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Audiovisual gamma stimulation for the treatment of neurodegeneration

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Alzheimer's disease (AD) is the most common type of neurodegenerative disease and a health challenge with major social and economic consequences. In this review, we discuss the therapeutic potential of gamma stimulation in treating AD and delve into the possible mechanisms responsible for its positive effects. Recent studies reveal that it is feasible and safe to induce 40 Hz brain activity in AD patients through a range of 40 Hz multisensory and noninvasive electrical or magnetic stimulation methods. Although research into the clinical potential of these interventions is still in its nascent stages, these studies suggest that 40 Hz stimulation can yield beneficial effects on brain function, disease pathology, and cognitive function in individuals with AD. Specifically, we discuss studies involving 40 Hz light, auditory, and vibrotactile stimulation, as well as noninvasive techniques such as transcranial alternating current stimulation and transcranial magnetic stimulation. The precise mechanisms underpinning the beneficial effects of gamma stimulation in AD are not yet fully elucidated, but preclinical studies have provided relevant insights. We discuss preclinical evidence related to both neuronal and nonneuronal mechanisms that may be involved, touching upon the relevance of interneurons, neuropeptides, and specific synaptic mechanisms in translating gamma stimulation into widespread neuronal activity within the brain. We also explore the roles of microglia, astrocytes, and the vasculature in mediating the beneficial effects of gamma stimulation on brain function. Lastly, we examine upcoming clinical trials and contemplate the potential future applications of gamma stimulation in the management of neurodegenerative disorders.

Keywords: Alzheimer's disease, gamma rhythms, neuromodulation, noninvasive brain stimulation, sensory stimulation, therapeutic potential

Introduction

Human brain function relies in the ability of neuronal networks to generate synchronous activity. Indeed, most brain regions can generate rhythmic activity at different frequencies depending on the task at hand. For instance, slow delta rhythms (0.5–4 Hz) are associated with memory consolidation and rest during sleep [1]. On the other hand, faster gamma rhythms (30–100 Hz) emerge when a memory is being actively recalled, and the activity of the memory-related cells requires precise coordination [2].

In Alzheimer's disease (AD) and other neurodegenerative disorders, the interplay between synchronous neuronal activity and cognition is profoundly impaired. Amyloid and tau pathology interfere with neural circuits generating oscillations that underlie cognition, which may occur long before cell death occurs. For instance, amyloid and tau accumulation can alter neuronal firing rates [3], axonal conduction speed [4], and synaptic connectivity [5]. As a result, the intricate

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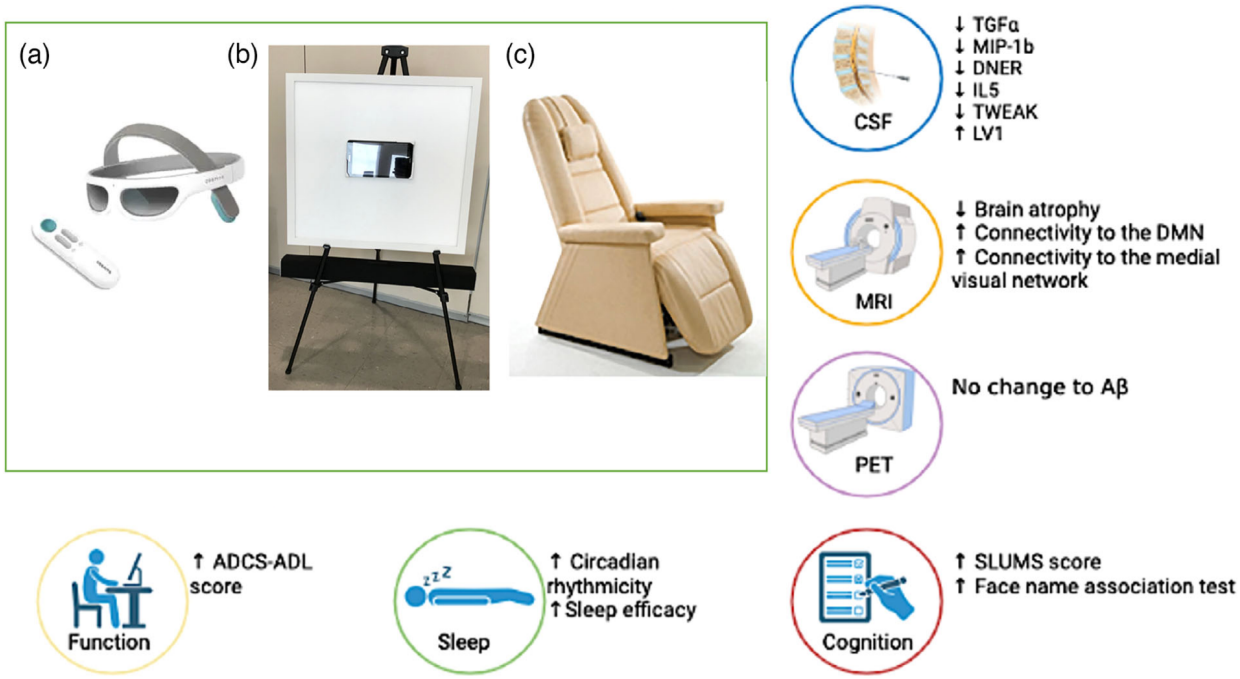

Fig. 1 *Effects of gamma sensory stimulation on patients with Alzheimer's disease: (A) Cognito's light and sound goggles, (B) MIT light and sound device, and (C) NextWave tactile stimulation chair (reference: Campbell). ADCS-ADL, Alzheimer's Disease Cooperative Study activities of daily living inventory; DMN, default mode network; DNER, delta and notch-like epidermal growth factor receptor; IL5, interleukin-5; MIP-1β, macrophage inflammatory protein 1β; SLUMS, Saint Louis University Mental Status; TGF-α, transforming growth factor alpha; TWEAK, tumor necrosis factor-related weak inducer of apoptosis.*

wiring of neural circuits is out of balance, and oscillations and cognition become affected [6].

Traditional clinical interventions often aim to preserve network activity and cognition by intervening at the level of molecular pathology. Recent research suggests that clinical intervention can occur at the level of brain rhythms. Specifically, brain rhythms at gamma frequency can be evoked by means of noninvasive stimulation—such as sound, light, magnetic, or low intensity electrical currents delivered between 30 and 50 Hz—and induce beneficial effects in the AD brain. Numerous preclinical studies have shown that such gamma stimulation attenuates amyloid and tau pathology and promotes neuroprotection in mouse models of AD and that this coincides with improvements in cognitive function [7–14]. Based on these findings, multiple clinical trials have focused on assessing the therapeutic potential of gamma stimulation and encouragingly show gamma stimulation can also ameliorate aspects of neurodegeneration and dementia in patients with AD [15–17].

In this review, we provide an overview of the potential therapeutic effects of gamma stimulation for AD. First, we provide an updated discussion on the effects of gamma stimulation on brain function, disease pathology, and cognition in human patients with AD. We then discuss preclinical findings on mouse models of AD, which have shed light into the mechanistic effects underlying the beneficial effects of gamma stimulation. Finally, we discuss the potential future of gamma stimulation in the treatment of neurodegenerative disorders.

The effects of gamma sensory stimulation in patients with Alzheimer's disease

There are several techniques currently available to evoke gamma frequency activity in the human brain, and one of the most widely used is sensory stimulation (Fig. 1). To lay the groundwork for assessing the therapeutic potential of gamma sensory stimulation in treating AD, safety, feasibility, and optimization studies have been done to determine the resonant frequencies and stimulation

characteristics that would most efficiently induce gamma oscillations in the human brain.

Feasibility and optimization of gamma sensory stimulation for patients with Alzheimer's disease

Using electroencephalography (EEG) to record the brain response, multiple groups have tested different visual and auditory stimulation paradigms to achieve the highest gamma activity or most widespread effects. Regarding visual 40 Hz stimulation, brighter lights induced the strongest gamma brain activity in cognitively normal cohorts [18, 19]. Different light wavelengths (i.e., colors) were tested, with some groups reporting enhanced gamma brain activity with red light [18], or enhanced connectivity with violet light as compared to white light [20]. Blue light was also used to induce 40 Hz brain activity and was shown to increase hippocampal and visual cortex activation using functional magnetic resonance imaging (fMRI) measures, though this study did not have a white light stimulation arm [21]. Of interest, invisible spectral flicker at 40 Hz was also shown to induce 40 Hz brain activity and was comparable in engaging cortical brain activation to flickering white light at the same frequency [22]. Invisible spectral flicker appears as constant white light, but due to two spectrally different compositions of light, the flicker is invisible.

In addition to the intensity and wavelength of the light, different studies have compared the efficacy of different gamma frequencies to induce the strongest gamma brain activity using visual stimulation. Lee et al. found that red light at 34–35 Hz induced stronger and more widespread cortical activity beyond the visual cortex as compared to 40–50 Hz in young adults [18]. Additionally, this group found that regardless of the color, light flickering at 32 and 34 Hz induced stronger gamma brain activity than higher flickering frequencies (i.e., 36, 38, and 40 Hz) in older adults [19].

Other EEG studies have also evaluated the efficacy of different sound conditions to induce gamma brain activity. Auditory stimulation at 40 Hz was found to induce the strongest neuronal response in the prefrontal cortex (PFC) using sinusoidal waves as compared to square wave sound [23]. Steady-state scalp EEG recordings also show that 40 Hz binaural click sounds are effective at inducing 40 Hz cerebral activity in widespread cortical regions, and this is associated with memory performance improvements [24–26].

