Gamma-patterned sensory stimulation reverses synaptic plasticity deficits in rat models of early Alzheimer's disease

Yin Yang[1], Tomas Ondrejcak[1], Neng-Wei Hu[1], Sadia Islam[1], Eugene O'Rourke[1], Richard Reilly[1], Colm Cunningham[1], Michael Rowan[1], and Igor Klyubin[1]

[1]Trinity College Dublin

February 6, 2023

Abstract

Non-invasive sensory stimulation in the range of the brain's gamma rhythm (30-100 Hz) is emerging as a new potential therapeutic strategy for the treatment of Alzheimer's disease (AD). Here we investigated the effect of repeated combined exposure to 40 Hz synchronized sound and light stimuli on hippocampal long-term potentiation (LTP) in vivo in three rat models of early AD. We employed a very complete model of AD amyloidosis, amyloid precursor protein (APP)-overexpressing transgenic McGill-R-Thy1-APP rats at an early pre-plaque stage, systemic treatment of transgenic APP rats with corticosterone modelling certain environmental AD risk factors and, importantly, intracerebral injection of highly disease-relevant AD patient-derived synaptotoxic beta-amyloid and tau in wild-type animals. We found that daily sessions of 40 Hz sensory stimulation fully abrogated the inhibition of LTP in all three models. Moreover, there was a negative correlation between the magnitude of LTP and the level of active caspase-1 in the hippocampus of transgenic APP animals which suggests that the beneficial effect of 40 Hz stimulation was dependent on modulation of pro-inflammatory mechanisms. Our findings support ongoing clinical trials of gamma-patterned sensory stimulation in early AD.

Short Communication

Gamma-patterned sensory stimulation reverses synaptic plasticity deficits in rat models of early Alzheimer's disease

Yin Yang[1,2], Tomas Ondrejcak[1], Neng-Wei Hu[1,2], Sadia Islam[3], Eugene O'Rourke[4], Richard B. Reilly[5], Colm Cunningham[3], Michael J. Rowan[1] and Igor Klyubin[1]

[1]Department of Pharmacology and Therapeutics and Institute of Neuroscience, Trinity College, Dublin 2, Ireland
[2]Department of Physiology and Neurobiology, School of Basic Medical Sciences, Zhengzhou University, 100 Science Avenue, Zhengzhou 450001, China
[3]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute and Trinity College Institute of Neuroscience, Trinity College, Dublin 2, Ireland
[4]Department of Electronic and Electrical Engineering and Trinity College Institute of Neuroscience, Trinity College, Dublin 2, Ireland
[5]School of Medicine and Trinity College Institute of Neuroscience, Trinity College, Dublin 2, Ireland

Correspondence: Igor Klyubin or Michael J. Rowan, Department of Pharmacology & Therapeutics, Watts Building, Trinity College, Dublin 2, Ireland. E-mail: klyubini@tcd.ie or mrowan@tcd.ie

Running Title (40 characters)
40 Hz stimulation and LTP in AD models

ACKNOWLEDGEMENTS
Research reported here was supported by Science Foundation Ireland (19/FFP/6437) and Health Research Board Ireland (ILP-POR-2019-051) to Michael J. Rowan, and Natural Science Foundation of Henan Province (212300410259) and China Scholarship Council (CSC, 202007045047) to Yin Yang, and National Natural Science Foundation of China (U2004134) and Zhengzhou University (140/3231029) to Neng-Wei Hu. We thank Dr Yingjie Qi for his input during pilot studies and Prof A. C. Cuello (McGill University, Montreal) for providing McGill-R-Thy1-APP breeding stock.

Word counts: 2,901

Gamma-patterned sensory stimulation reverses synaptic plasticity deficits in rat models of early Alzheimer's disease

Abstract

Non-invasive sensory stimulation in the range of the brain's gamma rhythm (30-100 Hz) is emerging as a new potential therapeutic strategy for the treatment of Alzheimer's disease (AD). Here we investigated the effect of repeated combined exposure to 40 Hz synchronized sound and light stimuli on hippocampal long-term potentiation (LTP) *in vivo* in three rat models of early AD. We employed a very complete model of AD amyloidosis, amyloid precursor protein (APP)-overexpressing transgenic McGill-R-Thy1-APP rats at an early pre-plaque stage, systemic treatment of transgenic APP rats with corticosterone modelling certain environmental AD risk factors and, importantly, intracerebral injection of highly disease-relevant AD patient-derived synaptotoxic beta-amyloid and tau in wild-type animals. We found that daily sessions of 40 Hz sensory stimulation fully abrogated the inhibition of LTP in all three models. Moreover, there was a negative correlation between the magnitude of LTP and the level of active caspase-1 in the hippocampus of transgenic APP animals which suggests that the beneficial effect of 40 Hz stimulation was dependent on modulation of pro-inflammatory mechanisms. Our findings support ongoing clinical trials of gamma-patterned sensory stimulation in early AD.

KEYWORDS

Alzheimer's disease aqueous brain extract, amyloid precursor protein transgenic rats, beta-amyloid, tau, long-term potentiation, 40 Hz sensory stimulation

Abbreviations: Alzheimer's disease, AD; amyloid precursor protein, APP; beta-amyloid, Aβ; excitatory postsynaptic potentials, EPSPs; high-frequency stimulation, HFS; intracerebroventricular, i.c.v.; intraperitoneal, i.p.; long-term potentiation, LTP; NOD-, LRR- and pyrin domain-containing protein 3, NLRP3; strong HFS, sHFS; subcutaneous, s.c.

