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Alzheimer's & Dementia

Featured Article

Gamma rhythm low field magnetic stimulation alleviates neuropathologic changes and rescues memory and cognitive impairments in a mouse model of Alzheimer's disease

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Abstract

Introduction: The abnormal amyloid β (Aβ) accumulation and Aβ-related neural network dysfunction are considered central to the pathogenesis of Alzheimer's disease (AD) at the early stage. Deep-brain reachable low field magnetic stimulation (DMS), a novel noninvasive approach that was designed to intervene the network activity in brains, has been found to alleviate stress-related cognitive impairments.

Methods: Amyloid precursor protein/presenilin-1 transgenic mice (5XFAD) were treated with DMS, and cognitive behavior and AD-like pathologic changes in the neurochemical and electrophysiological properties in 5XFAD mice were assessed.

Results: We demonstrate that DMS treatment enhances cognitive performances, attenuates Aβ load, upregulates postsynaptic density protein 95 level, and promotes hippocampal long-term potentiation in 5XFAD mouse brain. Intriguingly, the gamma burst magnetic stimulation reverses the aberrant gamma oscillations in the transgenic hippocampal network.

Discussion: This work establishes a solid foundation for the effectiveness of DMS in treating AD and proposes a future study of gamma rhythm stimulation on reorganizing rhythmic neural activity in AD brain.

Keywords: Alzheimer's disease; Deep-brain reachable low field magnetic stimulation; Treatment; Aβ; Gamma oscillations; Animal model

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder in elderly people and is the main cause of approximately two of three cases of dementia [1]. Extracellular amyloid β (Aβ) assemblies, which result in senile plaques, are considered to be the pathologic hallmark of AD [2]. Because the neuronal hyperactivity and aberrant

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network function occur at an early stage of pathologic alterations even before the formation of Aβ positive plaques, they are thought to be crucial events leading to mild cognitive impairment or AD [3–12]. As such, noninvasive methods aimed to restore both integrity and function of specific neural network are emerging as a useful new therapeutic tool to supplement or replace most drugs that were designed to clear Aβ deposits from brains of patients with AD, but failed in clinical trials because of toxicity and/or limited efficacy [13,14].

Transcranial magnetic stimulation (TMS) has been introduced to study brain function several decades ago [15]. Recently, magnetic stimulation was applied in clinic to treat brain diseases, for example, depression, stress, AD, and other neurodegenerative diseases [16–18]. Although TMS demonstrated symptomatic improvement in patients with AD [19–23], the underlying mechanisms remain elusive. Moreover, there is an increasing need to develop more portable and safer alternative magnetic devices for AD and other neurodegenerative disorders. Deep-brain reachable low field magnetic stimulation (DMS) was therefore developed in response to this need. This method generates a magnetic field with output pulses of higher-frequency, lower-intensity, and wider scope. As a noninvasive approach, DMS affects brain structures in a deep layer, such as the hippocampus, an area critical for learning and memory and principally impaired at the very early stage of AD. In fact, DMS promoted hippocampal neurogenesis and neuroplasticity in a stress rodent model [24]. Together, increasing evidence implies that DMS has potential protective effects on AD brains. Therefore, in this study, we sought to investigate the possible impact of DMS on AD in a mouse model of the disease.

Aβ precursor protein/presenilin-1 double-transgenic mice (5XFAD), which can rapidly develop Aβ deposition and AD-like behaviors, were used in the present study to evaluate effects of DMS on AD. We found that DMS treatment for 8 weeks improved cognitive performances, reduced amyloid burden, and restored the loss of a prime synaptic protein, that is, postsynaptic density protein 95 (PSD95), in the cortex and hippocampus of the transgenic mice. Parallel with these results, DMS reinstated the long-term potentiation (LTP) in the hippocampus of 5XFAD mice. Remarkably, the AD-relevant neuronal gamma oscillations in the hippocampus of 5XFAD mice were reversed by gamma burst magnetic stimulation. These results indicate that DMS is effective in alleviating symptoms of a rodent model of AD and might be a novel noninvasive therapeutic strategy for AD.

2. Methods

2.1. Animals and equipment

Amyloid precursor protein/presenilin-1 double transgenic mice (5XFAD; 006554, Jackson Laboratory) aged 4 months were used in this study. The use and care of animals were in strict accordance with the Chinese regulations involving animal protection and were approved by the Animal Ethics Committee of the Capital Medical University. All mice used in the study were female because of more severe and early AD-like phenotypic changes in female than male mice.

The magnetic equipment (designed and made by Beijing Aldans Biotech Co, Ltd), including two 360 mm-diameter coils, was connected to a magnetic field generator and outputs a time-varying magnetic field. Every 2-second output was composed of several rhythmical trains spiking in intervals of 27, 25, 23, 21, or 19 ms and formed the intermittent gamma burst stimulation at 30 to 40 Hz. The train was composed of six pulses with 130 μs width and 1000 Hz frequency. These 2-second runs were separated by an 8-second resting interval. Moreover, the shape of magnetic fields was changed every 4 minutes (between linear gradient and approximate distribution), and the rhythm was gradually increased every 8 minutes (30, 32.25, 34.5, 37, and 40 Hz). Successive trains of DMS for 40 minutes were administered daily for continuous 8 weeks. Female 5XFAD mice and their littermates aged 4 months were randomly divided into four groups: wild-type (WT) and 5XFAD mice treated with sham magnetic field or DMS. The specific parameters were set up based on a previous study [24]. Mice in their cages without metal covers were placed between the DMS coil pairs (Fig. 1A).

2.2. Behavioral assessment

Behavioral tests were carried out 24 h after the last DMS treatment, when the mice were aged 6 months. The following behavioral tasks were sequentially performed: novel object recognition, open field tests, and morris water maze (MWM). There was a 1-day interval between tasks. All experiments were performed during a period from 9 AM to 5 PM. The experimenters were blinded to the treatment of animals. For novel object recognition test, mice were allowed to explore one familiar object and one novel object for 5 minutes. The number of novel object contacting and novel object recognition index (time for novel object/time for familiar object + time for novel object) was calculated. Animals were observed for 30 minutes to assess locomotor function in open field test.