Several groups show compelling evidence of true brain activation in response to 40 Hz visual and/or auditory stimulation. In an elegant experiment, Zhang et al. showed appropriate ipsilateral cortical responses to 40 Hz light flicker when participants' left or right eye was blocked from light stimulation [27]. Intracranial EEG (iEEG) recordings also show induced neural activity in superficial as well as deep brain structures when participants were stimulated with 40 Hz light [28] or synchronized 40 Hz light and sound [29]. In both these studies, iEEG leads were surgically placed in patients with medically intractable epilepsy for surgical planning purposes. Subcortical areas of the brain affected in AD—such as the hippocampus, gyrus rectus, posterior insula, and amygdala—are of particular interest and were found to have induced 40 Hz brain activity during synchronized 40 Hz light and sound stimulation [29]. Furthermore, Khachatryan et al. reported that the addition of a visual attention task seemed to increase bilateral hippocampal gamma activity on iEEG during 40 Hz light flicker [28]. Neither study showed any indication that the gamma sensory stimulation (also referred to as Gamma ENtrainment Using Sensory stimulation, GENUS) triggered any epileptiform activity, even in these patients with medically intractable epilepsy.

Safety and tolerability were assessed both after a single stimulation session in the lab as well as longitudinally with devices that were used daily at home. All published studies of gamma sensory stimulation in humans have shown that the stimulation paradigms using light or sound or a combination of both have been well-tolerated with no significant adverse effects. In patients with mild cognitive impairment (MCI) due to AD or mild AD dementia, daily usage of 40 Hz light and sound devices ranging from 10 days to 6 months was well-tolerated and adherable with minimal adverse effects reported [29–32].

Taken together, these studies show that 40 Hz sensory stimulation is safe, tolerable and can be designed to efficiently induce gamma activity in multiple regions of the human brain. Various characteristics of 40 Hz light flicker or 40 Hz sound seem to improve the efficiency of cerebral response including specific thresholds for the luminance of the light, or decibels and waveform of the sound.

Importantly, should 40 Hz gamma sensory stimulation prove to be beneficial as a therapeutic for AD disease, it seems clear that the stimulation can be modified into a form factor that can be integrated into daily usage.

To investigate the therapeutic potential of this intervention, multiple studies have assessed its effects on brain activity, molecular pathology, and cognition in patients with AD. Table 1 summarizes the clinical studies that applied gamma frequency brain stimulation in patients with AD. We will proceed to discuss these advances.

Effects of gamma sensory stimulation on cerebral cortical activation

Multiple studies have shown that gamma sensory stimulation can increase cerebral cortical activity in different brain areas as measured by fMRI, positron emission tomography (PET), and EEG recordings. In a study of 42 cognitively normal adults, a single session of 40 Hz blue light increased hippocampal and visual cortex activation as measured by task-based fMRI [21]. However, after this single stimulation, there were no changes in response accuracy or response time despite increased brain activation. Using PET, regional cerebral blood flow was found to peak with 40 Hz binaural auditory stimulation as compared to 12 different frequencies ranging between 12 and 60 Hz in 18 cognitively normal adults, and increased amplitude of steady-state EEG activity at 40 Hz correlated with increased cortical activity [26]. Specific auditory regions of the pontocerebellum were selectively activated with this 40 Hz auditory stimulation, which further supports a cerebral-specific response to gamma sensory stimulation [26].

Effects of gamma sensory stimulation on brain connectivity

Brain connectivity is a measure of synchronization between brain regions or how different parts of the brain are working in relation to another. These connections are established during specific cognitive tasks, and dysfunction or desynchronizations in these systems are known to be implicated in AD [33–35]. Several studies using gamma sensory stimulation show increased connectivity in both cognitively normal adults and participants with AD. You et al. tested several rhythmic visual stimulation paradigms at 0, 6, 10, 15, and 40 Hz and found that in 33 cognitively normal adults, this light flicker stimulation increased connectivity between the dorsolateral prefrontal cortex (DLPFC) and visual cortices [36]. A light stimulation of 40 Hz induced the fastest integration in local regions forming the most effective functional networks in this study, which taken together, can facilitate improved attention.

Studies using combined 40 Hz light and sound stimulation longitudinally also showed enhanced connectivity in the default mode network (DMN) in patients with MCI due to AD or mild AD dementia. The DMN is a group of interconnected brain structures thought to play a crucial role during quiet wakefulness, which show altered connectivity in AD [33–35]. In a delayed-start study, He et al. showed that 40 Hz light and sound stimulation increased connectivity between the posterior cingulate cortex (PCC) and the precuneus in patients with MCI after 8 weeks of daily stimulation [31]. Functional connectivity between these two areas of the brain is weakened in AD, but after 8 weeks of 40 Hz gamma sensory stimulation, this connectivity in important nodes in the DMN was strengthened. To confirm these findings, it is essential to conduct additional testing involving a more extensive sample, an extended treatment period, and a blinded, placebo-controlled approach. Furthermore, in a placebo-controlled, randomized control trial of patients with mild AD dementia ($n = 15$), Chan et al. also showed globally enhanced connectivity to the PCC after 3 months of daily stimulation with 40 Hz light and sound stimulation [29].

Effects of gamma sensory stimulation on neurodegeneration

Chan et al. evaluated the effects of gamma sensory stimulation on the biomarkers of neurodegeneration in patients with mild AD. After 3 months of daily stimulation, 40 Hz light and sound stimulation reduced the rate of brain atrophy as measured by ventricular volume on structural magnetic resonance imaging (MRI), whereas the placebo group continued to experience brain atrophy as expected in the natural progression of mild AD dementia [29]. In addition, the placebo group also had hippocampal atrophy as expected while the active group receiving 40 Hz light and sound had no significant change in hippocampal size. These results suggest that 40 Hz sensory stimulation may slow neurodegeneration in patients with mild AD dementia, but definitive trials are necessary to confirm these findings.

Table 1. *Summary of clinical studies that applied gamma frequency stimulation to Alzheimer's disease (AD) patients.*

Citation	Modality	Stimulus type	Participants	Duration	Key points
Clements-Cortes et al. [38]	Vibrotactile	40 Hz	AD $n = 18$ (6 mild, 6 moderate, and 6 severe)	30 min twice a week for 6 weeks	Improvement for mild-to-moderate patients in cognition (St. Louis University Mental Status Test). Researcher observations suggest increased awareness of surroundings. No improvements in severe AD
Ismail et al. [32]	Light	40 Hz	AD $n = 5$; MCI $n = 1$	1-h twice daily for 10 days	No amyloid reduction on a postintervention PiB PET on day 11. No EEG was done to check entrainment
Chan et al. [29]	Light and sound	40 Hz	Mild AD $n = 15$	1-h daily for 3 months	Promote gamma brain activity, preservation of hippocampal volume and decrease in ventricular dilation, strengthened functional connectivity in the default mode network, and improvement in cognitive and circadian rhythmicity
Cimenser et al. [30]	Light and sound	40 Hz light and sound vs. sham	AD mild-to-moderate $n = 22$	1-h daily for 6 months	Improved activities of daily living and sleep
He et al. [31]	Light and sound	40 Hz	MCI due to AD $n = 10$	1-h daily for 4 or 8 weeks	Stimulation was safe, tolerable, and adherable. It strengthened functional connectivity and had an influence on cytokines and immune factors within the nervous system
Kehler et al. [49]	tACS	40 HZ tACS/sham tACS (left DLPFC) + brain exercises	AD $n = 17$	30 min twice a day, 4 weeks	Brain exercises (regardless of tACS) improved cognition postintervention. Only tACS-group maintained improvement at 4-week follow-up
Zhou et al. [53]	tACS	40 Hz and sham tACS (temporal lobes)	AD $n = 50$	30 sessions (20 min), 6 weeks	Cognitive improvement at the end of treatment. MMSE remained high at 12 weeks. Decrease of Aβ 40:42 ratio that correlated with cognitive improvement

(*Continued*)

Table 1. *(Continued)*

Citation	Modality	Stimulus type	Participants	Duration	Key points
Sprugnoli et al. [42]	tACS	40 Hz tACS (temporal lobe)	AD $n = 15$	10 session (1 h—uni) or 20 (1 h—bilateral)	Increase in blood perfusion in the temporal lobes bilaterally. This positively correlated with improvements in episodic memory performance and increased gamma spectral power
Benussi et al. [47]	tACS	40 Hz and sham tACS (medial parietal cortex and precuneus)	MCI/AD $n = 20$	Single session (1 h)	Improvement in episodic and associative memory. Restoration of SAI (indirect measure of cholinergic neurotransmission)
Bréchet et al. [43]	tACS	40 Hz tACS (left angular gyrus)	AD $n = 2$	70 sessions (20 min), 14 weeks	Improvement in autobiographical memory, episodic memory, and memory index scores. Demonstrates safety and feasibility of home-based gamma tACS treatment
Benussi et al. [44]	tACS	40 Hz and sham tACS (precuneus)	AD $n = 60$	Single session (1 h)	Improvement in episodic and associative memory. Significant correlation between an enhancement of episodic memory and indirect measures of cholinergic neurotransmission
Liu et al. [46]	tACS	40 Hz tACS + sound stimulation (DLPFC and supraorbital area)	AD $n = 1$	15 sessions (20 min), 3 weeks	Cognitive improvement after 15 sessions and continued improvement at 4 months of follow-up
Kim et al. [45]	tACS and tDCS	40 Hz and sham tACS, and tDCS (DLPFC)	MCI $n = 20$	Single session (30 min)	40 Hz tACS improved executive function compared to sham. tDCS led to a more subtle improvement in executive function relative to tACS
Liu et al. [46]	TMS	40 Hz and sham rTMS (angular gyrus)	AD $n = 37$	12 sessions (30 min), 4 weeks	Improvement in cognitive function that lasted up to 8 weeks; prevention of gray matter volume loss; increased local, long-range, and dynamic connectivity within the brain, enhancing information flow and integration

Abbreviations: AD, Alzheimer's disease; DLPFC, dorsolateral prefrontal cortex; EEG, electroencephalography; MCI, mild cognitive impairment; MMSE, mini-mental state examination; PiB PET, C-Pittsburgh compound-B positron emission tomography; p-tau, phosphorylated tau; SAI, short-latency afferent inhibition; tDCS, transcranial direct current stimulation.