1 | INTRODUCTION

After many years of intensive preclinical and clinical research there is growing hope that effective disease-modifying therapies for Alzheimer's disease (AD) are now an achievable goal. Agents that directly pharmacologically target major neuropathological hallmarks of the disease including beta-amyloid (Aß) plaques, tau-containing neurofibrillary tangles and neuroinflammation are the main focus of attention (Cummings *et al.*, 2022; van Dyck *et al.*, 2023). In addition, the therapeutic potential of non-pharmacological approaches, including those targeting brain network dysfunction, is gaining considerable traction (Chen *et al.*, 2022; Manippa *et al.*, 2022). Abnormal brain network activity in patients with AD include a marked reduction in gamma band electroencephalogram oscillations (γ, 30-100 Hz) (Casula *et al.*, 2022) a feature that also has been observed in many animal models (Driver *et al.*, 2007; Iaccarino *et al.*, 2016; Bazzigaluppi *et al.*, 2018). Considering the well-documented role of gamma rhythm in cognition (Griffiths *et al.*, 2019), much recent research has focussed on the development of potential AD treatments based on non-invasive electrical and sensory brain stimulation protocols that induce or restore gamma (Mably & Colgin, 2018; Traikapi & Konstantinou, 2021). Moreover, recent small clinical studies indicate reasonable safety and good tolerability of gamma-patterned sensory stimulation (He *et al.*, 2021; Chan *et al.*, 2022).

Preclinical studies in amyloid precursor protein (APP)- and tau-transgenic mouse models showed that gamma range sensory stimulation, in particular visual and auditory stimulation at 40 Hz, alone or combination, beneficially affected behavioural deficits in these animals (Adaikkan *et al.*, 2019; Martorell *et al.*, 2019). Somewhat surprisingly, the behavioural improvement was accompanied by marked reductions in Aß plaque and phosphorylated tau accumulation as well as neuroinflammatory markers in the brains of the transgenic mice.

Although the studies in transgenic mice indicate that 40 Hz sensory stimulation may have disease-modifying properties little is known about the action of such stimulation in other animal AD models. Here, we investigated whether therapeutically relevant visual and auditory stimulation synchronized at 40 Hz could reverse the inhibition of long-term potentiation (LTP), an electrophysiological correlate of learning and memory, in APP-transgenic rats at an early, pre-plaque, stage of amyloidosis. Because Aß oligomer-mediated pro-inflammatory mechanisms are strongly implicated in causing the LTP deficit in these rats (Qi *et al.*, 2014; Zhang *et al.*, 2017; Qi *et al.*, 2018), we also examined caspase-1 cleavage, a measure of NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome activation, one of the key elements of the brain's innate immune response to Aß (Heneka *et al.*, 2013; Rand & Cooper, 2021). Furthermore, we employed disease-relevant aqueous extract of AD brain to test the ability of 40 Hz sensory stimulation to reverse the persistent LTP deficit induced by intracerebral injection of patient-derived synaptotoxic Aß and tau.

2 | MATERIALS AND METHODS

2.1 | Animals

All experiments were carried out in accordance with the approval of the Health Products Regulatory Authority, Ireland. Animals had free access to food and water and a 12-h lights on/off cycle. Male transgenic rats (5-7 months old, Figure 1 and Figure 2) expressing human APP751 with Swedish and Indiana mutations under the control of the murine Thy1.2 promoter (McGill-R-Thy1-APP) (Leon *et al.*, 2010) and their wild-type Wistar Han littermates were genotyped commercially by Transnetyx (Cordova, TN, USA) using real-time PCR. Some McGill-R-Thy1-APP rats (Figure 2) received 3 doses of corticosterone (10 mg/kg/day, Sigma) in polyethylene glycol 400 (1 ml/kg, Sigma) as vehicle subcutaneously (s.c.) (Qi *et al.*, 2021). Male Lister Hooded wild-type rats (3-5 months old) were used for experiments with AD human brain aqueous extract from the temporal cortex of an 87-year-old man (Figure 4), prepared and characterized as described previously (Ondrejcak *et al.*, 2018), that contained both synaptotoxic Aß and tau (T. Ondrejcak *et al*., unpublished observations). Human brain tissue was used in accordance with the guidelines of Trinity College Dublin Faculty of Health Science Ethics Committee (under approval 16014).

2.2 | Surgery

The rats were anaesthetized with urethane (1.5g/kg, intraperitoneal, i.p.) and core body temperature was maintained at 37.5 $\pm$ 0.5°C. As previously described (Klyubin *et al.*, 2014), both recording (targeted at 3.8 mm posterior to bregma and 2.5 mm lateral to midline) and stimulating (targeted at 4.6 mm posterior to bregma and 3.8 mm lateral to midline) electrodes, constructed from Teflon coated tungsten twisted-pair wires (75 µm inner core diameter, 112 µm external diameter), were positioned in the stratum radiatum of area CA1. Screw electrodes located over the contralateral cortex were used as reference and earth.

To investigate the effect of AD extract in Lister Hooded wild-type rats, intracerebroventricular (i.c.v., coordinates: 0.5 mm posterior to bregma and 1.2 mm right of midline, depth 3.8mm) injections were carried out under recovery anaesthesia using a mixture of ketamine (60 mg/kg) and medetomidine (0.4 mg/kg) (both i.p.) (Klyubin *et al.*, 2014). Electrophysiological recordings were carried out under urethane anaesthesia 21 days later.

2.3 | *In vivo* electrophysiology

Excitatory postsynaptic potentials (EPSPs) were recorded from the stratum radiatum in the CA1 area of the dorsal hippocampus in response to stimulation of the ipsilateral Schaffer collateral/commissural pathway (Klyubin *et al.*, 2014). Test stimuli were delivered to the Schaffer-collateral/commissural pathway every 30 s to evoke field EPSPs that were 50% maximum amplitude. To induce LTP we used either our standard high-frequency stimulation (HFS) protocol consisting of 1 set of 10 trains of 20 stimuli at test pulse intensity at 200 Hz with an inter-train interval of 2 s, or, in the case of

Figure 2, a strong (sHFS) protocol consisting of 3 sets of 10 trains of 20 stimuli at high intensity (75% maximum) at 400 Hz with an inter-train interval of 2 s and an inter-set interval of 5 min.

