: 0.34/0.16, median: 0.62/0.44, SD: 2.51/3.40); however, at week 12, an indication of difference is observed (mean: 0.34/–2.03, median: 1.33/–2.01, SD: 3.26/1.18). A similar pattern is seen for the ventricular volume, with no difference at week 6 (mean: –0.76/–0.48, median: –0.44/–0.34, SD: 1.84/1.13), but a possible indication of difference at week 12 (mean: –0.36/2.52, median: –0.58/1.84, SD: 1.89/2.05). For completeness of the study, Supplementary Figures are supplied for the entire study.

DISCUSSION

During the recruitment process of this two-stage trial, about 2 out of 3 potential participants were rejected mainly due to the eligibility criteria, which is in line with other studies [6]. Although there were larger variations in disease severity at the inclusion point in STAGE II, diagnosis of possible mild to moderate AD was still achievable and confirmed by an unaffiliated treating neurologist with no direct involvement in the recruitment process.

Primary outcomes with 40Hz ISF intervention (safety and feasibility)

The primary objective of this randomized placebo-controlled study was to investigate the safety and feasibility of a novel non-invasive intervention using 40 Hz IS in two population groups, first in healthy elderly participants followed by a separate participants group with mild to moderate AD. The number of participants in the active groups who experienced at least 1 AE was low (STAGE I: 2 out of 3, STAGE II: 2 out of 5), and mild compared to treatment with cholinesterase inhibitors, which have 76 % of participants experiencing at least 1 AE and 11% dropout due to AEs [31]. This study had no dropouts due to AEs. Studies with bright light therapy in patients with AD shows marginally fewer AEs than this study [32, 33]. This difference could be explained by a shorter study duration with bright light therapy. Studies using 40 Hz induction with stroboscopic light in healthy volunteers [34] and patients with AD [12] have not reported on AEs or report similar number of AEs as this study [17]. Interestingly, the healthy elderly participants in STAGE I reported more AEs than the patients with AD in STAGE II despite a smaller sample and a significantly shorter intervention period. The cause of this is outside the scope of this study.