The effects of gamma sensory stimulation on cerebral amyloid are of particular interest given the robust and reproducible reductions in amyloid levels shown in mouse models of AD [7–9]. In a small, single-arm pilot study, five patients with mild AD dementia and one patient with MCI who used a 40 Hz light for 1 h twice daily for 10 days showed no change in cerebral amyloid levels as measured by PET scanning with Pittsburgh B compound (PiB) to visualize amyloid plaque [32]. This is admittedly a small study, and it is unclear whether one would expect any significant decrease in amyloid after only 10 days of stimulation. In addition, two of the participants in this small study had to wait 3 months for their follow-up PET PiB scan, which may have affected these outcomes; two of the participants had higher increases in amyloid than the others between pre- and post-stimulation scans (+10.6%, +16.5% globally). In fact, there were minor reductions in two participants (−4.1%, −12.5%), whereas the last two participants in this study only had marginal increases in amyloid (+4.1%, +5.4%). Evaluating the effects of gamma sensory stimulation in mouse models of AD suggests that the dense core of amyloid plaques are more resistant to removal by gamma sensory stimulation, whereas the less dense surrounding plaque is most affected by gamma-enhanced clearance mechanisms [7–9]. The PiB ligand may not be sensitive enough to make this distinction in vivo.

The effects of 40 Hz sensory stimulation were evaluated on fluid biomarkers of AD in a small delayed-start study in which 10 participants with MCI due to AD used 40 Hz light and sound daily for either 4 or 8 weeks. After this short stimulation period, no changes were found in cerebrospinal fluid (CSF) levels of amyloid-beta 42 ($A\beta_{42}$), phosphorylated tau (p-tau), total tau (t-tau) nor the ratio of t-tau/$A\beta_{42}$ [31]. However, after 8 weeks of daily 40 Hz sensory stimulation, there was a significant decrease in certain cytokines including TWEAK (tumor necrosis factor-related weak inducer of apoptosis) and down-trending levels of transforming growth factor alpha, macrophage inflammatory protein 1β, delta and notch-like epidermal growth factor receptor, and interleukin (IL)-5 [20]. There was modulation of a weighted profile of cytokines and immune factors to suggest that gamma sensory stimulation may down-regulate harmful cytokines that are involved in microglial and astrocytic activation.

Functional outcomes of gamma sensory stimulation

The effect of gamma sensory stimulation on sleep is of particular interest given the known relationship among light exposure, sleep quality, and circadian rhythmicity. Furthermore, poor sleep is also linked with worsened cognitive impairment and incidence of dementia in AD. While controlling for light levels between the placebo and 40 Hz light and sound stimulation groups (i.e., same lux levels between both groups, 1 h of stimulation daily), Chan et al. found in their pilot study that patients with mild AD dementia had improved circadian rhythmicity after 3 months of daily 40 Hz sensory stimulation [29]. Cimenser et al. also found that 40 Hz light and sound improved sleep in a placebo-controlled, randomized control trial in patients with AD ($n = 22$) after 6 months of daily stimulation [30]. In both studies, circadian rhythmicity using intra-daily stability or activity/rest periods were captured by actigraphy data, disturbances in which are shown to correlate with accelerated progression from MCI to dementia in AD [37].

In patients with mild AD, gamma sensory stimulation seemed to improve performance on cognitive tests [29, 38] and activities of daily living [31]. In a small pilot study of 19 patients with AD (6 mild, 6 moderate, and 6 severe dementia), 40 Hz tactile stimulation via the NextWave chair for 30 min improved scores on global cognition on the Saint Louis University Mental Status test and an observed emotion rating scale in patients with mild-to-moderate dementia due to AD, but not in severe AD [38]. The pilot study done by Chan et al. showed improved performance accuracy on a face-name associative delayed recall memory test after 3 months of daily 40 Hz light and sound stimulation in patients with mild AD [29]. As compared to the placebo group, 40 Hz light and sound improved scores on the AD cooperative study activities of daily living scale after 6 months of daily stimulation, which reflects patient's competence with basic and instrumental activities of daily living [30].

It is essential to emphasize that the research of the therapeutic potential of sensory gamma stimulation in AD patients is in its early stages. Many of the published studies have limitations, such as small sample sizes, short treatment durations, and the absence of long-term impact assessments. Furthermore, some of these trials lack a placebo condition, making it difficult to arrive at definitive conclusions. Despite these present constraints, this

research offers highly promising data and encourages the pursuit of further investigation.

In fact, in light of the promising results obtained using gamma sensory stimulation in patients with AD, interest has grown toward investigating the efficacy of other stimulation methods to induce gamma brain activity in this population. Among these, the use of noninvasive electric and magnetic brain stimulation to induce gamma activity in the brain of patients with AD has shown highly promising results.

The effects of noninvasive electric and magnetic gamma stimulation in patients with Alzheimer's disease

Mounting evidence has shown that electric and magnetic stimulation can be used to modulate cortical activity at different frequencies [39]. Transcranial alternating current stimulation (tACS) and transcranial magnetic stimulation (TMS) have been proposed as alternative approaches to sensory stimulation to study the effects of gamma stimulation in AD. tACS delivers sinusoidal alternating electric currents between two electrodes placed on the scalp. It can be delivered at any frequency, and it can modulate neuronal activity locally underneath the stimulating electrode and cause compensatory changes in connected neuronal networks [39]. TMS uses magnetic fields to induce electrical currents in specific regions of the brain. Using repetitive TMS, trains of magnetic pulses can be delivered to a targeted brain region and induce cortical oscillations at specific frequencies [39]. The ability of tACS to entrain neural activity has been highly debated; however, multiple studies performed in awake monkeys and humans have confirmed that low current tACS do entrain cortical neurons and the dose of the stimulation matters [40].

Preclinical studies have applied 40 Hz tACS in the frontal cortex of 5XFAD [13] mice or the hippocampus of APP/PS1 mice [11, 14] and have shown reductions in amyloid pathology as well as long-lasting improvements in synaptic plasticity, brain connectivity and memory performance. To lay the groundwork for assessing the therapeutic potential of gamma tACS and TMS stimulation in treating AD in human patients, different studies have explored whether this intervention can induce gamma brain activity in different areas of the brain in a safe and feasible manner and whether this has a therapeutic beneficial effect (Fig. 2). Table 1 summarizes the clinical studies that applied gamma frequency brain stimulation in patients with AD, including tACS and TMS.

Inducing gamma activity in patients with Alzheimer's disease using tACS and TMS

Several studies demonstrate that gamma 40 Hz brain activity can be induced in specific areas of the brain using tACS and TMS in a safe manner. Analysis of EEG recordings after tACS sessions in AD and MCI patients have shown induced 40 Hz brain activity in the temporal lobes [41, 42], angular gyrus [43], precuneus [44], and DLPFC [45] when targeting these areas. Moreover, some of these studies have reported that 40 Hz tACS can modulate neuronal activity at the theta, alpha, and beta frequency range in distant brain areas that are functionally connected to the site of stimulation [43–45]. Increases in 40 Hz power at the site of stimulation have also been reported after gamma TMS treatment targeting the angular gyrus [46]. Importantly, these studies demonstrate that 40 Hz tACS and TMS stimulation is feasible and can be safely delivered to patients with AD and MCI, with no adverse events and strong adherence to the treatment. Growing interest has then arisen to investigate the therapeutic potential of this intervention by assessing its effects on cognition, molecular pathology, and brain activity in AD patients. We will proceed to discuss these advances.

Cognitive effects of gamma tACS and TMS

Effects of 40 Hz tACS and TMS on memory. Multiple studies have explored the potential of 40 Hz tACS to improve memory function in patients with MCI and AD. A few of these studies have focused on brain regions that constitute the DMN and are associated with memory processing. These studies have demonstrated treatment-related enhancements in memory processing. Two randomized, double-blind, sham-controlled, cross-over studies explored the effect of a single 1-h 40 Hz tACS session targeting the medial parietal cortex and precuneus in 20 patients with MCI/AD [44, 47], and targeting the precuneus in 60 patients with AD [44]. These studies found a significant improvement in episodic and associative memory (Rey auditory verbal learning test—RAVLT and face-name association task—FNAT) in the active 40 Hz tACS condition relative to sham. Interestingly, 40 Hz tACS also increased cholinergic transmission (measured indirectly using TMS) and this increase correlated positively with the reported