2.3 | 40 Hz sensory stimulation

Rats housed in transparent cages were placed in a light- and sound-insulated modified rat cage holding cabinet (Scantainer) with background dimmed light during their 12-h light on phase for either one- or two-hour daily session. The cabinet contained an Arduino-controlled system composed of light-emitting diodes and audio speakers similar to that previously described (Singer *et al.*, 2018). The light (pulse power 260 µW) was synchronized with the sound (pulse intensity 80 dB) and applied at 40Hz or, as a control, at a random frequency (10-80Hz) continuously for 1 (Figure 2 and Figure 3) or 2 h (Figure 1). In the case of corticosterone-treated animals (Figure 2), the 40 Hz sensory stimulation was applied during the 12-h light off phase, with no background light inside the modified Scantainer. LTP experiments were carried out one day after the last sensory stimulation session.

2.4 | Western blot

Western blotting was performed as previously described (Chen *et al.*, 2020; Zhang *et al.*, 2022). Briefly, rats were decapitated after electrophysiological recording and the brains were taken out immediately. Hippocampi were separated on the ice-cold surface and frozen immediately. Proteins were extracted by homogenizing in CHAPS lysis buffer and concentrations were determined by BCA protein assay (ThermoFisher, 23227). Proteins were separated in 4 to 12% midi protein gels (ThermoFisher, WG1402A) by electrophoresis and transferred to PVDF membrane (Merck Millipore, IPVH00010). Then the membranes were blocked with 5% non-fat milk (Cell Signaling Technology, 9999S) for 1 h in room temperature and incubated with primary antibodies for overnight in 4° C. The primary antibody was anti-Caspase-1 (p20) (AdipoGen, AG-20B-0042, 1:1000). After washing with TBST, the membranes were incubated with the corresponding HRP-conjugated secondary antibodies including anti-rabbit IgG (Dako, P0448, 1:2000), anti-mouse IgG (Dako, P0447, 1:1000). Finally, the bands were detected with enhanced chemiluminescence reagents (Merck Millipore, WBKLS0500) and quantified using Image Lab software (Version 6.1.0 build 7, Bio-Rad Laboratories, Inc.). Values shown are normalized to beta-actin and then expressed relative to levels of p20 in wild-type animals.

2.5 | Statistical analysis

The strength of synaptic transmission is expressed as a percentage of the EPSP amplitude recorded over a 30 min baseline recording period. The magnitude of LTP was measured at 3 h post-HFS and expressed as the mean ± SEM % baseline. No data were excluded. Control experiments were interleaved randomly throughout experimental sets. Sample sizes were chosen to ensure adequate statistical power comparable to previously published papers. Experimental data were normally

distributed. For statistical analysis and graphical display EPSP amplitude measurements were grouped into 10 min epochs. One-way ANOVA followed by Bonferroni's multiple comparison tests was used to compare the magnitude of LTP between multiple groups. Paired and unpaired Student's t-tests were used to compare within one group and between two groups, respectively. One-way ANOVA was also used to analyse the Western blots and a parametric Gaussian distribution was assumed for correlation analysis (with Pearson's r). A p-value of <0.05 was considered statistically significant. Statistical analyses were performed in Prism 9.5.0.

2 | **RESULTS**

To investigate any potential beneficial effects of light and sound stimulation synchronized at 40 Hz we studied pre-plaque transgenic animals first (Figure 1A,B). Consistent with our previous reports (Qi *et al.*, 2014; Qi *et al.*, 2019), whereas 200 Hz HFS induced robust synaptic LTP in the hippocampus of wild-type rats (Untreated WT, p=0.0059, compared with pre-HFS baseline), LTP was only transient in age-matched pre-plaque APP transgenic rats, being completely blocked at 3 h post-HFS (Untreated TG, p=0.1775, compared with pre-HFS baseline and p=0.0167, compared with Untreated WT). A two-week treatment consisting of two-hour daily sessions completely abrogated the LTP deficit in transgenic rats (40 Hz TG, p=0.0071, compared with pre-HFS baseline and p>0.9999, compared with Untreated WT, p=0.0009, compared with Untreated TG, Figure 1B). Reducing the 40 Hz sensory stimulation session to 1h/day had similar beneficial effects in transgenic rats (148±7%, p=0.0029, compared with pre-HFS baseline and p=0.0006, compared with Untreated TG, n=4).

Because of our previous discovery of the involvement of pro-inflammatory mechanisms in LTP inhibition in McGill APP transgenic rats (Qi *et al.*, 2018), we also measured caspase-1 cleavage in the same animals post-mortem (Figure 1A,B). Using Western blotting for caspase-1 p20 subunit we observed a modest, but not statistically significant, increase in the level of activated caspase-1 in Untreated TG with respect to Untreated WT and 40 Hz TG groups (p=0.0652, one-way ANOVA, Figure 1C). We wondered if the ability of HFS to trigger LTP in these animals was related to the extent of caspase-1 activation in individual rats across the three experimental groups. Interestingly, there was a highly significant negative correlation between the level of active caspase-1 in the dorsal hippocampus and the magnitude of LTP (Figure 1D).