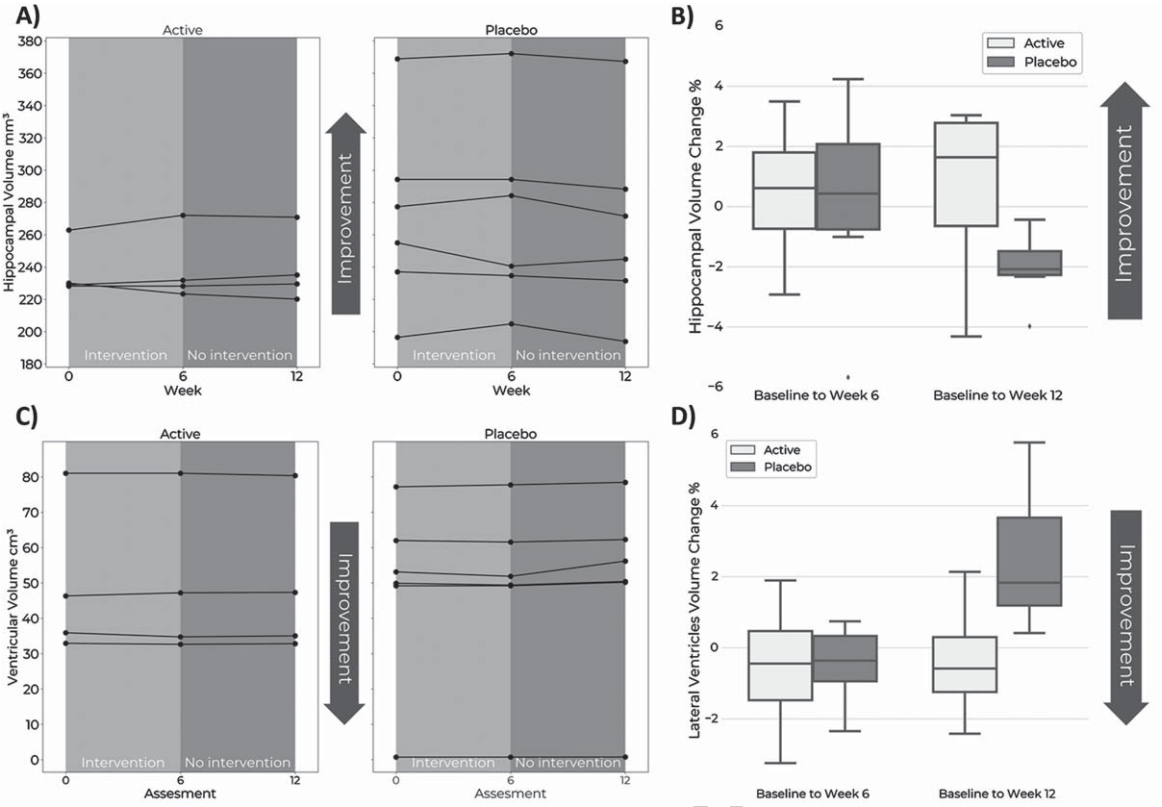

Fig. 4. Volume of the hippocampi and the lateral ventricles for participants in STAGE II. A) Spaghetti plot of hippocampal volume, 2/4 participant had a greater volume at week 6 than at baseline in the active group compared to 4/6 in the placebo group. At week 12, 3/4 in the active group and 0/6 in the placebo group had a greater volume of the hippocampi than at baseline. B) Boxplots of changes in hippocampal volume relative to baseline at week 6 (Active mean: 0.34 SD: 2.51, Placebo mean: 0.16 SD: 3.4) and week 12 (Active: mean: 0.35 SD: 3.26, Placebo: mean: 2.02 SD: 1.18). C) Spaghetti plot of ventricular volume, 3/4 had a decreased ventricular volume at week 6 than at baseline in the active group, compared to 3/6 in the placebo group. At week 12 3/4 participants in the active group had smaller volume of the ventricles than at baseline, compared to 0/6 in the placebo group. D) Boxplot of changes in ventricular volume relative to baseline at week 6 (Active mean: –0.76 SD: 1.84, Placebo mean: –0.48 SD: 1.13) and week 12 (Active mean: –0.36 SD: 1.89, Placebo mean: 2.52 SD: 2.05).

Still, one might speculate that this is caused by AEs usually presenting in the beginning of a new intervention [35], or that AD patients fail to report all incidents due to memory impairment. This study did not actively search for non-symptomatic AEs as it could have benefitted from a more thorough evaluation of potential non-symptomatic AEs such as amyloid related imaging abnormalities [36] or evaluation of eye health as certain bandwidth of light have potential effect on the retina [37].

Feasibility was assessed by evaluating adherence to the intervention. Adherence to this intervention required more from the participants compared to oral treatments, as the participants must remember to start the intervention and then remain adherent for the 1-h of intervention. The simplest estimation of adherence is the number of days with use of the LTS which is similar to adherence to oral treatments with cholinesterase inhibitors for AD [38]. However, participants in this study were also required to remain in front of the LTS for 1 h, which was assessed by the time present. All participants (STAGE I and II) had a mean time present of >50 min (max 60 min), with no meaningful difference between the active and placebo groups. Time present will never reach the full 60 min, as the detection of presence is sensible to movement and change in lighting conditions. Participants were also encouraged to direct their gaze to the LTS as much as possible, as in other studies [12, 17, 34]. Participants were able to direct their gaze for most of the 60 min and for the majority of the time present, which may be interpreted as the intervention are relatively little associated with discomfort. Regardless of the measure of adherence, there were no significant differences between the active and placebo groups, which may be explained by the lack of perceptual dif-

ference between the placebo light and the active 40 Hz ISF [24], indicating that 40 Hz ISF is associated with similar amount of discomfort as the placebo which is analogous to bright light therapy. Participants preferred to direct their gaze to the lower part of the LTS, which is likely caused by all participants incorporating the intervention with their breakfast, thus having food and drinks between themselves and the LTS. Whether direct gaze at the device is needed for 40 Hz entrainment is unknown. Preliminary results from our group indicate that gaze directed in the proximity of the device gives the highest amplitude of entrainment (unpublished).