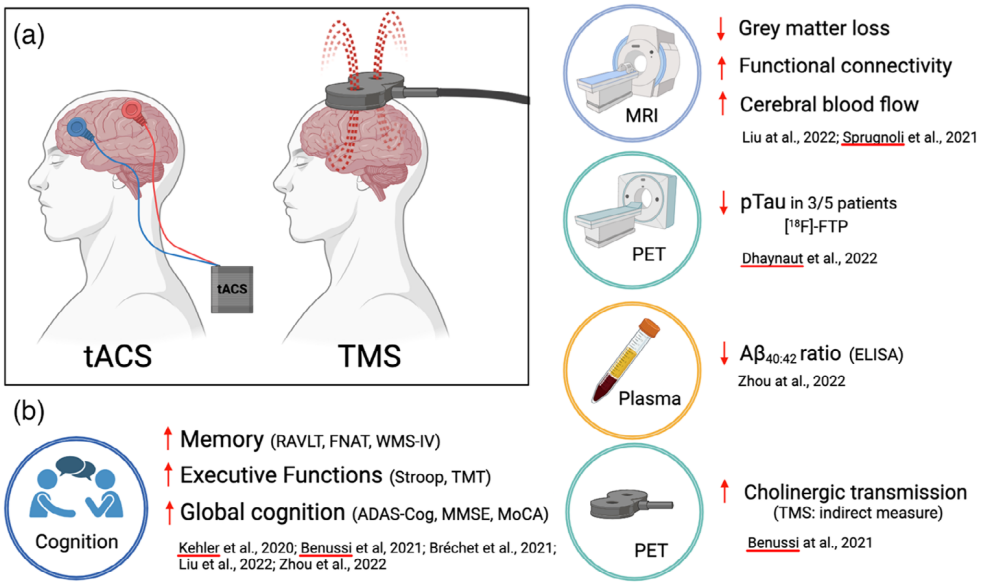

Fig. 2 *Effects of 40 Hz transcranial alternating current stimulation (tACS) and transcranial magnetic stimulation (TMS) on patients with Alzheimer's disease. (A) Schematic of tACS and TMS devices. tACS involves the application of sinusoidal alternating electric currents between two scalp electrodes. It is capable of operating at various frequencies and can influence neural activity in the immediate vicinity of the stimulating electrode, leading to compensatory adjustments in interconnected neural networks [49]. TMS, on the other hand, employs magnetic fields to generate electrical currents within specific brain regions. Through repetitive TMS, sequences of magnetic pulses can be directed at a targeted area of the brain, thereby inducing cortical oscillations at designated frequencies [49]. (B) Reported effects of 40 Hz tACS and TMS treatments on Alzheimer's disease (AD) patients. [$_{18}$F]-FTP, [18F]-Flortaucipir tau tracer; Aβ, amyloid-beta; ADAS-Cog, Alzheimer's disease assessment scale cognitive section; FNAT, face-name association task; WMS-IV, Wechsler Memory Scale-IV; MMSE, mini-mental state examination; MoCA, Montreal Cognitive Assessment; MRI, magnetic resonance imaging; PET, positron emission tomography; p-tau, phosphorylated tau; RAVLT, Rey auditory verbal learning test; TMT, trail making test.*

cognitive enhancement. Preliminary data on two patients with AD who received 70 20-min 40 Hz tACS sessions targeting the left angular gyrus across 14 weeks showed substantial improvements in autobiographical memory after the treatment [43]. Similarly, a pilot study performed on eight patients with AD showed that daily 20-min 40 Hz tACS sessions for 14 weeks targeting the left angular gyrus improved memory in the Memory Index Score. These improvements were sustained at a 3-month follow-up in which no tACS treatment was provided [48]. These last two studies [43, 48] did not incorporate a control condition, making it impossible to rule out the potential influence of a placebo effect. Furthermore, they cannot exclude the possibility of a learning effect associated with the memory tasks employed at various time intervals.

In contrast to these findings, other studies have targeted brain regions that are thought to be implicated in executive function and behavioral control. Benussi et al. reported that a single 1-h session of 40 Hz tACS over the DLPFC improved executive function but did not have a positive effect on memory performance (measure by the RAVLT and FNAT) in 12 patients with AD [44]. A treatment duration of 1 h might not be sufficient to produce a significant effect, but together these findings suggest that the cognitive effects of gamma tACS stimulation may be site specific. Stimulation of the medial parietal cortex, precuneus and angular gyrus seems to improve memory function in patients with MCI and AD, whereas the effects of targeting the DLPFC need further assessment in larger sample size cohorts ongoing longer treatment.

Preliminary results also suggest that combining 40 Hz tACS with additional interventions can lead to long-term improvements in memory processing. In a pilot study performed in 17 patients with mild-to-moderate AD, Kehler et al. combined brain exercises with either 40 Hz tACS targeting the DLPFC or no-tACS [49]. They found that after 4

weeks of training, both the 40 Hz tACS and no-tACS groups showed improvement in memory performance (Wechsler Memory Scale-IV), but only the tACS group maintained this improvement at a 1-month follow-up. Similarly, a case study in a patient with moderate AD, which combined 40 Hz auditory stimulation with 40 Hz tACS targeting the DLPFC, reported improvements in auditory verbal learning test scores after 15 20-min daily sessions, which continued to improve at a 4-month follow-up [46]. These studies support the notion that 40 Hz tACS can improve memory function in patients with MCI and AD, that it has the potential to provide long-lasting cognitive enhancing effects, and that combining it with exercise or sensory stimulation may be of further benefit.

Effects of 40 Hz tACS and TMS on executive function. The potential of 40 Hz tACS to improve executive function in patients with MCI and AD has also been explored. In a sham-controlled, double-blind, repeated-measures study performed on 20 patients with MCI, Kim et al. administered 40 Hz or sham tACS and tDCS for 30 min in the DLPFC [45], a region which is known to play a crucial role in executive function [50, 51]. Gamma-tACS significantly improved executive function scores (Stroop and Trail Making Test, TMT) relative to the sham condition, whereas no difference was present between gamma-tDCS and the sham condition. In contrast to this study, Benussi et al. explored the effect of a single 1 h session of 40 Hz tACS targeting the precuneus (an area known to play a crucial role in memory [52]) on tests measuring executive functions and memory in 60 patients with AD [44]. The authors found a significant improvement in memory processing after 40 Hz tACS, but no significant difference of 40 Hz tACS versus sham on tests measuring executive function (digit span backward, TMT, and clock drawing). Together, these results support the idea that the cognitive effects of 40 Hz tACS stimulation may be site specific, and therefore, the choice of target may have relevant implications. Targeting the DLPFC with 40 Hz tACS seems to be efficient at improving executive function in patients with MCI, whereas targeting the precuneus may not have these beneficial effects in patients with AD.

Effects of 40 Hz tACS and TMS on global measurements of cognition. Multiple studies have measured the effects of 40 Hz tACS or TMS on global measures of cognition at the end of treatment or at follow-up. Three of these studies have assessed the effect of administering 40 Hz tACS in the temporal lobes. In a randomized sham-controlled study performed on 50 patients with AD, Zhou et al. administered 30 20-min sessions across 6 weeks of 40 Hz or sham tACS targeting the bilateral temporal lobes [53]. They found significant global cognitive improvement at the end of the treatment as measured by using the Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog) and the mini-mental state examination (MMSE). Moreover, the improvement in the MMSE scores was maintained at a 12-week follow-up. In contrast to these findings, two pilot studies found no improvement in the global cognition of 20 1-h 40 Hz tACS sessions delivered to the temporal lobes of 5 [41] or 15 [42] patients with mild-to-moderate AD. However, these pilot studies were not designed to test the cognitive effects of the stimulation and reportedly lack the power, sample size, and placebo control necessary to accurately test changes in global measurements of cognition.

Beneficial effects on global cognition have also been found by other studies using different stimulation methods and targeting regions outside the temporal lobe. In a pilot home-based study performed on two patients with AD, Bréchet et al. found that 70 20-min gamma-tACS sessions targeting the left angular gyrus across 14 weeks improved global cognitive function as measured bi-weekly with the Mini/5 min, a nonvisual version of the MoCA [43]. Although preliminary and underpowered, this study suggests that home-based approaches to administer gamma-tACS are feasible and safe. Similarly, in their case study, Liu et al. showed that 15 20-min daily sessions combining 40 Hz auditory stimulation and tACS targeting the DLPFC improved global measurements of cognition (MMSE, ADAS-Cog, and MoCa) and that these improvements were maintained at a 4-month follow-up [46]. Finally, Liu et al. demonstrated the relevance of TMS stimulation [46]. In this sham-controlled study, 37 patients with AD received 12 30-min sessions across 4 weeks of 40 Hz TMS targeting the angular gyrus or sham. They found improvements in global cognitive functions (MMSE, MoCA, and ADAS-Cog) after active TMS treatment, and these improvements remained at an 8-week follow-up.

Overall, these results suggest that gamma frequency stimulation through noninvasive techniques may have potential to improve cognitive performance in patients with AD and MCI and

that these improvements seem to remain for several weeks after the end of treatment. Results vary depending on the targeted brain region, length of the stimulation, and the specific cognitive process used as read-out. Although it is premature to make definitive assertions regarding the significance of these various parameters, additional double-blind, sham-controlled studies with larger sample sizes are warranted. Nevertheless, the current pilot studies appear to reveal meaningful trends. For instance, longer duration of stimulation seems to produce more favorable and robust outcomes for the treatment. Moreover, combining 40 Hz noninvasive electrical stimulation with exercise or sensory stimulation has the potential of strengthening the long-term cognitive improvements. Additionally, the cognitive effects of 40 Hz tACS stimulation seem to be site specific, and taking advantage of the benefit that tACS and TMS allow the selection of specific brain targets would be highly relevant. Specifically, targeting areas such as the medial parietal cortex, precuneus, and angular gyrus seems to improve memory function in patients with MCI and AD, whereas targeting the DLPFC seems to have a stronger beneficial effect on executive function.

Effects of gamma tACS and TMS on Alzheimer's pathology and microglia

A few studies have investigated the effects of 40 Hz tACS on Aβ and p-tau load as well as microglial activity. Dhaynaut et al. used PET imaging to study the effects of administering twenty 1-h sessions of 40 Hz tACS targeting the bi-temporal lobes to five patients with mild-to-moderate AD on measurements of Aβ, p-tau, and microglia [41]. PET imaging was performed longitudinally before and after the 40 Hz tACS treatment via C-Pittsburgh Compound B to quantify Aβ load, [^{18}F]-Flortaucipir([^{18}F]-FTP) to quantify p-tau burden, and [^{11}C]-PBR28 to assess microglia activation. The results revealed a significant decrease in p-tau pathology following 40 Hz tACS in three of five patients in the temporal lobes and especially in the entorhinal cortex. The levels of Aβ were not affected by the treatment, and only one in four patients showed a decrease in microglial activation posttreatment. It is important to note that [^{11}C]-PBR28 appears to attach itself to the proinflammatory phenotype of microglia, and to a certain degree, to the anti-inflammatory phenotype [41]. The absence of exclusive binding to a specific microglial phenotype might have restricted the identification of microglia activation. Additionally, this study involved a limited sample size and lacks a placebo control, thus presenting a difficulty in forming conclusive findings.