While AD can have a strong genetic component, in most cases environmental factors, such as lifetime stress, are known to significantly exacerbate symptoms and likely accelerate disease progression. Recently, we reported that brief exposure of APP transgenic rats to elevated glucocorticoids triggers a persistent exacerbation of LTP deficits (Qi *et al.*, 2021). We, therefore, examined the effects of 40 Hz sensory stimulation in APP transgenic rats that previously had been briefly exposed to elevated levels of corticosterone (Figure 2B-D). Consistent with our previous report (Qi *et al.*, 2021), a strong 3x400 Hz HFS protocol induced similar magnitude LTP in untreated

wild-type and transgenic animals (Untreated TG, p=0.0004, compared with pre-sHFS baseline and p=0.9846, compared with Untreated WT, Figure 2A). We also confirmed our previous findings (Qi *et al.*, 2021) that three daily injections of corticosterone (3 x 10 mg/kg daily) selectively and persistently inhibited sHFS-induced LTP in transgenic rats for at least four weeks (TG CORT, p=0.3248, compared with pre-sHFS baseline and p=0.0014, compared with WT CORT, Figure 2C,D). Importantly, 1 h daily 40 Hz sensory stimulation for 2 weeks (Figure 2B) reversed the additional LTP deficit in corticosterone-injected transgenic rats (TG CORT 40 Hz, p=0.0023, compared with pre-sHFS baseline and p=0.7069, compared with Untreated WT, Figure 2C,D).
Finally, we employed a highly disease-relevant, non-transgenic, model (Klyubin *et al.*, 2014), in which wild-type rats received a single i.c.v. injection of aqueous extract of AD brain under recovery anaesthesia and LTP inducibility was tested under non-recovery anaesthesia three weeks later (Figure 3A). Previously, we found that this particular extract of AD brain caused an Aß- and tau-dependent persistent inhibition of LTP, being prevented by immunodepletion of either Aß or tau (T. Ondrejcak *et al*., unpublished observations). Remarkably, daily 1 h long sensory stimulation at 40 Hz reversed the LTP inhibition in this model as well (40 Hz, p=0.0011, compared with pre-HFS baseline, and p>0.9999, compared with Untreated, Figure 3B). In contrast, control random frequency (10-80 Hz) visual and auditory stimulation didn't reverse inhibition of LTP in AD brain extract-injected rats (Random, p=0.4676, compared with pre-HFS baseline, p=0.0005 compared with Untreated, and p=0.0002 compared with 40 Hz, Figure 3B).

4 | **DISCUSSION**

Our results provide strong evidence that combined auditory and visual stimulation synchronized at 40 Hz has the ability to reverse LTP deficits in three rat models of early AD, transgenic APP overexpression, gene x environmental corticosterone interaction and delayed effect of AD brain extract. Indeed, gamma rhythms that support synaptic plasticity under physiological conditions (Zarnadze *et al.*, 2016) are impaired in AD patients (Guntekin *et al.*, 2022) and normalising any deficits likely will be important for restoring normal synaptic functioning. Apart from uncovering the beneficial effect of 40 Hz sensory stimulation in transgenic APP rats, a very complete model of AD amyloidosis (Leon *et al.*, 2010), our findings in AD brain extract-injected wild-type animals help close the gap between preclinical studies and therapeutic implications because these aqueous extracts contain highly disease-relevant patient-derived synaptotoxic Aß and tau (Hong *et al.*, 2018; Ondrejcak *et al.*, 2018).
How then might gamma-patterned sensory stimulation alleviate AD-associated hippocampal LTP deficits? One possibility is that repeated 40 Hz sensory stimulation promotes clearance of synaptotoxic Aß by microglia. In transgenic plaque-bearing mice prolonged presentation of either 40 Hz light or sound stimulation reduced both soluble and insoluble Aß load (Martorell *et al.*, 2019), probably by microglial engulfment (Singer *et al.*, 2018), and decreased the number of Aß-positive neurons

(Park *et al.*, 2020). Interestingly, combined visual and auditory 40 Hz stimulation, similar to what we used in this study, was more effective in clustering of microglia around plaques than either alone (Martorell *et al.*, 2019). Additionally, in pre-plaque 3xTg-AD mice 40 Hz light flicker significantly reduced the number of Aß-positive neurons in the hippocampus as well (Park *et al.*, 2022).

A possibly related mechanism, gamma-patterned brain stimulation may have triggered anti-inflammatory processes. Our data showing a negative correlation between LTP magnitude and caspase-1 activation (Figure 1D) are consistent with this hypothesis. Indeed, pre-plaque 3xTg-AD (Park *et al.*, 2022) and Tau P301S (Adaikkan *et al.*, 2019) mice had a reduced inflammatory profile after exposure to 40 Hz light flicker. Moreover, 40 Hz transauricular vagal nerve electrical stimulation reduced hippocampal expression of NLRP3, caspase-1, interleukin-1ß and interleukin-18 which was accompanied by microglia-associated reduction in both soluble and insoluble Aß deposition in APP/PS1 mice (Yu *et al.*, 2022).

It is also possible that 40 Hz sensory stimulation interacted with tau in the case of the model using patient-derived synaptotoxic Aß and tau (Figure 3). In tau overexpressing neurofibrillary tangle-bearing mice 40 Hz light flicker improved synaptic integrity markers such as brain-derived neurotrophic factor (BDNF) and synaptophysin (Adaikkan *et al.*, 2019; Park *et al.*, 2020). On the other hand, Jeong *et al* reported that 40 Hz transcranial electrical stimulation reversed impaired hippocampal LTP in the absence of changes in BDNF expression in young 5xFAD mice in the absence of tau deposition (Jeong *et al.*, 2021).