This study also examined clinically relevant outcomes of cognition and brain atrophy. This study found that participants exposed to the active intervention improved about 2-3 points on the ADAS-Cog + EF&FA, while the placebo group did not improve. The cause of the observed improvement in cognitive performance for the active treatment groups may be a training effect [39]. However, the impact of practice effects on the results is mitigated by comparing cognitive change with a parallel placebo group [40]. ADAS-Cog + EF&FA has a wider scoring range than the original ADAS-Cog and a higher sensitivity to change [27]. Consequently, absolute change in ADAS-Cog and ADAS-Cog + EF&FA are not equal. The 2-3 point improvement in ADAS-Cog + EF&FA found in this study is probably not enough for clinical relevance as a 3-4 point change in ADAS-Cog, with a narrower range of scoring, is proposed as target for clinical relevance [41].

The other exploratory outcome brain atrophy revealed no change at week 6, which is likely because 6 weeks is a too short timeframe to observe changes in atrophy. At week 12, the placebo group showed a median atrophy progression of approximately 2%, while the active group had a median of no change or slight improvement. The median atrophy progression in the placebo group at week 12 is comparable to the atrophy rate found in other studies which have found a 4.66% annual hippocampal atrophy rate [42] and a 5.7% change in ventricular volume at 6 months [43]. One study using 40 Hz stroboscopic light and 40 Hz sound, reports comparable rates of atrophy in the active group [19].

The results from this two-stage trial not only provide insight on the safety and feasibility of ISF in healthy volunteers and AD patients but also allow first insight on clinically relevant outcomes of cognition and brain atrophy. Thereby, paving the way for a larger study with longer duration to assess whether ISF could possibly be a novel treatment option for AD.

The main limitation of this study is the small sample size, and consequently insufficient statistical power for investigating effects of the 40 Hz ISF on AEs, cognition, and brain atrophy. However, as all detected AEs were mild and comparable to other studies with exposure to light, it is expected that negative effects of 40 Hz ISF are limited. In the recruitment phase of this study, a small number of potential participants declined participation. These individuals may have considered the intervention too cumbersome, possibly causing an overestimation of adherence. As this study was not powered to show significant difference in the exploratory outcomes, interpretation of the observed difference between active and placebo must be cautious. Another limitation of this study is the relative low entrainment power of 40 Hz ISF compared to stroboscopic light [25]. However, it remains untested whether the amplitude of the entrained signal needs to be as high as possible or above a certain, unknown threshold for the neuroprotective effect. Indeed some studies indicate that the spatial distribution of the entrained 40 Hz signal is more important than the amplitude of the entrainment [13, 19]. ISF has only previously been examined in young and healthy volunteers, and whether or not entrainment of 40 Hz activity is possible in elderly patients with AD remains untested. However, age only has a minor effect on entrainment of 40 Hz activity [44].

In conclusion, this study found that treatment with 40 Hz ISF is safe and associated with no significant risks of AEs nor any substantial discomfort. Importantly, all participants were able to incorporate use of the LTS in their daily life with minimal interference. The exploratory outcomes indicated that 40 Hz ISF may have a potential beneficial effect on cognition and atrophy rate, though this must be tested in studies with sufficient statistical power. Thus, ISF offers yet another modality of 40 Hz entrainment, with the benefit of being non-invasive and with the possibility of future placebo-controlled trials.

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CONFLICT OF INTEREST

MPA and MH have received indirect funding from Optoceutics through management of collaborative grants. MAH and CRBJ are employees at Optoceutics ApS, but some of the work has been as student projects at Technical University Denmark. MSC, MN, ERD, and PMP have partial ownership of Optoceutics ApS. MPA is an Editorial Board Member of this journal but was not involved in the peer-review process nor had access to any information regarding its peer-review. AOB, KHM, TWK, KM, and PH have no conflicts of interest.

DATA AVAILABILITY

According to the approvals from the regulatory authority's data from this study are not subject to sharing. Specific request may be made to the corresponding author, who will share when appropriate and within legal permissions.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-221238.

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