The effect of 40 Hz tACS on Aβ pathology has also been studied through measurements of plasma Aβ levels. In a randomized-controlled trial, Zhou et al. administered a 6-week 40 Hz tACS intervention targeting the temporal lobes bilaterally in 50 patients with mild-to-moderate AD [53]. They measured the serum levels of Aβ_{40}, Aβ_{42}, and A$\beta_{40:42}$ ratio with enzyme-linked immunoassays (ELISA) at baseline and after treatment. The authors report a significant reduction in A$\beta_{40:42}$ ratio after stimulation in the active group that was absent in the sham group; however, no reduction of Aβ_{40}, Aβ_{42} was reported. Additionally, the authors found a significant correlation between the A$\beta_{40:42}$ ratio and the ADAS-Cog score during baseline and after stimulation in the active group. Lower A$\beta_{40:42}$ ratio has been associated with lower cortical amyloid burden and improved cognitive function [53].

Although preliminary, these results provide further evidence supporting the potential promise of gamma-tACS interventions for modulating AD-related pathology and cognitive deficits. The sample size of these studies is relatively small, and they lack a sham condition, rendering it challenging to establish firm conclusions. Nevertheless, there is an intriguing possibility that 40 Hz tACS stimulation may have differential effects on amyloid and tau pathology depending on the brain area targeted by the stimulation and the AD stage of the participants. Specifically, in early stages of the disease, tau depositions are concentrated in the temporal lobes, whereas amyloid shows a diffuse deposition across several brain regions [41, 54, 55]. This scenario could potentially explain why 40 Hz tACS delivered over the temporal lobes of patients with early AD was more effective at reducing tau pathology compared to amyloid [41].

Effects of gamma tACS and TMS on structural and functional brain imaging

Structural and functional brain imaging has also provided important insights into the therapeutic potential of noninvasive gamma stimulation in the context of AD. In a sham-controlled study performed in 37 patients diagnosed with probable AD, Liu et al. administered 12 30-min sessions of 40 Hz or sham TMS (targeting the bilateral angular gyrus) across 4 weeks [46]. Although the sham group

showed a significant reduction in MRI gray matter volume in the temporal and parietal cortices, precuneus, thalamus, and insula after the intervention relative to baseline, this reduction was not detected in the active-stimulation group. This suggests that 40 Hz TMS stimulation may prevent gray matter volume loss. Additionally, to assess whether the intervention had an effect on functional information integration, the authors measured MRI local functional connectivity [56], and global functional connectivity strength [57]. They found that 40 Hz TMS enhanced local functional integration in the angular gyrus, and global functional integration in the angular gyrus and the left middle frontal gyrus. These results suggest that 40 Hz TMS has the potential of enhancing information flow and integration in the AD population.

Brain imaging studies also support the therapeutic promise of 40 Hz tACS. In two open-label studies, Sprugnoli et al. measured the effects of gamma tACS on measurements of cerebral perfusion and cognition in 15 patients with mild-to-moderate AD [42]. Patients were presented with 40 Hz tACS targeting the temporal lobes for 1-h daily across 2 or 4 weeks. Whole brain cerebral-blood-flow was measured using arterial spin labeling, a perfusion-sensitive MRI imaging technique. The results revealed that 40 Hz tACS induced a significant increase in blood perfusion in the temporal lobes bilaterally from baseline assessment to postintervention assessment performed after 2 or 4 weeks of treatment. These physiological changes were positively correlated with episodic memory performance and induced gamma brain activity as measured by EEG.

In general, the study of eliciting gamma brain activity through noninvasive electric and magnetic brain stimulation as a potential treatment for MCI and AD is in its early stages. To establish their effectiveness and clinical relevance conclusively, more randomized, double-blind, and sham-controlled studies with larger sample sizes are required. Nevertheless, despite the current limitations, existing research indicates that these interventions hold promise in modulating AD-related pathology, eliciting structural and functional changes in brain regions affected by AD, and enhancing cognitive function in this population.

Furthermore, Benussi et al. have identified the APOE genotype and the extent of cognitive impairment as the most reliable indicators for predicting the response to gamma-tACS treatment [44]. Consequently, it is important for future research to consider diverse disease stages and varying genetic profiles in order to assess the clinical significance of gamma-tACS and TMS in the context of AD. Additionally, it would be of clinical significance to compare different stimulation frequencies to establish the specific benefits of gamma frequency in producing these positive effects.

Moreover, both tACS and TMS offer the advantage of targeting specific brain regions and adjusting the intensity of stimulation. Although no specific parameters or locations have been pinpointed as more advantageous, it appears that these variables do influence the treatment's functional effects. Consequently, conducting further studies to address this unresolved question is also of great importance.

Preclinical evidence of the mechanisms underlying the beneficial effects of gamma stimulation in Alzheimer's disease

Understanding the mechanisms that underlie the beneficial effects of gamma stimulation can be valuable when contemplating the potential adoption of sensory and electrical gamma stimulation in therapeutic and clinical contexts. Preclinical studies have yielded invaluable information concerning these potential mechanisms.

Accumulating studies in mice have demonstrated that gamma sensory stimulation can reduce amyloid and tau pathology [7–9]. Noninvasive 40 Hz visual stimulation attenuated amyloid burden in the visual cortex of 5XFAD mice [7], reduced tau phosphorylation in the visual cortex of the P301S mice [9], and reduced amyloid and tau levels in the hippocampus of 3xTg mice [10] and APP/PS1 mice [58]. Noninvasive auditory stimulation at 40 Hz attenuated amyloid pathology in the auditory cortex of 5XFAD mice, and in tauopathy mouse models, it alleviated tau burden [8]. Furthermore, combining audio and visual 40 Hz stimulation reduced amyloid in multiple cortical regions including areas beyond sensory cortices [8]. Suk et al. also showed that whole-body vibrotactile stimulation not only decreased brain pathology in the primary somatosensory and primary motor cortex of P301S and CK-p25 mice, but it also improved motor function [12], a unique function of tactile stimulation that had not been observed after audiovisual stimulation. A recent publication did

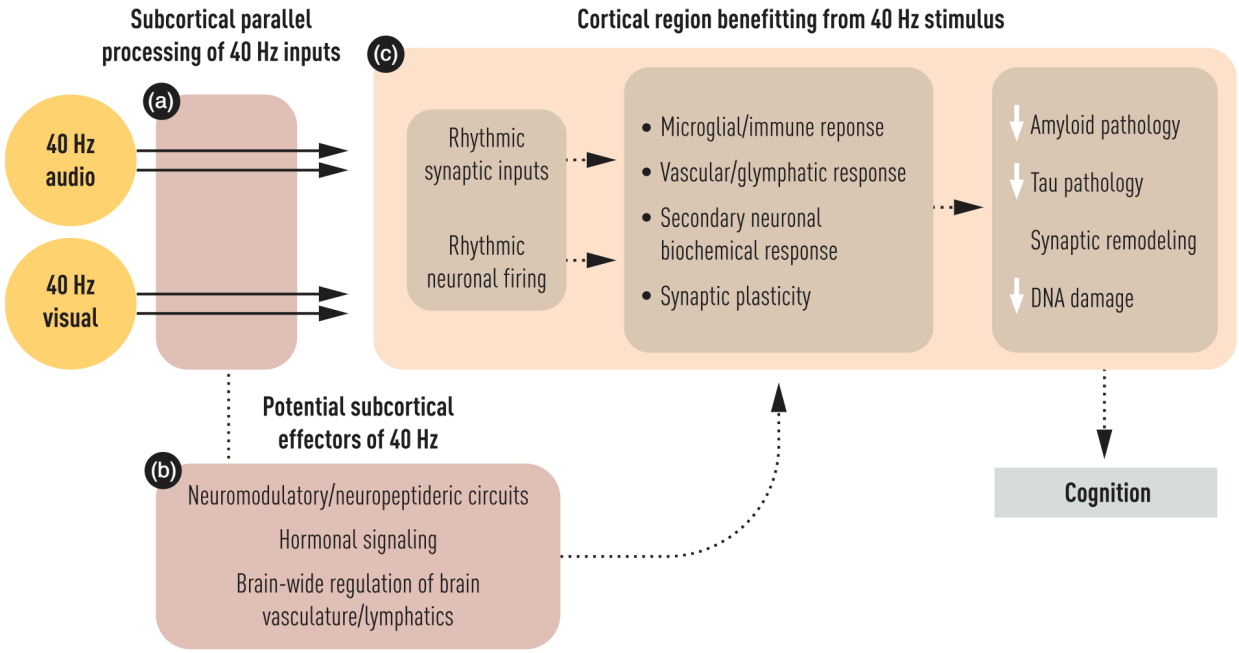

Fig. 3 *Mechanisms underlying the beneficial effects of gamma stimulation in AD schematic showing known and hypothetical mechanisms mediating the clinical effects of 40 Hz stimulation. (A) Sensory stimulation is transduced in the sensory organs and the signals progress through the canonical sensory pathways. Note that sensory processing is highly parallelized (indicated by parallel black arrows); hence, 40-Hz stimulation not only is relayed to cortex via thalamus (e.g., Schneider et al. for unimodal visual stimulation [65]) but also travels via parallel pathways to multiple subcortical regions and can influence other modalities via regions such as the thalamic reticular nucleus. (B) Hypothetical involvement of subcortical circuits that process sensory stimulation in parallel to cortical signaling. (C) A region that responds to 40 Hz stimulation (e.g., amyloid reduction and neuroprotection) may do so due to direct subthreshold synaptic currents (e.g., evoking hemodynamic changes or synaptic plasticity, changes in neuronal firing) or due to nonlocal events (e.g., global increase in CSF flow and neuromodulators). CSF, cerebrospinal fluid.*

not report a significant reduction in amyloid burden after 40 Hz visual stimulation for 1 h [59], but this publication used nonstandard methodologies (such as amyloid ELISA on fixed tissue, and pooling amyloid data across different mouse models, sexes, and ages), all of which would be expected to strongly increase the variability and decrease statistical power [60]. Beyond the attenuation of amyloid and tau, gamma sensory stimulation has been reported to offer neuroprotection by reducing neuroinflammation [8, 9], preventing brain atrophy and preserving neuronal and synaptic density across multiple regions of the brain [9].