In MWM test, mice were habituated in a circular swimming pool (1.2 m in diameter) containing a visible platform. The consecutive 5-day training was performed and the escape latency was recorded. A probe trial (60 second) with withdrawal of the platform 24 hour after the last training was applied, and the number of crossing the original platform and swimming time spent in the target quadrant were recorded by a video tracking system (for details, see Supplementary Materials and Methods).

2.3. Histology and staining

After completion of the behavioral tests, mice were anesthetized by an intraperitoneal injection of 6% chloral hydrate (400 mg/kg) and perfused transcardially with cold saline.

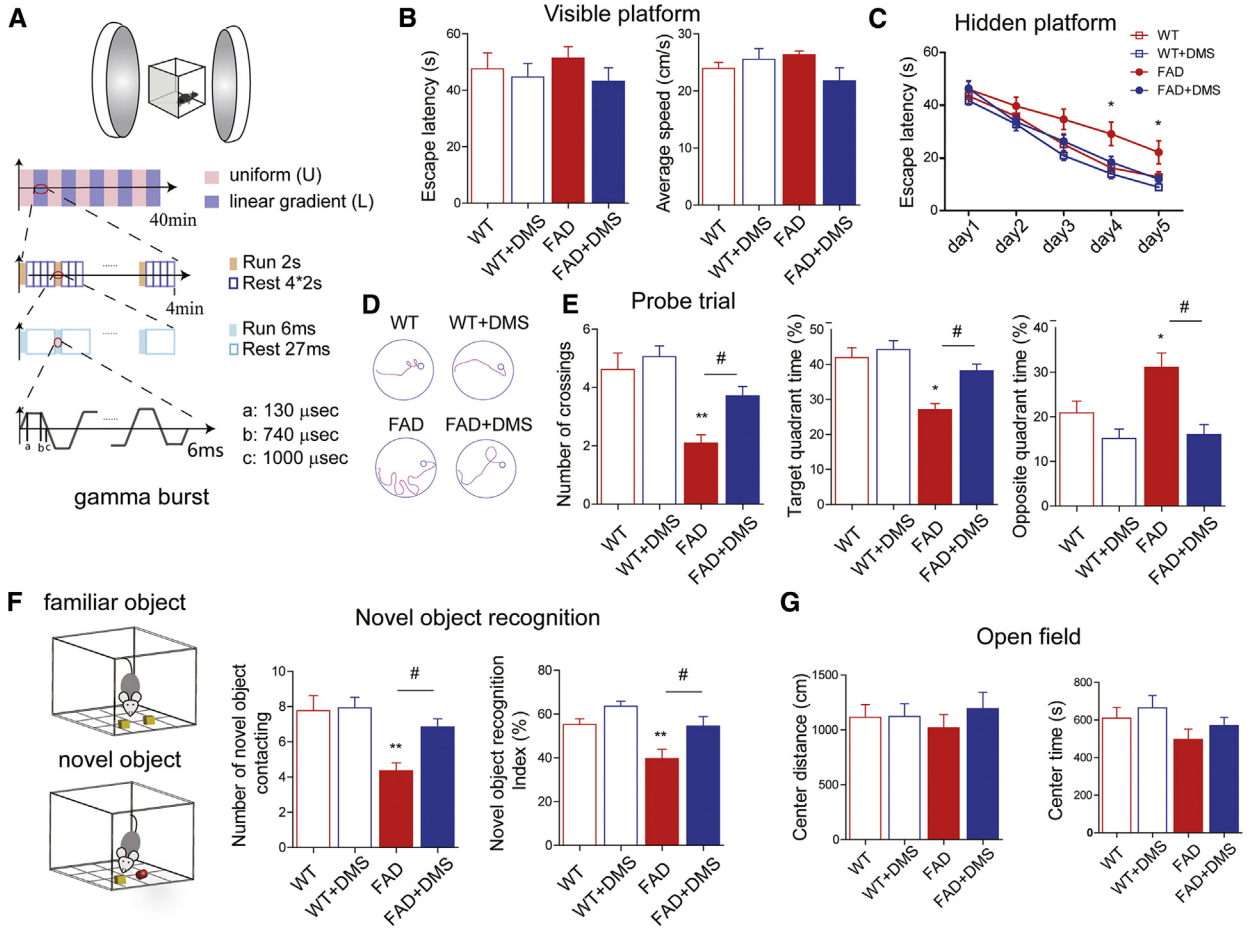


Fig. 1. DMS improved cognitive performances of 5XFAD mice. (A) A schematic diagram illustrating the operational principle of DMS (every 2-second output is composed of rhythmical trains spiking in intervals of 27, 25, 23, 21, or 19 ms and constitutes the intermittent gamma burst stimulation at 30–40 Hz rhythm.). (B–E) Effects of DMS on spatial learning and memory detected by MWM. The escape latency and swimming speed were observed in a visible platform first (B). Escape latency in a hidden platform was then recorded on every training day (C). A probe trial was applied after 5 days of training. The numbers and the quadrant time crossing over the target platform, and the quadrant time crossing the opposite platform (D and E) were recorded and used in statistical analysis (number of crossings: 4.615 ± 0.57 [WT], 5.063 ± 0.37 [WT + DMS], 2.100 ± 0.27 [FAD], 3.714 ± 0.32 [FAD + DMS], $F_{(3,50)} = 8.95$, $P < .05$, ANOVA; target quadrant time: 42.04 ± 2.78% [WT], 44.3 ± 2.49% [WT + DMS], 27.07 ± 1.78% [FAD], 38.22 ± 1.88% [FAD + DMS], $F_{(3,50)} = 9.66$, $P < .05$, ANOVA). (F) Effects of DMS on the number of novel object contacting and novel object recognition index in a novel object recognition test (the number of novel objects contacting in 5 minutes: 7.769 ± 0.86 [WT], 7.938 ± 0.59 [WT + DMS], 4.364 ± 0.45 [FAD], 6.857 ± 0.45 [FAD + DMS], $F_{(3,50)} = 8.419$, $P < .05$, ANOVA; the index of discrimination: 55.2 ± 2.63% [WT], 63.64 ± 2.20% [WT + DMS], 39.65 ± 4.27% [FAD], 54.49 ± 4.36% [FAD + DMS], $F_{(3,50)} = 8.151$, $P < .05$, ANOVA). (G) Locomotion activity in an open field test (center distance: $F_{(3,50)} = 1.642$; center time: $F_{(3,53)} = 1.173$, ANOVA). WT and 5XFAD (FAD) mice were treated with or without DMS for 8 weeks. All values are presented as the mean ± SEM. $^{*}P < .05$ versus WT, day × group interaction ($P > .05$) (two-way repeated measures ANOVA) (C). $^{*}P < .05$, $^{**}P < .01$ versus WT; $^{\#}P < .05$ versus FAD (two-way ANOVA with Bonferroni post hoc test) (B, E–G). WT ($n = 13$), FAD ($n = 11$), WT + DMS ($n = 16$), FAD + DMS ($n = 14$). Abbreviations: ANOVA, analysis of variance; DMS, deep-brain reachable low field magnetic stimulation; MWM, morris water maze; SEM, standard error of the mean; WT, wild type.