The attempts to restore/influence gamma power in AD are still at an investigational stage as no changes in AD biomarkers in humans after such treatment have been reported to date (Manippa *et al.*, 2022). However, gamma-patterned AD treatment has a clear clinical potential as gamma entrainment in sensory cortex can propagate into deeper brain areas in rodents (Adaikkan *et al.*, 2019; Martorell *et al.*, 2019) and humans (Jones *et al.*, 2019). Given the importance of corticosteroids in mediating circadian rhythms (Oster *et al.*, 2017), our data on beneficial effects of 40 Hz sensory stimulation in corticosterone-injected APP transgenic rats (Figure 2) are in accord with findings that similar sensory stimulation improves the circadian clock in APP/PS1 mice (Yao *et al.*, 2020) and sleep in AD patients (Cimenser *et al.*, 2021) support this approach. In conclusion, results of this study may lead to development of effective non-invasive treatment as a new potential disease-modifying therapy for AD.

ACKNOWLEDGEMENTS

Research reported here was supported by Science Foundation Ireland (19/FFP/6437 and 14/IA/2571) and Health Research Board Ireland (ILP-POR-2019-051) to Michael J. Rowan, and Natural Science Foundation of Henan Province (212300410259) and China Scholarship Council (CSC, 202007045047) to Yin Yang, and National Natural Science Foundation of China (U2004134) and Zhengzhou University (140/3231029) to Neng-Wei Hu. We thank Dr Yingjie Qi for his input during pilot studies and Prof A. C. Cuello (McGill University, Montreal) for providing McGill-R-Thy1-APP breeding stock.

CONFLICTS OF INTEREST

The authors declare no financial interests or potential conflicts of interest.

AUTHORS CONTRIBUTIONS

Yin Yang: conceptualization; investigation; formal analysis; writing – original draft preparation; writing – review & editing. Tomas Ondrejcak: conceptualization; writing – review & editing. Neng-Wei Hu: conceptualization; writing – review & editing. Sadia Islam: methodology; writing – review & editing. Eugene O'Rourke: conceptualization; resources; writing – review & editing. Richard Reilly: conceptualization; resources; writing – review & editing. Colm Cunningham: conceptualization; writing – review & editing. Michael J. Rowan: conceptualization; writing – original draft preparation; writing – review & editing. Igor Klyubin: conceptualization; investigation; formal analysis; writing – original draft preparation; writing – review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Adaikkan, C., Middleton, S.J., Marco, A., Pao, P.C., Mathys, H., Kim, D.N.W., Gao, F., Young, J.Z., Suk, H.J., Boyden, E.S., McHugh, T.J. & Tsai, L.H. (2019) Gamma entrainment binds higher-order brain regions and offers neuroprotection. *Neuron*, **102**, 929-943. https://doi.org/10.1016/j.neuron.2019.04.011

Bazzigaluppi, P., Beckett, T.L., Koletar, M.M., Lai, A.Y., Joo, I.L., Brown, M.E., Carlen, P.L., McLaurin, J. & Stefanovic, B. (2018) Early-stage attenuation of phase-amplitude coupling in the hippocampus and medial prefrontal cortex in a transgenic rat model of Alzheimer's disease. *Journal of Neurochemistry*, **144**, 669-679. https://doi.org/10.1111/jnc.14136

Casula, E.P., Pellicciari, M.C., Bonni, S., Borghi, I., Maiella, M., Assogna, M., Minei, M., Motta, C., D'Acunto, A., Porrazzini, F., Pezzopane, V., Mencarelli, L., Roncaioli, A., Rocchi, L., Spampinato, D.A., Caltagirone, C., Santarnecchi, E., Martorana, A. & Koch, G. (2022) Decreased frontal gamma activity in Alzheimer's disease patients. *Annals of Neurology*, **92**, 464-475. https://doi.org/10.1002/ana.26444

Chan, D., Suk, H.J., Jackson, B.L., Milman, N.P., Stark, D., Klerman, E.B., Kitchener, E., Fernandez Avalos, V.S., de Weck, G., Banerjee, A., Beach, S.D., Blanchard, J., Stearns, C., Boes, A.D., Uitermarkt, B., Gander, P., Howard, M., 3rd, Sternberg, E.J., Nieto-Castanon, A., Anteraper, S., Whitfield-Gabrieli, S., Brown, E.N., Boyden, E.S., Dickerson, B.C. & Tsai, L.H. (2022) Gamma frequency sensory stimulation in mild probable Alzheimer's dementia patients: Results of feasibility and pilot studies. *PLoS One*, **17**, e0278412. https://doi.org/10.1371/journal.pone.0278412

Chen, F., Jiang, G., Liu, H., Li, Z., Pei, Y., Wang, H., Pan, H., Cui, H., Long, J., Wang, J. & Zheng, Z. (2020) Melatonin alleviates intervertebral disc degeneration by disrupting the IL-1beta/NF-kappaB-NLRP3 inflammasome positive feedback loop. *Bone Res*, **8**, 10. https://doi.org/10.1038/s41413-020-0087-2

Chen, X., Shi, X., Wu, Y., Zhou, Z., Chen, S., Han, Y. & Shan, C. (2022) Gamma oscillations and application of 40-Hz audiovisual stimulation to improve brain function. *Brain Behav*, **12**, e2811. https://doi.org/10.1002/brb3.2811

Cimenser, A., Hempel, E., Travers, T., Strozewski, N., Martin, K., Malchano, Z. & Hajos, M. (2021) Sensory-evoked 40-Hz gamma oscillation improves sleep and daily living activities in Alzheimer's disease patients. *Frontiers in Systems Neuroscience*, **15**, 746859. https://doi.org/10.3389/fnsys.2021.746859

Cummings, J., Lee, G., Nahed, P., Kambar, M.E.Z.N., Zhong, K., Fonseca, J. & Taghva, K. (2022) Alzheimer's disease drug development pipeline: 2022. *Alzheimers & Dementia-Translational Research & Clinical Interventions*, **8**, e12295. https://doi.org/10.1002/trc2.12295