A tantalizing question that arises at this point and is crucial for exploring the clinical use of gamma stimulation is as follows: How can externally driven rhythmic engagement of regional brain activity reduce the accumulation of amyloid, tau, and the death of synapses on a cellular level? There is not yet a definitive answer for this question, but the original sensory gamma stimulation papers provided evidence for the first time that increased gamma synchrony led to molecular and biochemical changes in various brain cell types and sparked numerous mechanistic insights from studies in rodents and humans (Fig. 3). Ultimately, this line of research holds the promise of refining 40 Hz treatment based on mechanistic insights and advancing our understanding of basic brain health.

The overarching mechanistic question is how neuronal activity produced by 40 Hz stimulation is translated into beneficial effects across the brain. This chain of events can be broken down into multiple smaller questions which we will discuss next. A first key question is how gamma stimulation translates into neuronal activity and how it spreads throughout the brain. A second key question is where and how neuronal activity is translated into beneficial effect. Finally, a tantalizing question—and arguably the clinically most relevant one—is

what cellular and molecular mechanisms are directly responsible for the beneficial effects of gamma stimulation in a given brain region.

The brain-wide neuronal response to gamma stimulation

There are two distinct aspects of neural activity that have been examined using invasive intracranial recordings in rodents in response to gamma stimulation. Several studies have recorded neuronal spiking in response to sensory gamma stimulation. These recordings describe the local number and timing of action potentials of individual neurons. An overlapping set of studies have examined the global or local field potentials (LFP). These measures reflect synchronous electrical potentials—including the activity resulting from synaptic signals—of a population of neurons within a brain region [61]. As we will discuss in the next section, it is important to keep in mind that the relationship between neuronal activity and beneficial effects at the cellular level may be complex and involve nonlocal components.

Most of the physiological data acquired from invasive recordings in rodents focuses on mice undergoing unimodal 40 Hz visual stimulation (despite audiovisual stimulation prevailing in the clinics). In mice, visual stimulation evokes a strong intracranial 40 Hz LFP response in the visual cortex [7, 9, 59] and, according to one study, also in other cortical areas [9]. Interestingly, recent studies suggest that 40 Hz sensory stimulation may not engage the same circuitry that generates endogenous gamma oscillations but rather induce externally driven gamma activity [59, 62, 63]. In the hippocampus, three studies reported a significant increase in LFP 40 Hz power response [7, 9, 64], whereas one study did not [59]. Importantly, the study that did not report elevated LFP power found evidence of neuronal spiking responses. Such neuronal responses cannot occur without considerable synaptic input, which should affect the electrical field potential. Thus, differences in these studies are likely due to probe orientation, a weaker hippocampal LFP response compared to the visual cortex leading to decreased statistical power to detect an effect, and different analysis methods [60]. In the neuronal spiking response to visual stimulation, neuronal phase locking is strong in the visual thalamus and layer four of the visual cortex, but weaker in other layers of the visual cortex and the hippocampus, although studies again diverge on the extent of hippocampal engagement [9, 59, 65]. Differences between studies could be caused by variable mouse genotypes, ages, stimulation parameters, and differences in data analysis (e.g., different measures of phase-locking) [9]. Interestingly, all studies agree that inhibitory interneurons show a stronger phase-locking response than excitatory pyramidal cells, suggesting that these neurons may warrant closer examination as a potential mediator of beneficial effects.

Although all the above studies use unimodal visual stimulation, most human studies and ongoing animal studies use audiovisual stimulation. Martorell et al. recorded putative single units and LFP in the auditory cortex, hippocampal CA1, and PFC in response to auditory stimulation alone or combined audiovisual stimulation [8]. Interestingly, they found that phase locking of neuronal spiking occurred in all brain regions during audiovisual stimulation and unimodal auditory stimulation. However, only audiovisual stimulation increased median firing rate. Similarly, auditory stimulation alone caused a clear average evoked potential in the LFP in all brain regions but only increased average LFP gamma power in the auditory cortex. In contrast, audiovisual stimulation led to an increase in LFP gamma power in all regions.

In summary, 40 Hz stimulation leads to robust rhythmic neuronal firing along the canonical sensory processing cascade up to the primary visual cortex. For unimodal visual stimulation, the rhythmic neuronal firing then becomes weaker as the stimulation propagates to other regions, such as the hippocampus. However, compared to spiking responses of excitatory neurons, rhythmic synaptic input likely spreads further throughout the brain, and rhythmic spiking of interneurons is more robust. These are important insights highlighting promising candidate mechanisms. Moreover, combined audiovisual stimulation may enhance the spread of 40 Hz responses, albeit more data are needed to substantiate this on the level of neuronal firing or LFP. Finally, little is known about the neuronal response in brain regions outside the canonical visual pathway that may nevertheless be key effectors of the 40 Hz response. Examples for such regions are subcortical neuromodulatory regions that can powerfully modulate all cells in the brain, or the inhibitory neurons of the thalamic reticular nucleus, which could be key for multisensory stimulation.

The translation of neuronal firing into beneficial cellular and molecular effects

Although it is tempting to speculate that a neuron directly benefits from being engaged in the neuronal response to gamma stimulation, it is important to note that no experiment has established yet whether any type of local neuronal activity is required for a brain region to benefit from gamma stimulation. Several potential cellular and molecular mechanisms could be initiated nonlocally, such as a change in CSF flow, or the volume release of neuropeptides or other diffusible signals.

Interestingly, one noteworthy candidate mechanism for gamma stimulation could act both locally and via longer distances. Specifically, neurotransmitter release from dense core vesicles has been suggested to occur predominantly at high frequency firing rates [66]. These vesicles are released by long-range, nonlocal neuromodulatory axons and by local interneurons. Long-range neuromodulators could include neuropeptides and catecholamines released by brainstem serotonergic and noradrenergic circuits or basal forebrain cholinergic neurons. Local forebrain interneurons release modulatory substances such as vasoactive intestinal polypeptide (VIP), acetylcholine, or somatostatin. The possibility of neuropeptide release by local interneurons is especially intriguing given recent findings by Schneider et al. that interneurons seem to respond more strongly to 40 Hz stimulation [65].

If local neuronal activity is required, there are generally two distinct aspects that could be important for the therapeutic effects of gamma stimulation in any given brain region. First, a brain region can receive synaptic inputs at gamma range, and therefore, its neurons experience rhythmic current flow at their dendrites. This type of input can be reflected in the electrical field potential that can be measured using noninvasive EEG and invasive LFP recordings (see previous section). Second, rhythmic synaptic input can modulate neural firing, which can be measured using invasive electrodes.

Although the modulation of neuronal firing is crucial for the computational output of a circuit, it is conceivable that it is less relevant from a therapeutic standpoint to alleviate pathology and cognitive decline. The first reason for this is that a much larger fraction of neurons responds to any given input by subthreshold currents compared to spiking. For instance, Moore and Nelson reported that out of 24 cortical neurons, 20 showed subthreshold responses to sensory stimulation, whereas only seven also responded with spiking [67]. A second reason is that synaptic glutamate release, but not local action potentials, is a key driver for neurometabolic coupling [68], which is likely involved in mediating the beneficial effects of gamma stimulation (see below). Finally, subthreshold synaptic inputs are a key substrate for synaptic plasticity. Indeed, two separate studies in mice and humans have recently suggested that 40 Hz stimulation can enhance synaptic plasticity [64, 69].

Nevertheless, the role of local neural firing should be evaluated carefully. Especially the role of inhibitory interneurons seems worth examining, as they are more strongly affected by gamma stimulation and have powerful tools to affect other cell types, such as neuropeptides (e.g., VIP) or small molecules (e.g., nitric oxide) [70]. Future studies using additional experimental systems such as voltage sensor imaging could be employed to closely examine firing of local interneurons.

Neuronal and synaptic mechanisms behind the beneficial effects of gamma stimulation

Gamma stimulation affects several aspects of neuronal biology pertinent to AD, culminating in protective effects against the loss of neurons and synapses in mouse models of neurodegeneration [9], tauopathy [9], and stroke [64]. Although certain aspects of this neuroprotection may be secondary to other effects (e.g., enhanced clearance of extracellular tau/amyloid), several experiments suggest that 40 Hz directly affects neurons.

Adaikkan et al. performed bulk RNA sequencing on isolated NeuN+ nuclei from primary visual cortex of CK-p25 and P301S mice that had received either no stimulation or 6 weeks of 40 Hz light flicker [9]. What stood out in this analysis was that in both models of neurodegeneration, genes linked to synaptic transmission and intracellular transport were downregulated compared to wild-type littermates but were upregulated after 40 Hz stimulation. These results were confirmed when the authors turned to a phosphoproteomic (LC–MS/MS) analysis. Interestingly, this analysis included a group of wild-type mice that received 40 Hz stimulation, in which the authors also found that 40 Hz stimulation affected proteins related to synaptic function and vesicle transport, suggesting

that 40 Hz stimulation directly affects these neuronal processes even in the absence of neurodegeneration.

Further evidence for an effect of 40 Hz stimulation on synaptic function comes from a study applying 40 Hz stimulation in a mouse model of cerebral ischemia [64]. Like the above study, the authors conducted a proteomic analysis and found that 40 Hz stimulation prominently affected proteins related to synaptic function. Moreover, the authors found that 40 Hz stimulation rescued hippocampal LTP and short-term plasticity.

In addition to synaptic function and intracellular transport, 40 Hz stimulation has been shown to be protective against DNA damage. Not only was DNA damage reduced by 40 Hz in the CK-p25 mouse model of neurodegeneration, but there was also an upregulation of proteins involved in protecting against negative consequences of DNA-damage, such as histone H3F3 [9].