Brains were rapidly removed and bisected through the midsagittal plane into hemispheres. After removal of the brainstem and cerebellum, the left hemispheres were fixed in 4% paraformaldehyde in phosphate-buffered saline and used in detection of amyloid plaques and glial reaction. The right hemispheres were used in biochemical assays. Briefly, every 10 consecutive brain sections (30 μm) were subjected to immunostaining for detection of Aβ-positive plaques (anti-Aβ antibody) or glial reaction (anti–Iba-1 or glial fibrillary acidic protein [GFAP] antibody). The images were observed under a light or confocal microscope. The area occupied by Aβ- or glia-positive staining was measured using Image Pro Plus 6.0 and the value was normalized to total hippocampus or cortex area of 10× image. The mean value calculated across images from each brain was considered one sample (Supplementary Materials and Methods).

2.4. Biochemical assays

The cortex and hippocampus were dissected from right hemispheres for biochemical assays. For Aβ detection, the soluble and insoluble fractions were collected and quantified by human Aβ40 and Aβ42 enzyme linked immunosorbent assay (ELISA) kits. The Aβ level was normalized to the total

protein level of the tissue. The Western blot assay was used to detect the protein levels of synaptophysin (SYP) and PSD95 (Supplementary Materials and Methods).

2.5. Hippocampal slice preparation and recording

The multielectrode dish (Panasonic, MED 64 planar microelectrodes) was prepared as described previously [25]. The perforant pathway and dentate gyrus (DG) of the hippocampus were selected for stimulation and recording sites, respectively. After stabilizing synaptic responses for 15 minutes, an input-output curve was first determined for each group via the measurements of spell out (field excitatory postsynaptic potential) amplitude or slope in response to a series of stimulation intensities starting at 10 μA. A baseline was then recorded for additional 15 minutes. The 50% maximal stimulation intensity was set for baseline recording. LTP was induced by the theta-burst stimulation (TBS), which consisted of five bursts, each containing four pulses at 100 Hz with an interburst interval of 200 ms (5×TBS). After 5× TBS, the test stimulus was repeatedly delivered once every 1 minute for 60 minutes for observations of any changes in LTP magnitude and duration. Traces were obtained and analyzed using an MED64 System soft program (Alpha Med Science Inc, Osaka, Japan).

2.6. In vivo electrophysiology

After anesthesia with pentobarbital sodium (50 mg/kg, intraperitoneally), mice were placed in a stereotaxic apparatus (Stoelting, USA) and subjected to craniotomy (8 mm × 10 mm). Electrophysiological recordings were conducted by linear array multicontact U-Probe electrodes (PLX-UP-16-16ED-50-260-40-20(640)-15T-900-C16o25, U-Probe-Item 33574-50, Plexon, USA) containing 16 recording contacts (0.3–1.3 MΩ at 1 kHz) with an intercontact spacing of 50 μm to obtain spell out (local field potentials [LFP]) in vivo. Data were analyzed off-line using software from Offline Sorter V3, Neuroexplorer 4 (Plexon) and MATLAB 7.5 software by MathWorks. The low and high gamma bands were set at 35 to 55 and 65 to 110 Hz, respectively. Theta oscillations were extracted by applying a 4 to 12 Hz finite impulse response passband with zero-phase shift filter function in MATLAB (Supplementary Materials and Methods).

2.7. Statistical analysis

All data are shown as the mean ± standard error of the mean. Escape latencies in MWM were analyzed using a repeated measures analysis of variance (ANOVA). Other data were analyzed using Student's *t*-test for two-group comparisons or two-way ANOVA followed by Bonferroni post hoc for comparisons among more than two groups. The statistical analysis was performed with SPSS 13.0 (IBM, Armonk, NY, USA) and GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA, USA). $P < .05$ was considered statistical significance.

3. Results

3.1. DMS reverses cognitive impairments in 5XFAD mice

First, we tested hippocampus-dependent spatial learning and memory behaviors after DMS treatment. In MWM tests, we found no changes in escape latency and average speed in the visible platform test between WT and 5XFAD mice (Fig. 1B), indicating a minimal difference in the swimming ability and visual discrimination between normal and 5XFAD mice. Remarkably, in the hidden platform test, 5XFAD mice exhibited a significantly prolonged escape latency in training days 4 and 5 compared with WT mice (Fig. 1C, two-way repeated measures ANOVA, $P < .05$). DMS treatment prevented the increase in escape latency in 5XFAD mice. At 24 h after the last hidden platform training, the platform was removed and a probe trial was performed to evaluate the memory retrieval ability. As expected, both the number of crossings over the previous platform location and the percentage of time spent in the previous target quadrant in 5XFAD mice were less than that of WT mice (Fig. 1D and E). DMS was able to recover the reduction in these behavioral activities in 5XFAD mice to a level insignificantly different from WT mice. These results demonstrate that DMS is effective in improving spatial learning and memory in 5XFAD mice.

To substantiate the effectiveness of DMS in alleviating cognitive impairments in 5XFAD mice, we performed novel object recognition as well. As shown in Fig. 1F, DMS treatment increased the tendency of 5XFAD mice to interact more with a novel object than with a familiar object.

To exclude the possibility that the improvement in cognitive performances in 5XFAD mice by DMS was because of enhancement of movement ability, we used the open field test to detect the spontaneous locomotor behavior activity in these mice. There was no significant difference in the total distance traveled and the time spent in the central region among the groups (Fig. 1G), indicating that DMS had no effect on the locomotor activity in AD mice.