Driver, J.E., Racca, C., Cunningham, M.O., Towers, S.K., Davies, C.H., Whittington, M.A. & LeBeau, F.E.N. (2007) Impairment of hippocampal gamma (gamma)-frequency oscillations in vitro in mice overexpressing human amyloid precursor protein (APP). *European Journal of Neuroscience*, **26**, 1280-1288. https://doi.org/10.1111/j.1460-9568.2007.05705.x

Griffiths, B.J., Parish, G., Roux, F., Michelmann, S., van der Plas, M., Kolibius, L.D., Chelvarajah, R., Rollings, D.T., Sawlani, V., Hamer, H., Gollwitzer, S., Kreiselmeyer, G., Staresina, B., Wimber, M. & Hanslmayr, S. (2019) Directional coupling of slow and fast hippocampal gamma with neocortical alpha/beta oscillations in human episodic memory. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 21834-21842. https://doi.org/10.1073/pnas.1914180116

Guntekin, B., Erdal, F., Bolukbas, B., Hanoglu, L., Yener, G. & Duygun, R. (2022) Alterations of resting-state gamma frequency characteristics in aging and Alzheimer's disease. *Cognitive Neurodynamics*, **4**, s11571. https://doi.org/10.1007/s11571-022-09873-4

He, Q.L., Colon-Motas, K.M., Pybus, A.F., Piendel, L., Seppa, J.K., Walker, M.L., Manzanares, C.M., Qiu, D.Q., Miocinovic, S., Wood, L.B., Levey, A.I., Lah, J.J. & Singer, A.C. (2021) A feasibility trial of gamma sensory flicker for patients with prodromal Alzheimer's disease. *Alzheimers & Dementia-Translational Research & Clinical Interventions*, **7**, e12178. https://doi.org/10.1002/trc2.12178

Heneka, M.T., Kummer, M.P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., Griep, A., Axt, D., Remus, A., Tzeng, T.C., Gelpi, E., Halle, A., Korte, M., Latz, E. & Golenbock, D.T. (2013) NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature*, **493**, 674-678. https://doi.org/10.1038/nature11729

Hong, W., Wang, Z., Liu, W., O'Malley, T.T., Jin, M., Willem, M., Haass, C., Frosch, M.P. & Walsh, D.M. (2018) Diffusible, highly bioactive oligomers represent a critical minority of soluble Abeta in Alzheimer's disease brain. *Acta Neuropathol*, **136**, 19-40.https://10.1007/s00401-018-1846-7

Iaccarino, H.F., Singer, A.C., Martorell, A.J., Rudenko, A., Gao, F., Gillingham, T.Z., Mathys, H., Seo, J., Kritskiy, O., Abdurrob, F., Adaikkan, C., Canter, R.G., Rueda, R., Brown, E.N., Boyden, E.S. & Tsai, L.H. (2016) Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature*, **540**, 230-235. https://doi.org/10.1038/nature20587

Jeong, W.H., Kim, W.I., Lee, J.W., Park, H.K., Song, M.K., Choi, I.S. & Han, J.Y. (2021) Modulation of long-term potentiation by gamma frequency transcranial alternating current stimulation in transgenic mouse models of Alzheimer's disease. *Brain Sciences*, **11**, 1532. https://doi.org/10.3390/brainsci11111532

Jones, M., McDermott, B., Oliveira, B.L., O'Brien, A., Coogan, D., Lang, M., Moriarty, N., Dowd, E., Leo, Q.L., Mc Ginley, B., Dunne, E., Newell, D., Porter, E., Elahi, M.A., O' Halloran, M. & Shahzad, A. (2019) Gamma band light stimulation in human case studies: groundwork for potential Alzheimer's disease treatment. *Journal of Alzheimers Disease*, **70**, 171-185. https://10.3233/Jad-190299

Klyubin, I., Ondrejcak, T., Hayes, J., Cullen, W.K., Mably, A.J., Walsh, D.M. & Rowan, M.J. (2014) Neurotransmitter receptor and time dependence of the synaptic plasticity disrupting actions of Alzheimer's disease A beta in vivo. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **369**, 20130147. https://doi.org/10.1098/rstb.2013.0147

Leon, W.C., Canneva, F., Partridge, V., Allard, S., Ferretti, M.T., DeWilde, A., Vercauteren, F., Atifeh, R., Ducatenzeiler, A., Klein, W., Szyf, M., Alhonen, L. & Cuello, A.C. (2010) A novel transgenic rat model with a full Alzheimer's-like amyloid pathology displays pre-plaque intracellular amyloid-beta-associated cognitive impairment. *Journal of Alzheimers Disease*, **20**, 113-126. https://doi.org/10.3233/Jad-2010-1349

Mably, A.J. & Colgin, L.L. (2018) Gamma oscillations in cognitive disorders. *Current Opinion in Neurobiology*, **52**, 182-187. https://doi.org/10.1016/j.conb.2018.07.009

Manippa, V., Palmisano, A., Filardi, M., Vilella, D., Nitsche, M.A., Rivolta, D. & Logroscino, G. (2022) An update on the use of gamma (multi)sensory stimulation for Alzheimer's disease treatment. *Frontiers in Aging Neuroscience*, **14**, 1095081. https://doi.org/10.3389/fnagi.2022.1095081

Martorell, A.J., Paulson, A.L., Suk, H.J., Abdurrob, F., Drummond, G.T., Guan, W., Young, J.Z., Kim, D.N.W., Kritskiy, O., Barker, S.J., Mangena, V., Prince, S.M., Brown, E.N., Chung, K., Boyden, E.S., Singer, A.C. & Tsai, L.H. (2019) Multi-sensory gamma stimulation ameliorates Alzheimer's-associated pathology and improves cognition. *Cell*, **177**, 256-271. https://doi.org/10.1016/j.cell.2019.02.014