Nonneuronal mechanisms behind the beneficial effects of gamma stimulation

Beyond the role of neurons, several nonneuronal mechanisms have been found to support the beneficial effects of gamma stimulation in AD. Glial cells, such as microglia and astrocytes as well as vascular cells, are some of these potential mechanisms.

Effects of gamma stimulation on microglia and immune profiles. Several studies highlight a response of 40 Hz sensory stimulation on microglia. Iaccarino et al. reported changes in microglia morphology in 40 Hz light stimulation in 6-month-old 5XFAD mice, including enlarged soma and shortened processes [7]. Similar morphological changes were observed after auditory stimulation and multisensory audiovisual stimulation promoted a microglial clustering phenotype [8]. These morphological transformations may be associated with alterations in phagocytosis, and new research on the plasticity of microglial states will provide insight into how distinct patterns of neuronal activity modulate transitions between cellular states for distinct microglial subpopulations [71, 72]. Similar changes were also observed in CK-p25 and P301S mice that received 6 weeks of 40 Hz light flicker [9]. It remains unclear to what extent the microglial response to 40 Hz sensory stimulation is responsible for cognitive effects, clearance of amyloid, and neuronal synchrony. Future studies are needed to disambiguate the multiple attributes of microglia to brain health related to AD function and 40 Hz stimulation. For example, it is not clear whether the microglial response to sensory stimulation is related to amyloid phagocytosis or synaptic remodeling. Given the multiple functions of microglia in sculpting virtually every aspect of brain health, including neurovascular coupling [73], supporting healthy myelination [74], promoting glial interactions, and promoting interactions with distinct neuronal subtypes [75], it will be interesting to see how distinct brain rhythms interface with microglia function.

In addition to modulating microglia, gamma stimulation might also modulate peripheral immune biology. For example, Garza et al. found 40 Hz visual stimulation promoted a distinct cytokine profile [76]. Specifically, the 40 Hz flicker was found to increase IL-6 and IL-4, M-CSF, and MIG. A 40-Hz flicker was also found to upregulate phosphosignaling within the NFκB [77] and MAPK pathways. Although the consequence of these immune molecules on cognition is unclear and likely dependent on several convergent and overlapping factors of brain health, these results suggest that noninvasive sensory stimulation might modulate immune-related profiles relevant to brain health. For example, given that IL4 was increased by noninvasive stimulation, it is interesting to note that several lines of evidence implicate IL4 in normal learning and memory processes, such as the recent discovery that IL4 regulates T cell effects on contextual fear memory [68]. The upregulation of M-CSF might be relevant to microglial homeostasis, given that M-CSF binds to CSF1R [78], which is crucial to the survival of microglia. Collectively, these findings highlight a role for 40 Hz stimulation to rapidly modulate immune signaling pathways.

Effects of gamma stimulation on the vasculature and astrocytes. Other mechanisms that seem to underly the beneficial effects of gamma stimulation in AD are changes in vascular and waste clearance systems. Vascular cells are critical for promoting neurovascular coupling, maintaining barrier properties of the blood–brain barrier, and providing neurons with the nutrients necessary for brain health [10]. Martorell et al. found that chronic 40 Hz auditory stimulation increased the diameter of blood vessels in the auditory cortex and hippocampus of 5XFAD mice [8]. This increase was

observed 24 h poststimulation, suggesting that it is not a transient response. Changes in regional cerebral blood flow in relation to 40 Hz stimulation have also been reported in different human studies [26, 42, 79]. Vascular cells promote perivascular movement of fluid through the brain, collectively called the glymphatic system, which is thought to be impaired in aging and AD [80]. Hablitz et al. hypothesized that 40 Hz oscillations may promote glymphatic clearance [81], which may be responsible for the removal of amyloid.

Additionally, Martorell et al. found that chronic 40 Hz auditory stimulation increased glial fibrillary acidic protein and S100B-positive reactive astrocytes in 5XFAD mice. Astrocytes are important regulators of the vascular and glymphatic networks in the brain [82]. Water channels known as aquaporin-4 expressed along the astrocytic endfeet are thought to facilitate the movement of CSF into the brain and support the clearance of amyloid [83, 84]. Therefore, it is possible that sensory stimulation at 40 Hz might promote astrocytic and vascular changes that enhance removal of amyloid. Aquaporin-4 channels are critically involved in the movement of brain fluids of the glymphatic system, which facilitates the removal of metabolic products from the brain, including amyloid. Neuronal activity by noninvasive sensory gamma stimulation might attenuate amyloid burden by acting on glymphatic mechanisms. For example, factors released by neurons during gamma neuronal activity such as neuropeptides might act on receptors on astrocytes, thereby facilitating astrocytic intracellular reorganization of aquaporin channels. As aquaporin-4 signaling is tightly regulated by astrocytes, a neuron-astrocyte signaling mechanism recruited by distinct patterns of neuronal activity might regulate glymphatic clearance.

Additional clearance systems may be recruited by gamma stimulation. For example, the transcytosis of particles across the blood–brain barrier has been shown to be a player in clearing molecules from the brain. Distinct brain patterns can sculpt the expression of solute transporters in brain endothelial cells [85], potentially suggesting that 40 Hz sensory stimulation might modulate the expression of genes related to blood–brain barrier permeability governing the removal of metabolites from the brain. Indeed, Martorell et al. reported LRP1-related mechanisms may be recruited by auditory stimulation [8]. Collectively, these results highlight the diverse mechanisms by which neuronal activity shapes the response of various glial and vasculature cell types that sculpt clearance pathways in the brain and enhance brain health. Future work is needed to define how neuronal factors released during distinct brain activities recruit glial cells and activate clearance systems.

Clinical future of gamma stimulation

The induction of gamma brain rhythms has substantial potential as a therapeutic option for AD due to its promising effects on AD pathology and related behavioral and cognitive deficits in both mouse models and human patients. Ongoing clinical trials aim to optimize the therapeutic applications of gamma stimulation in the context of AD and related conditions. Table 2 summarizes these ongoing clinical trials. Several of these trials encompass a larger sample size and extended follow-up durations, incorporating wash-out intervals (NCT05655195 [86], NCT05544201 [87], NCT03657745 [88], and NCT04122001 [89]). These aspects aim to assess the enduring clinical effectiveness of this intervention over an extended period. A growing number of these clinical trials make use of devices to perform home-based gamma sensory or tACS stimulation (NCT05655195[86], NCT03657745 [88], and NCT05643326 [90]), which have the potential of increasing the adherence and accessibility of the intervention.

Additionally, previous studies have revealed that the combination of different gamma stimulation modalities (e.g., light and sound stimulation [8]) or different types of stimulation (e.g., gamma tACS, gamma sensory stimulation and cognitive training [49], or gamma sensory stimulation and exercise [10]) have the potential of maximizing the beneficial effects on AD. Current ongoing clinical trials are further exploring the benefits of combining tACS and sensory stimulation NCT05251649 [91] or tDCS and cognitive training NCT04122001 [89] on larger clinical samples, which will test the potential synergistic effects of these types of interventions.

Important efforts are also underway to identify optimal parameters for gamma stimulation. For instance, it is not currently known whether any specific brain region could be an ideal target for gamma tACS or TMS stimulation. Current clinical trials are therefore exploring whether targeting the area of maximal tracer uptake on amyloid PET imaging could maximize the benefits of the

Table 2. *Summary of ongoing clinical studies studying the effects of gamma frequency stimulation on Alzheimer's disease (AD) patients.*

CT. gov	Modality	Eligibility	Trial design	Outcomes
NCT05776641	40 Hz light and sound (MIT device)	Cognitively normal with cerebral amyloid deposition by PET-amyloid ($n = 50$)	Blinded RCT with two arms: placebo vs. 40 Hz light and sound for 1 h daily at-home stimulation for 1 year	- PET amyloid, tau - Brain structure (MRI) - Brain connectivity (fMRI, EEG) - Neuropsychological battery - Fluid biomarkers (CSF, blood)
NCT05206305	40 Hz light and sound (GammaSense Stimulation device)	Mild-to-moderate AD ($n = 20$) with cerebral amyloid (PET-amyloid or CSF)	Open label single group assignment for 1 h daily for 8 weeks	- Cortical network functioning evaluated with EEG and ERP - Cognition - Resting state fMRI
NCT03880240	40 Hz tACS	Mild to moderate AD ($n = 55$) with cerebral amyloid (PET-amyloid)	Blinded RCT with four arms: 2 or 4 weeks, once a day or twice a day, sham or 40 Hz tACS (in lab) for 1 h targeting the area of maximal amyloid PET uptake	- PET amyloid (up to 16 weeks) - PET tau - EEG gamma band - ADAS-Cog
NCT05655195	40 Hz light and sound (MIT device)	Mild AD ($n = 50$)	Blinded RCT with two arms (placebo and control) with 1 h daily at-home stimulation for 6 months	- EEG gamma band - Cognition - Fluid biomarkers - Resting state fMRI - CSF flow - Actigraphy
NCT05784298	40 Hz TMS	Mild AD ($n = 27$)	Randomized, double-blind, crossover design with sham vs. 40 Hz rTMS of the precuneus for 45 min (1 session). Each participant gets both types of stimulation	- Face-name associative memory test - Verbal fluency scores - RAVLT
NCT05643326	40 Hz TACS (at home)	Mild AD ($n = 30$)	Blinded RCT over the precuneus daily for 9 weeks (5 days a week)	- CDR - ADASCog - RAVLT - Face-name associative memory test - NPI-Q - EEG - Plasma biomarkers - fMRI

(*Continued*)