3.2. DMS mitigates AD-like pathology in 5XFAD mouse brains

To evaluate the effects of DMS on pathologic changes in amyloid, we performed immunostaining of brain sections with an Aβ antibody. Massive amyloid plaques were predominantly distributed in the cortex and hippocampus of 5XFAD mice, whereas amyloid plaques were undetectable in WT mouse brains (Fig. 2A and B). When DMS was administered, the average area occupied by Aβ positive plaques was decreased in 5XFAD mice. These data reveal the inhibitory effect of DMS on Aβ deposition in the brain of 5XFAD mice.

To determine whether the reduced deposition of amyloid plaques was because of a decrease in total Aβ levels in the brain, we carried out sandwich ELISA assays to quantify changes in soluble and insoluble forms of Aβ in the cortex and hippocampus of 5XFAD mice in response to DMS. The

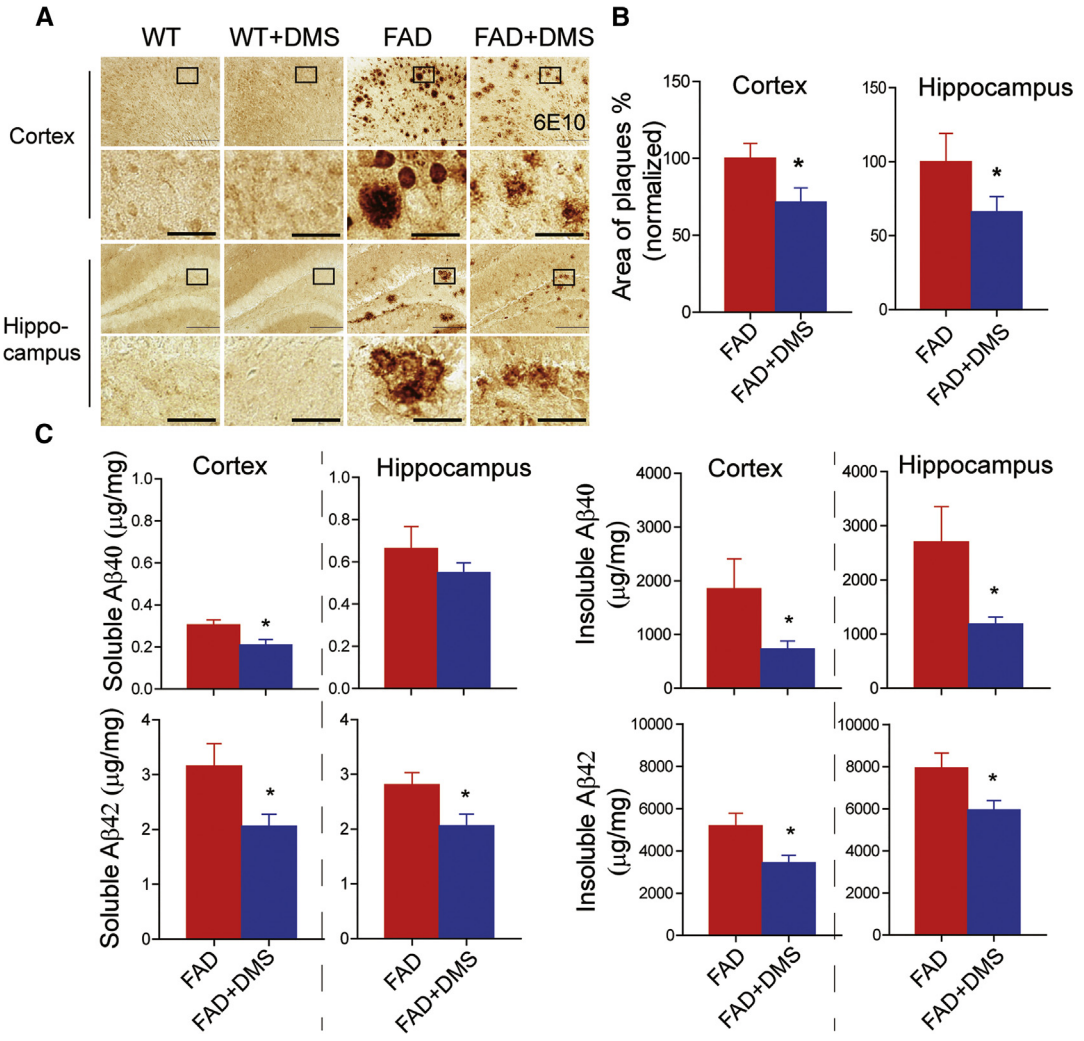


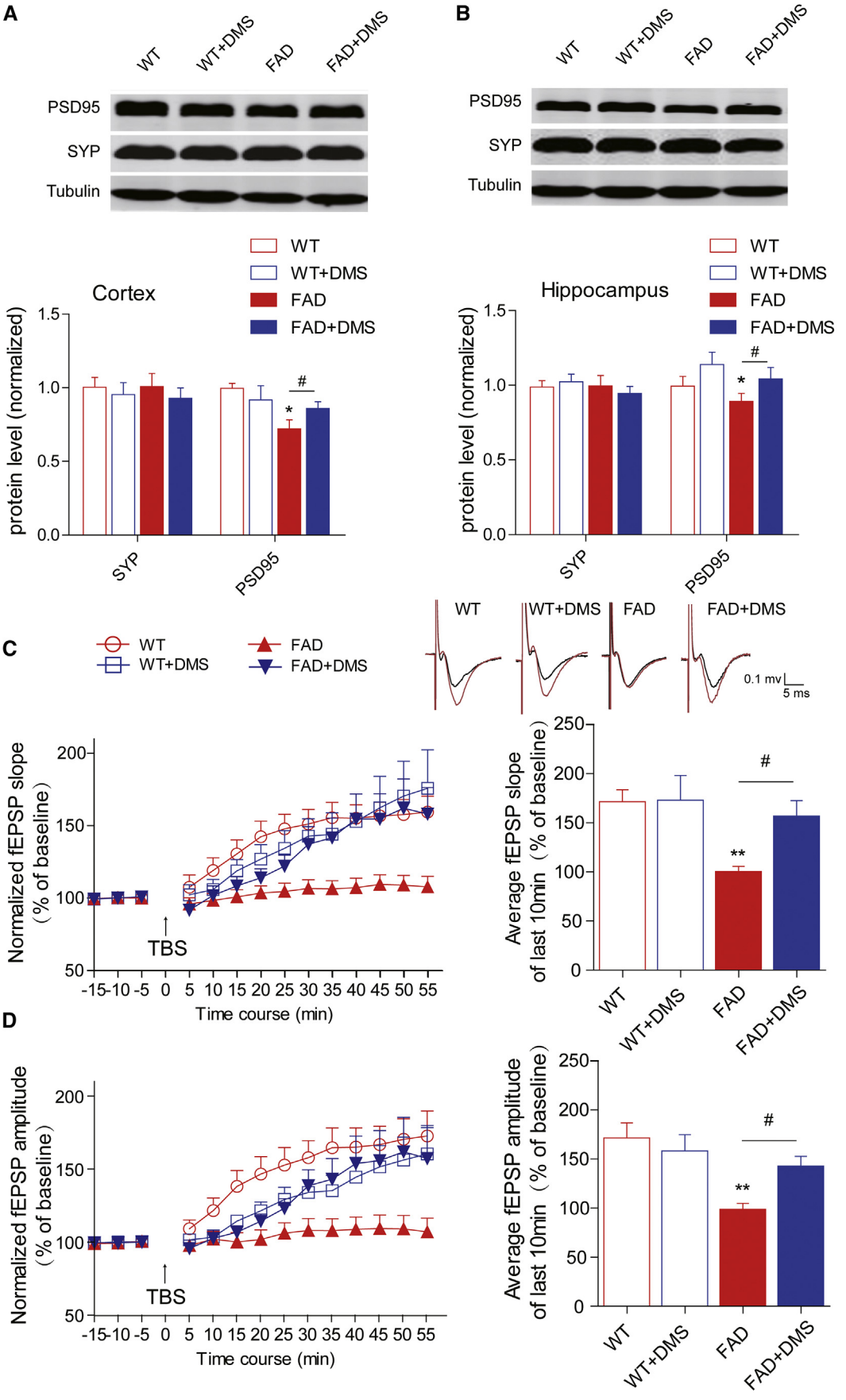
Fig. 2. DMS attenuated Aβ burden in the cortex and hippocampus of 5XFAD mice. (A) Representative immunostaining of Aβ-positive plaques in the cortex and hippocampus of WT and 5XFAD (FAD) mice with or without DMS treatment. (B) Effects of DMS on the area occupied by Aβ-positive plaques in the cortex and hippocampus (cortex: 71.6 ± 9.2% of FAD; hippocampus: 66.2 ± 10.33% of FAD, unpaired *t*-test, $P < .05$). (C) Effects of DMS on the expression of soluble and insoluble Aβ40 and Aβ42 in the cortex and hippocampus. All values are presented as the mean ± SEM ($n = 6$ mice/group). $^*P < .05$, unpaired student's *t*-test. Abbreviations: Aβ, amyloid β; DMS, deep-brain reachable low field magnetic stimulation; SEM, standard error of the mean; WT, wild type.

contents of both soluble and insoluble Aβ40 were reduced in the cortex. In the hippocampus, insoluble Aβ40 was reduced. Soluble Aβ40 showed a tendency of reduction in the hippocampus, although it did not reach a statistically different level. Similarly, levels of both soluble and insoluble Aβ42 were reduced in the cortex and hippocampus of 5XFAD mice treated with DMS compared with 5XFAD mice untreated with DMS (Fig. 2C). These data suggest that DMS alleviates the total burden of Aβ in the 5XFAD brain.