Ondrejcak, T., Klyubin, I., Corbett, G.T., Fraser, G., Hong, W., Mably, A.J., Gardener, M., Hammersley, J., Perkinton, M.S., Billinton, A., Walsh, D.M. & Rowan, M.J. (2018) Cellular Prion Protein Mediates the Disruption of Hippocampal Synaptic Plasticity by Soluble Tau In Vivo. *Journal of Neuroscience*, **38**, 10595-10606.https://10.1523/Jneurosci.1700-18.2018

Oster, H., Challet, E., Ott, V., Arvat, E., de Kloet, E.R., Dijk, D.J., Lightman, S., Vgontzas, A. & Van Cauter, E. (2017) The functional and clinical significance of the 24-Hour rhythm of circulating glucocorticoids. *Endocrine Reviews*, **38**, 3-45.https://doi.org/10.1210/er.2015-1080

Park, S.S., Park, H.S., Kim, C.J., Baek, S.S., Park, S.Y., Anderson, C., Kim, M.K., Park, I.R. & Kim, T.W. (2022) Combined effects of aerobic exercise and 40-Hz light flicker exposure on early cognitive impairments in Alzheimer's disease of 3xTg mice. *Journal of Applied Physiology*, **132**, 1054-1068. https://doi.org/10.1152/japplphysiol.00751.2021

Park, S.S., Park, H.S., Kim, C.J., Kang, H.S., Kim, D.H., Baek, S.S. & Kim, T.W. (2020) Physical exercise during exposure to 40-Hz light flicker improves cognitive functions in the 3xTg mouse model of Alzheimer's disease. *Alzheimers Research & Therapy*, **12**, s13195. https://doi.org/10.1186/s13195-020-00631-4

Qi, Y., Klyubin, I., Hu, N.W., Ondrejcak, T. & Rowan, M.J. (2019) Pre-plaque Aß-mediated impairment of synaptic depotentiation in a transgenic rat model of Alzheimer's disease amyloidosis. *Front Neurosci*, **13**, 861. https://doi.org/10.3389/fnins.2019.00861

Qi, Y.J., Klyubin, I., Cuello, A.C. & Rowan, M.J. (2018) NLRP3-dependent synaptic plasticity deficit in an Alzheimer's disease amyloidosis model in vivo. *Neurobiology of Disease*, **114**, 24-30. https://doi.org/10.1016/j.nbd.2018.02.016

Qi, Y.J., Klyubin, I., Harney, S.C., Hu, N., Cullen, W.K., Grant, M.K., Steffen, J., Wilson, E.N., Do Carmo, S., Remy, S., Fuhrmann, M., Ashe, K.H., Cuello, A.C. & Rowan, M.J. (2014) Longitudinal testing of hippocampal plasticity reveals the onset and maintenance of endogenous human A beta-induced synaptic dysfunction in individual freely behaving pre- plaque transgenic rats: rapid reversal by anti-A beta agents. *Acta Neuropathologica Communications*, **2**, s40478. https://doi.org/10.1186/s40478-014-0175-x

Qi, Y.J., Klyubin, I., Ondrejcak, T., Hu, N.W. & Rowan, M.J. (2021) Enduring glucocorticoid-evoked exacerbation of synaptic plasticity disruption in male rats modelling early Alzheimer's disease amyloidosis. *Neuropsychopharmacology*, **46**, 2170-2179. https://doi.org/10.1038/s41386-021-01056-9

Rand, D. & Cooper, I. (2021) Caspase-1: an important player and possible target for repair of the blood-brain barrier underlying neurodegeneration. *Neural Regeneration Research*, **16**, 2390-2392. https://doi.org/10.4103/1673-5374.313031

Singer, A.C., Martorell, A.J., Douglas, J.M., Abdurrob, F., Attokaren, M.K., Tipton, J., Mathys, H., Adaikkan, C. & Tsai, L.H. (2018) Noninvasive 40-Hz light flicker to recruit microglia and reduce amyloid beta load. *Nature Protocols*, **13**, 1850-1868. https://doi.org/10.1038/s41596-018-0021-x

Traikapi, A. & Konstantinou, N. (2021) Gamma oscillations in Alzheimer's disease and their potential therapeutic role. *Frontiers in Systems Neuroscience*, **15**, 782399. https://doi.org/10.3389/fnsys.2021.782399

van Dyck, C.H., Swanson, C.J., Aisen, P., Bateman, R.J., Chen, C., Gee, M., Kanekiyo, M., Li, D., Reyderman, L., Cohen, S., Froelich, L., Katayama, S., Sabbagh, M., Vellas, B., Watson, D., Dhadda, S., Irizarry, M., Kramer, L.D. & Iwatsubo, T. (2023) Lecanemab in early Alzheimer's disease. *New England Journal of Medicine*, **388**, 9-21. https://doi.org/10.1056/NEJMoa2212948

Yao, Y.L., Ying, Y., Deng, Q.Y., Zhang, W.J., Zhu, H.Z., Lin, Z.L., Zhang, S.L., Ma, J.X. & Zhao, Y.Y. (2020) Non-invasive 40-Hz light flicker ameliorates Alzheimer's-associated rhythm disorder via regulating central circadian clock in mice. *Frontiers in Physiology*, **11**, 294. https://doi.org/10.3389/fphys.2020.00294

Yu, Y., Jiang, X., Fang, X., Wang, Y., Liu, P., Ling, J., Yu, L., Jiang, M. & Tang, C. (2022) Transauricular vagal nerve stimulation at 40 Hz inhibits hippocampal P2X7R/NLRP3/caspase-1 signaling and improves spatial learning and memory in 6-month-old APP/PS1 mice. *Neuromodulation*, **1-12**, 011. https://doi.org/10.1016/j.neurom.2022.03.011