Table 2. *(Continued)*

CT. gov	Modality	Eligibility	Trial design	Outcomes
NCT05015478	40 Hz light	MCI ($n = 20$) vs. cognitively typical controls ($n = 20$)	Randomized, blinded, crossover design using 40 Hz light or placebo for 1 h for 1 session separated by 1 week washout period	To evaluate the effects on subjective sleepiness and cognition - Karolinska sleepiness scale - EEG - Working memory task
NCT05260177	40 Hz invisible spectral light	Mild to moderate AD ($n = 62$)	Blinded RCT using 40 Hz light or placebo for 1 h daily for 6 months	- EEG gamma band (primary) - Cognition - Fluid biomarkers - Resting state fMRI - MR spectroscopy - MR perfusion - Structural MRI - Sleep quality - Actigraphy - Plasma biomarkers
NCT05016219	40 Hz light	MCI ($n = 120$) with sleep disturbances (PSQI > 5)	Placebo (red) lights with 40 Hz flicker or random flicker vs. light source that stimulates the circadian system (blue) and 40 Hz flicker or random flicker for 2 h daily for 8 weeks. Outcomes measured at 8 weeks and after 4 weeks washout period	- Cognition (primary) - Urine melatonin - Actigraphy - DQoL - PSQI
NCT05326750	40 Hz tACS	Patients with AD, NPH, DLB, FTD ($n = 200$)	Blinded RCT with 40 Hz tACS over superior parietal cortex with stim 1x for 4 consecutive days with visits at 1 and 4 weeks after 4 sessions	- Cognition: RAVLT (primary) - Short latency afferent inhibition (SAI) (cholinergic neurotransmission) - Short interval intracortical inhibition (SICI) (gabaergic neurotransmission). - Intracortical facilitation (ICF) (glutamatergic neurotransmission)

(*Continued*)

Table 2. *(Continued)*

CT. gov	Modality	Eligibility	Trial design	Outcomes
NCT05544201	40 Hz tACS	Patients with MCI due to AD with sleep disturbances ($n = 99$)	Blinded RCT for 4 weeks with either HD-tDCS, 40 Hz HD-tACS, sham HD-tCS (three arms) with assessments at 4, 8, 12, and 24 weeks	- Sleep as measured by PSQI (primary) - Delayed recall of words using word-list learning test (primary) - Attention (attention network test) - Executive function (category verbal fluency test) - Saliva Ab40 and Ab42
NCT05251649	40Hz tACS + 40Hz sound	Patients with MCI due to AD with sleep disturbances ($n = 99$)	Blinded RCT 15 daily 20 min sessions with either 40Hz tACS (DLPFC and c/l supraorbital area) with 40Hz sound, 40Hz tACS, or 40Hz sound through earplugs for 5 min on and 1 min off at 3 weeks and 3 months	- ADAS-Cog (primary) - Cognition (MMSE, MoCA, AVLT, BNT-30, NPI, ADL) - fMRI - Brain structure (MRI)
NCT03657745	iPad app with 40 Hz light with cognitive therapy	MCI or dementia due to AD ($n = 2000$)	Prospective observational study evaluating use of AlzLife app for 30 min daily with exercise	- Cognition (performance on games; primary) - ADCS-ADL
NCT05710549	tACS (gamma, beta or actisham)	MCI ($n = 40$), CN younger ($n = 40$) and CN older ($n = 40$)	Single-blinded RCT evaluating hdEEG after 20 min of tACS across 3 lab sessions	- Spatiotemporal dynamic changes on hdEEG - Cognition (autobiographical memory, MoCA)
NCT04122001	HD-tDCS in combination with a word list learning intervention	AD or lvPPA with AD biomarkers (total $n = 60$)	Blinded, randomized, crossover study evaluating whether active HD-tDCS over angular gyrus vs. sham for 2 weeks in combination with a word list learning intervention (10 sessions per period) with 3-month washout	- Cognition (primary, recall of word list) - Cognition (secondary, RAVLT, MMSE, MST, language tests, digit span, spatial span) - Brain structure (MPRAGE) - GABA concentration via MRS - White matter tracts via DTI - Sleep via actigraphy

Abbreviations: ADAS-COG, Alzheimer's disease assessment scale cognitive section; DTI, diffusion tensor imaging; fMRI, functional magnetic resonance imaging; hdEEG, high-definition electroencephalography; HD-tDCS, high definition transcranial direct current stimulation; lvPPA, logopenic variant primary progressive aphasia; MCI, mild cognitive impairment; MMSE, Mini-Mental State Exam; MoCA, Montreal Cognitive Assessment; MPRAGE, Magnetization-Prepared Rapid Gradient-ECHO; MRS, magnetic resonance spectroscopy; MST, Mnemonic Similarity Task; NPI, neuropsychiatric inventory; PSQI, Pittsburgh Sleep Quality Index; RAVLT, Rey auditory-verbal learning test; RCT, randomized controlled trial; rTMS, repetitive transcranial magnetic stimulation; tACS, transcranial alternative current stimulation.

intervention (NCT03880240 [92]). Other clinical trials are also using different light parameters to develop novel ways to induce gamma brain activity that could have additional beneficial effects. One clinical trial (NCT05260177 [93]) aims to reduce the invasiveness of the light treatment by modulating the spectral composition of a white light to create a light flicker invisible to the human perception but capable of inducing gamma activity in the brain. Another clinical trial is exploring whether using blue light flickering at the gamma frequency range could have beneficial effects in AD and further improve circadian deficits in this population (NCT05016219 [94]).

Important advances are also being made in studying the potential of gamma stimulation in the treatment of other neurological disorders or conditions apart from AD. Preliminary studies using gamma stimulation techniques, such tACS or DBS, have shown promising results in alleviating motor symptoms and improving motor control in Parkinson's disease [95, 96]. However, more research is needed to fully understand the underlying mechanisms and determine the long-term effectiveness and safety of gamma stimulation as a treatment for Parkinson's disease. Additionally, ongoing clinical trials suggest that there could be potential for gamma stimulation in the treatment of conditions such as dementia with Lewy bodies, frontotemporal dementia (NCT05326750 [97]), logopenic variant PPA with AD pathology (NCT04122001 [89]), circadian or sleep disorders (NCT05016219 [94] and NCT05544201 [87]), and Down syndrome (NCT05196984 [98]).

Finally, current efforts are being made to study the effect of gamma stimulation on further outcome measures that could inform the relevance of this intervention for additional conditions. For example, rodent studies have shown that gamma deep-brain reachable low field magnetic stimulation facilitated the restoration of the myelin sheath and elevated the levels of neuregulin-1 and its receptor ErbB4 in the PFC of demyelinated mice [99]. Based on this, different clinical trials and research studies are exploring the effects of gamma stimulation on white matter tracts (e.g., using diffusion tensor imaging) (NCT04122001 [89]) and suggest that there is potential in the use of gamma stimulation for the treatment of diseases affecting white matter such as multiple sclerosis. Additionally, pilot studies have suggested that gamma stimulation modulates the activity of neuromodulators such as acetylcholine [44], which have an important role in different disorders such as AD and diseases of the neuromuscular junction. Ongoing clinical studies are using magnetic resonance spectroscopy to investigate the effects of gamma stimulation in the concentration of neurotransmitters and neuromodulators such as glutamate, GABA, and acetylcholine in the context of AD and other disorders (NCT05326750 [97] and NCT04122001 [89]). These advances will be highly relevant to determine potential future directions for gamma stimulation as a treatment for diverse diseases.

Concluding remarks

The field of gamma stimulation has undergone remarkable progress and expansion in recent years. What started in 2016 with optogenetic and visual stimulation in mice has expanded to a multitude of stimulation paradigms and a wide range of human clinical studies with promising results and is narrowing in on the mechanisms underlying this phenomenon.

Gamma stimulation paradigms have expanded from visual stimulation to include auditory, audiovisual, tactile stimulation, and transcranial noninvasive stimulation. A key advantage of multimodal stimulation is that there is more widespread engagement of the brain, and it may even produce supralinear dynamics of brain activation, such that the resulting activation may be greater than the sum of its parts. A second advantage is that auditory and tactile stimulation may be better suited for longer stimulation paradigms as they are better tolerated than visual stimulation in some patients.

In addition to sensory stimulation, a multitude of studies explored noninvasive transcranial stimulation at gamma frequency. These promising studies suggest that it may be possible to directly target specifically affected areas with gamma stimulation, offering tremendous potential to tailor gamma stimulation to each patient's individual need and overcome potential sensory comorbidities that would otherwise be a hurdle for sensory gamma stimulation.

The most important test for gamma stimulation is without doubt whether it is safe and beneficial for patients. So far, results from several small trials on sensory gamma stimulation suggest that it is safe and evokes rhythmic EEG brain

responses, and there are promising signs for AD symptoms and pathology. Specifically, long periods of audiovisual stimulation (>8 weeks) may reduce brain atrophy, strengthen the functional connectivity between regions that normally lose connectivity in AD, and improve associative memory. Similarly, studies on transcranial stimulation report the potential to benefit memory and global cognitive function even beyond the end of treatment. Moreover, this type of stimulation also benefits structural measures of AD pathology. To validate the clinical significance of these findings, it is imperative to undertake further studies with larger cohorts, prolonged treatment durations, and a blinded, placebo-controlled methodology.

Finally, the field has generated tantalizing leads on how gamma stimulation may translate into beneficial effects on the cellular and molecular level. Neuronal recordings highlight a potential role of interneuron and neurometabolic coupling. Moreover, it seems likely that gamma stimulation exerts its benefits by engaging brain microglia and the vasculature. These insights may soon pave the way toward enhancing the efficacy of gamma stimulation.

The next generation of studies on gamma stimulation is already underway. Most importantly, larger clinical studies are required to ascertain the long-term benefits of gamma stimulation. In animal models, the focus should be on delineating the mechanism of gamma stimulation and providing further proof of principle studies on what other applications gamma stimulation may have.

Author contributions

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Conflict of interest statement

Professor Tsai is a scientific cofounder and SAB member of Cognito Therapeutics.

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