Microglial activation is considered an important neuroinflammatory event and another crucial phenotype of AD brains associated with amyloid deposition [2,26]. To determine the effect of DMS on this AD-associated neuroinflammation response, the expression of Iba1 (the microglial marker) was assessed in brain sections from 5XFAD mice treated or untreated with DMS by using immunofluorescent staining. A large number of Iba1 immunoreactive cells were observed in clusters surrounding the senile plaques in the cortex and hippocampus of 5XFAD mice, whereas they were not evident in WT mice (Fig. S1A and B). DMS slightly but significantly reduced the level of Iba1 immunoreactivity in the cortex and hippocampus of 5XFAD. Similarly, the area occupied by GFAP positive cells was much larger in the cortex and hippocampus of 5XFAD mice than WT mice (Fig. S1C and D). DMS reduced GFAP immunoreactivity in the cortex of 5XFAD mice, although DMS did not induce a significant reduction in GFAP immunostaining in the hippocampus. These results indicate that DMS could partly mitigate an Aβ-mediated neuroinflammatory process in the 5XFAD mouse brain.

3.3. DMS restores PSD95 expression and hippocampal LTP in 5XFAD mice

To determine whether synaptic proteins in the AD transgenic brain were affected by DMS, we measured changes in


A
WT
WT+DMS
FAD
FAD+DMS
PSD95
SYP
Tubulin
B
WT
WT+DMS
FAD
FAD+DMS
PSD95
SYP
Tubulin
Cortex
protein level (normalized)
1.5
1.0
0.5
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SYP
PSD95
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*
Hippocampus
protein level (normalized)
C
WT
WT+DMS
FAD
FAD+DMS
0.1 mv
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Normalized fEPSP amplitude (% of baseline)
Average fEPSP amplitude of last 10min (% of baseline)

abundance of both a presynaptic protein, SYP, and a postsynaptic protein, PSD95, in the cortex and hippocampus. As shown in Fig. 3A and B, relative protein levels of SYP were changed neither in the cortex nor in the hippocampus of 5XFAD mice compared with WT mice. In contrast, PSD95 levels were decreased in the cortex and hippocampus of 5XFAD mice relative to WT mice. A significantly less decrease in PSD95 levels was seen after DMS administration in both regions, suggesting that DMS could restore PSD95 expression in the brain of 5XFAD mice.

To examine the effect of DMS on hippocampal LTP in normal and 5XFAD mice, we conducted field hippocampal LTP recordings in these mice. We found that the TBS failed to induce the increase in the slope and amplitude of perforant path-evoked field excitatory postsynaptic potential in the DG of 5XFAD mice (Fig. 3C and D), indicating a lack of LTP in 5XFAD mice. This depression of LTP was reversed by DMS. In addition, both the paired-pulse ratio, a measure of presynaptic efficacy, and input-output curves of field potential, an indicator of basal synaptic response were unchanged in hippocampal slices in all groups of mice (Fig. S2), consistent with our finding that no significant change was found in the expression of the presynaptic protein SYP. Taken together, DMS exhibits the ability to restore postsynaptic protein (PSD95) expression and synaptic plasticity in a form of LTP in 5XFAD mice.

3.4. DMS modulates gamma oscillations in the hippocampus of 5XFAD mice

The specific oscillatory patterns resulted from the combined activity of neural networks and gamma oscillations were closely related to cognitive function [27–30]. To determine whether 5XFAD mice present aberrant gamma oscillations and whether DMS alters the oscillatory rhythm, we performed in vivo electrophysiological experiments to record LFP in the hippocampal network of the transgenic and normal mice treated with or without DMS. We found that, in a discrete high-frequency range (65–110 Hz), the amplitude of gamma oscillations was reduced and increased in the left CA1 and DG, respectively, of 5XFAD mice compared with WT mice (Fig. 4A and B). No phase-amplitude coupling change was observed in the low-gamma range (35–55 Hz) and the theta frequency range (4–12 Hz) between FAD and FAD + DMS mice in these two hippocampal subdivisions. Of note, DMS reversed these changes in the left CA1 and DG. However, in DMS-treated FAD mice compared with DMS-untreated FAD mice, we did not observe changes in gamma oscillations in the right side of the CA1 and DG as well as in the CA2 and CA3 areas (either left side or right side) (Fig. S3–8).

4. Discussion

Previous work shows that magnetic stimulation can directly regulate neuronal circuits and improve brain functions [31]. Because the aberrant neural network triggered by abnormal Aβ assemblies is considered to be crucial and reversible at the early stage of AD [3,8,32], we selected the 5XFAD mice aged 4 months at which few Aβ plaques were developed in the hippocampus and spatial memory was normal [33] to evaluate the therapeutic effect of DMS on AD brains. Both the cognitive decline and the Aβ accumulation in brains of 5XFAD mice were effectively inhibited by DMS. Meanwhile, synaptic plasticity (LTP) in the 5XFAD mouse hippocampus was rescued. Intriguingly, we for the first time to our knowledge discovered that the aberrant oscillatory rhythmic activity in gamma band of the left CA1 and DG areas of the hippocampal network was reversed by the magnetic stimulation in the transgenic mice, implying that DMS could be a novel noninvasive approach to hinder AD progression via a mechanism involving the regulation of neural network activity (Fig. 5).

Although magnetic stimulation has been reported to be effective in treating neuropsychiatric disorders, including depression, stress, and AD [23], underlying mechanisms and its effects on AD-like pathology remain to be investigated. Here, we conducted several behavioral tests to systematically determine the effect of DMS on cognitive deficits in AD mice. Consistent with the previous finding of improved spatial cognitive ability in the aging mice by TMS [34,35], hippocampus-dependent cognitive performance of 5XFAD mice measured by MWM was ameliorated after DMS application in the present study. Furthermore, we tested novelty recognition behavior. These data together indicate that the memory acquisition, consolidation, and retrieval dependent on hippocampal-neocortical connections [36] were enhanced in 5XFAD mice by DMS treatment.

Intriguingly, Aβ-specific plaques and total levels of Aβ40 and Aβ42 in the cortex and hippocampus of 5XFAD mice

Fig. 3. DMS restored reduction of PSD95 and rescued hippocampal LTP in 5XFAD mice. (A and B) Effects of DMS on synaptophysin (SYP) and PSD95 expression in the cortex (A) and hippocampus (B). Representative immunoblots are shown above the quantification graphs. All values that were normalized to WT mice are presented as the mean ± SEM and were analyzed by two-way ANOVA with Bonferroni post hoc test (cortex: 0.859 ± 0.02 [FAD + DMS] vs. 0.720 ± 0.03 [FAD], $F_{(3,12)} = 13.32$; hippocampus: 1.041 ± 0.04 [FAD + DMS] vs. 0.891 ± 0.02 [FAD], $F_{(3,12)} = 8.742$. $n = 4$ mice/group). $^{*}P < .05$ versus WT, $^{\#}P < .05$ versus FAD. (C and D) Effects of DMS on the slope (C) and amplitude (D) of fEPSPs recorded in the dentate gyrus. fEPSPs were induced by theta-burst stimulation (TBS, indicated by arrows). Inset traces, representative traces of fEPSP before and after the induction of potentiation. All values are presented as the mean ± SEM and were analyzed by two-way ANOVA with Bonferroni post hoc test (slope normalized to baseline: 171.6 ± 12% [WT], 173.1 ± 25% [WT + DMS], 100.5 ± 5% [FAD], 156.8 ± 16% [FAD + DMS], $F_{(3,43)} = 5.809$; amplitude: 171.5 ± 15% [WT], 158.3 ± 17% [WT + DMS], 98.8 ± 6% [FAD], 142.8 ± 10% [FAD + DMS], $F_{(3,43)} = 7.918$. $n = 10$ slices/6 mice for WT, $n = 11$ slices/7 mice for WT + DMS, $n = 15$ slices/6 mice for FAD, and $n = 11$ slices/6 mice for FAD + DMS). $^{**}P < .01$ versus WT. $^{\#}P < .05$ versus FAD. Abbreviations: ANOVA, analysis of variance; DMS, deep-brain reachable low field magnetic stimulation; fEPSPs, field excitatory postsynaptic potential; LTP, long-term potentiation; SEM, standard error of the mean; WT, wild type.

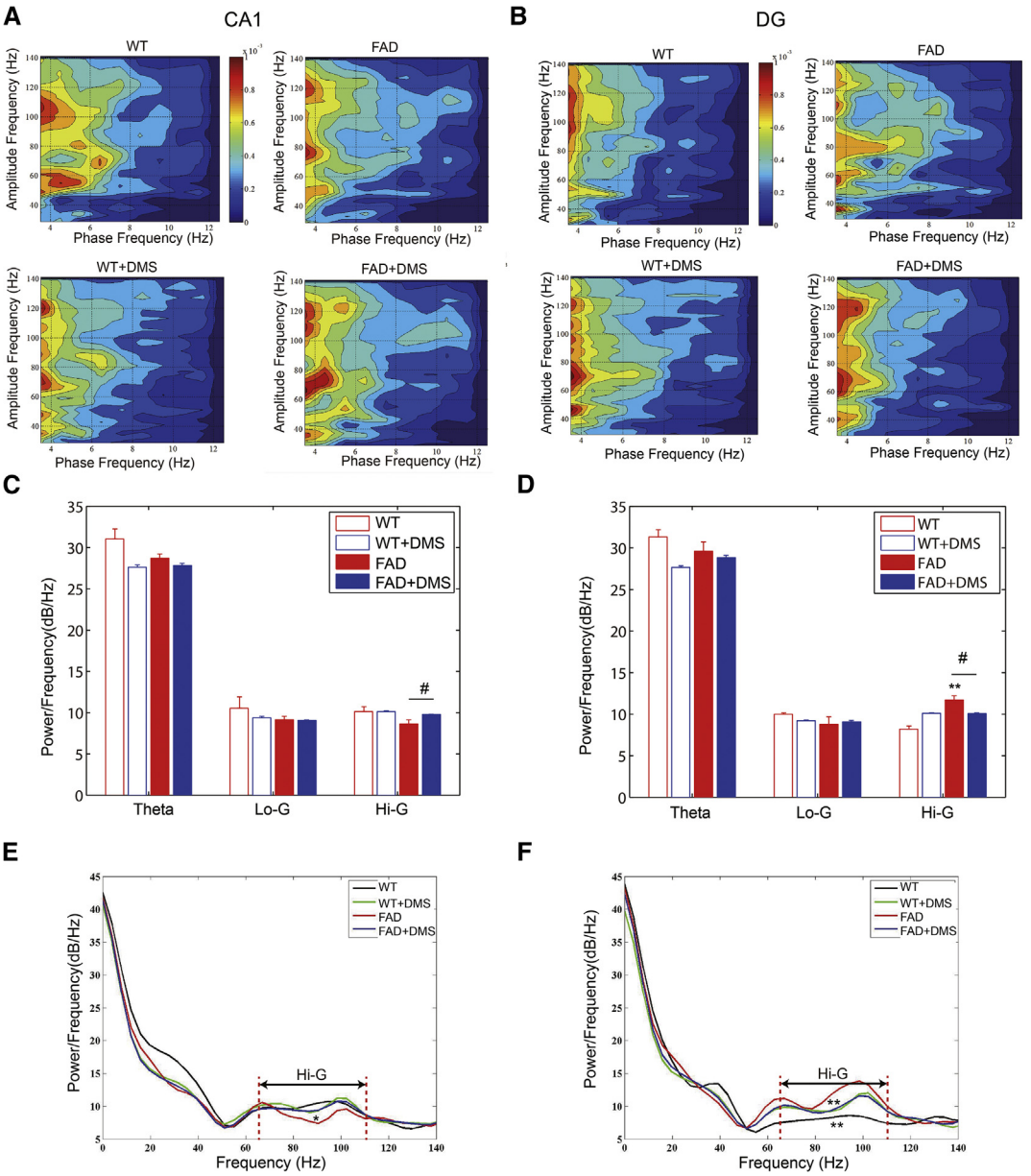

Fig. 4. The modulation of high-frequency LFP oscillations by DMS in the CA1 and DG. (A and B) Representative phase-to-amplitude comodulograms illustrating effects of DMS on high-frequency LFP oscillations in the CA1 (A) and DG (B) of four groups of mice. A pseudocolor scale for modulation index values is shown next to the lower-right panel. (C and D) Effects of DMS on LFP power in different frequency bands recorded in the CA1 (C) and DG (D). All values are presented as the mean ± SEM and were analyzed by two-way ANOVA with Bonferroni post hoc test. $^{**}P < .01$ versus WT, $^{\#}P < .05$ versus FAD. (E and F) Effects of DMS on power spectrum density (PSD) in the CA1 (E) and DG (F). LFP recordings were performed in four groups of mice ($n = 6$ mice/group). All values are presented as the mean ± SEM and were analyzed by two-sample *t*-test with Bonferroni's correction across LFP sub-bands. $^{*}P < .05$, $^{**}P < .01$ versus FAD mice. Abbreviations: ANOVA, analysis of variance; DG, dentate gyrus; DMS, deep-brain reachable low field magnetic stimulation; fEPSPs, field excitatory postsynaptic potential; SEM, standard error of the mean; WT, wild type.

were significantly attenuated by DMS. This suggests that the amyloid overproduction and deposition may be prevented. This prevention is particularly possible when the young transgenic mice at their early stage of AD-like pathology were used and examined [37]. Given the association of glial activation with Aβ plaques in AD brains [26], we analyzed microglia and astrocyte responses in these mice. Although dramatic microglial activation and astrogliosis in the cortex and hippocampus of 5XFAD mice were found, DMS had a limited effect on gliosis. This led us to speculate that DMS may not exert its anti-AD effects via a mechanism involving antigliosis. Other mechanisms may thus underlie the therapeutic action of DMS. Nevertheless, reserved glial responses in AD process may contribute to the compensatory remodeling of neural network function as suggested by Wyss-Coray and Mucke [38].

A number of studies revealed that synaptic depression caused by oligomeric Aβ assemblies preceded neuronal loss

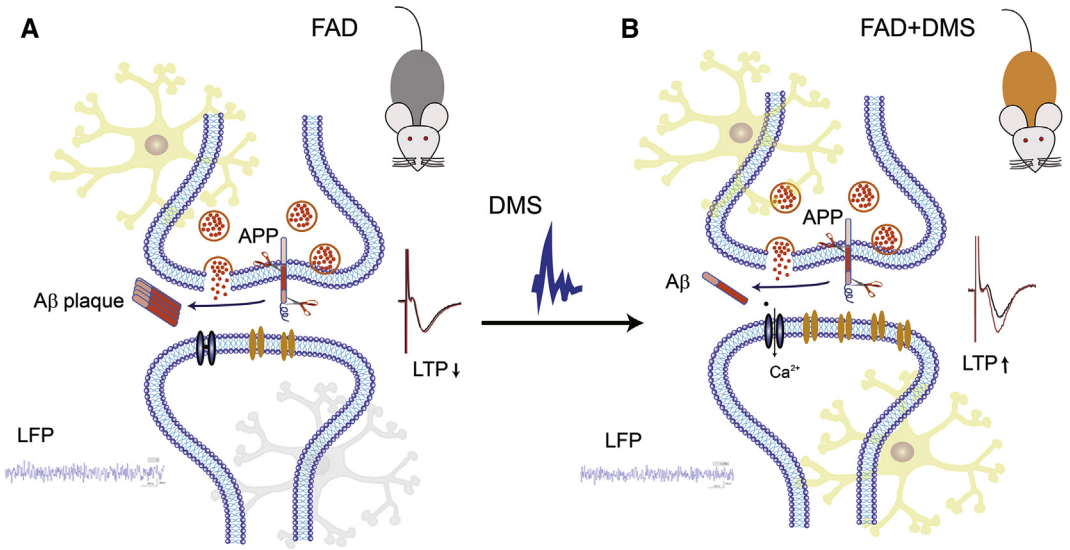

Fig. 5. A working model in which DMS alters AD-like changes in AD transgenic mice. (A and B) Synaptic activities in AD transgenic mice before (A) and after (B) DMS application. In 5XFAD transgenic brains, a series of pathologic changes occur, including deposition of Aβ-positive plaques, impairment of hippocampal LTP derived from postsynaptic degeneration, and disruption of hippocampal gamma oscillations probably because of Aβ-induced aberrant neural network. DMS treatment for 8 weeks enhances cognitive performances, decreases the Aβ load in transgenic mouse brains, and promotes hippocampal LTP. Reestablishing homeostasis of gamma oscillations in the hippocampus of 5XFAD mice by DMS may be involved in the restoration of cognitive function. Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, amyloid β; DMS, deep-brain reachable low field magnetic stimulation; LTP, long-term potentiation.

at a very early stage of AD and that cognitive decline correlated more closely with synaptic depletion than with the plaques [9,39–43]. Consistent with a previous report [37], PSD95 levels were decreased in the cortex and hippocampus of 5XFAD mice, although SYP levels were unchanged. Notably, the reduction in PSD95 levels was reversed by DMS, which seems to be the first evidence, to our knowledge, supporting the effectiveness of DMS in alleviating synaptic impairments in an AD model. In aging mice, PSD95 levels were also upregulated by TMS [34]. The subsequent LTP results strengthen a notion that DMS can reinstate the normal synaptic connections, which are required for cognitive behaviors [36,44,45].

Neuronal oscillations because of rhythmically synchronized neuronal activity are proposed to play an important role in information processing, and the gamma oscillation with low frequency (25–50 Hz) or high frequency (65–140 Hz) has been hypothesized to allow cortical-hippocampal circuit-dependent cognition and its ensuing behavior [27,29,30,46–48]. Increasing evidence shows that defected gamma oscillations in the hippocampus occurred at the early stage of AD and were associated with subsequent cognitive impairments and synaptic depression [10,42,47,49]. Aβ, as a central player controlling neuronal and network activity [7,11,42], may be involved in aberrant network activity. Our positive results from behavioral, neurochemical, and electrophysiological experiments point out a question as to whether magnetic stimulation in a gamma burst pattern could trigger a synchronous rhythm in specific brain regions. Magnetic stimulation indeed exerts its effects by inducing electrical currents in biological tissues especially in the brain because there is a set of synaptic network formed by various neurons [50]. Thus, it is possible that AD-linked areas in the brain are subject to the regulation by gamma burst magnetic stimulation. In support of this, we found that gamma burst magnetic stimulation reversed aberrant changes in a high frequency band range (65–110 Hz) of gamma oscillations in the left DG and CA1 of 5XFAD mice.

Recently, the therapeutic effect of TMS has also been observed in another AD transgenic mouse model via a mechanism involving the large conductance calcium-activated potassium (BK) channels [35]. Although both TMS [31] and DMS (this study) lowered Aβ levels and improved learning behavior in AD mouse models, we provide additional evidence supporting that DMS could act on oscillatory neural network to intervene the development of AD. Moreover, the pattern of magnetic stimulation in our study differs from that of TMS. TMS focuses on the touched area with a higher strength (more than 1 T) and cannot reach the hippocampus directly [31]. Unlike TMS, DMS involves a time-varying magnetic field with a lower strength (only 1/500 T) that can generate an induced square-wave electric field and might reach the hippocampus because of its whole-head penetration [51,52].

In sum, our observations at multiple levels collectively support that the imbalance between excitation and inhibition in neuronal circuitry, which has been suggested to be the causal factor of cognitive deficits in AD animals [6,14,53,54], could be rescued by the gamma burst magnetic stimulation. Thus, DMS can act as an effective "buffer machine" to normalize cognitive function and prevent AD process. However, it should be pointed out that studies on DMS in terms to its effect on AD are still at its infant stage. More in-depth experiments need to be conducted in the future to unravel the central substrates of DMS and molecular mechanisms underlying the therapeutic effect of DMS on AD.

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Author contributions: J.Z. and Y.Q. performed the biochemical, behavioral, and long-term potentiation tests. X.W. carried out the field recording experiment and L.C. analyzed the electrophysiological data. Y.Z. and X.W. designed the study and with the help of all other authors prepared the manuscript.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.trci.2017.07.002.

RESEARCH IN CONTEXT

1. Systematic review: The abnormal amyloid β (Aβ) accumulation and Aβ-related neural network dysfunction are considered central to the pathogenesis of Alzheimer's disease (AD) at the early stage. Deep-brain reachable low field magnetic stimulation (DMS), a novel noninvasive approach that was designed to intervene the network activity in brains, has been found to alleviate stress-related cognitive impairments. Yet, whether DMS protects brains against AD is still unclear.
2. Interpretation: Our findings suggest that DMS reverses multiple abnormal changes in a mouse model of AD. Thus, DMS might be a novel, promising, and noninvasive therapeutic strategy for AD.
3. Future directions: The results from this work establish a solid foundation for the effectiveness of DMS in treating AD and create a new area for future studies. Examples of future studies may include investigations of (1) the mechanisms underlying the DMS action in reorganizing rhythmic neural activity, (2) the cell type of neurons involved in the rhythmic conversion, and (3) the potential therapeutic effect of DMS in patients with AD.

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