Zarnadze, S., Bauerle, P., Santos-Torres, J., Bohm, C., Schmitz, D., Geiger, J.R.P., Dugladze, T. & Gloveli, T. (2016) Cell-specific synaptic plasticity induced by network oscillations. *Elife*, **5**, 14912. https://doi.org/10.7554/eLife.14912

Zhang, D.N., Qi, Y.J., Klyubin, I., Ondrejcak, T., Sarell, C.J., Cuello, A.C., Collinge, J. & Rowan, M.J. (2017) Targeting glutamatergic and cellular prion protein mechanisms of amyloid beta-mediated persistent synaptic plasticity disruption: Longitudinal studies. *Neuropharmacology*, **121**, 231-246. https://doi.org/10.1016/j.neuropharm.2017.03.036

Zhang, Y.Y., Yang, Y., Hu, Z.T., Zhu, M.Y., Qin, S.Y., Yu, P.P., Li, B., Xu, J.T., Ondrejcak, T., Klyubin, I., Rowan, M.J. & Hu, N.W. (2022) Long-term depression-inducing low frequency stimulation enhances p-tau181 and p-tau217 in an age-dependent manner in live rats. *Journal of Alzheimers Disease*, **89**, 335-350. https://doi.org/10.3233/Jad-220351

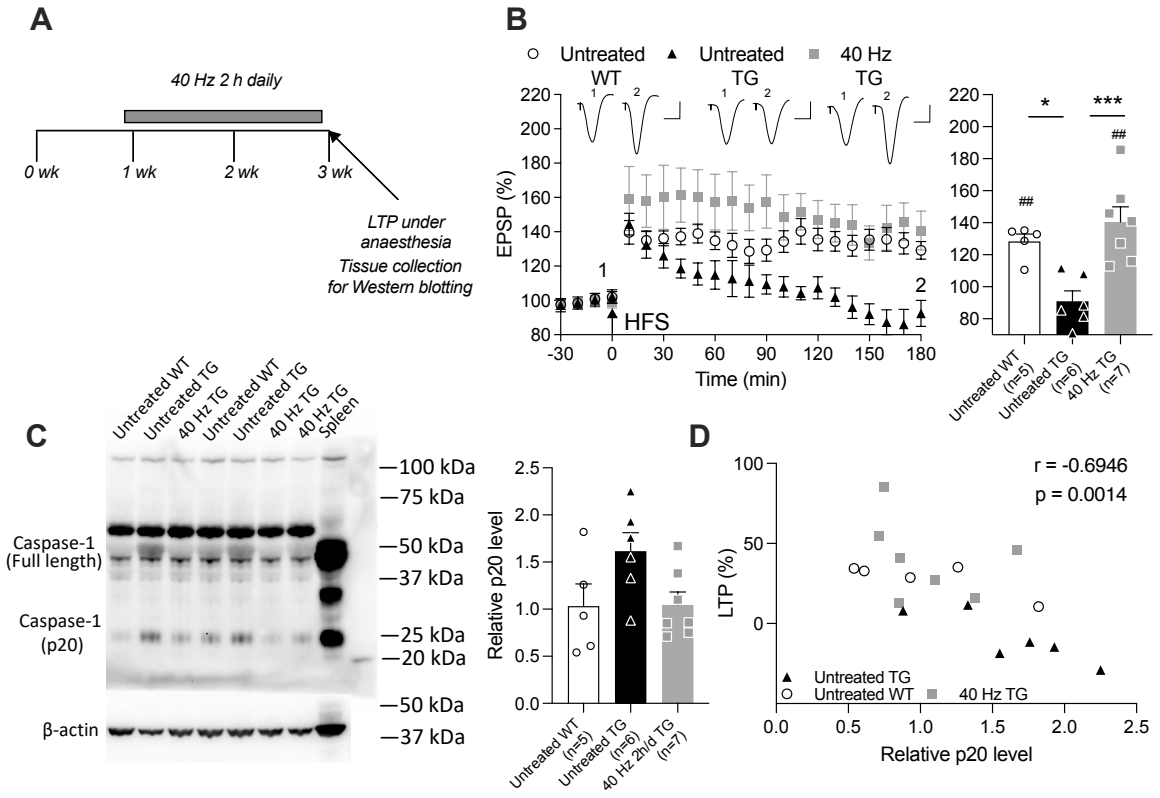

FIGURE 1. Daily 40 Hz sensory stimulation abrogates the LTP deficit in pre-plaque APP transgenic rats. **(A**) Schematic diagram of the treatment regimen. Animals were treated daily (2 h/day) for two weeks with auditory and visual stimulation synchronized at 40 Hz. The following day the animals were anaesthetised and LTP was measured *in vivo*. Post-mortem samples of the hippocampus were collected for subsequent Western blotting. (**B**) High-frequency stimulation (HFS)-induced LTP was impaired in transgenic (Untreated TG) rats compared with age-matched wild-type (Untreated WT) littermates. Sensory stimulation at 40 Hz enhanced LTP in TG animals to a level indistinguishable from untreated WT rats. The summary bar chart of LTP magnitude during the last 10 min is in the right-hand panel. Insets show representative field EPSP traces at the times indicated. Calibration bars: vertical, 1 mV; horizontal, 10 ms. Values are mean ± SEM. $^{\#\#}p < 0.01$ compared with pre-HFS, paired t-test; $*p < 0.05$, $***p < 0.001$, one-way ANOVA followed by Bonferroni's multiple-comparison tests. (**C**) Representative Western blotting and quantification analysis of the level of active caspase-1 (p20) in the dorsal hippocampus, normalized to beta-actin and then expressed relative to levels of p20 in WT animals. As a positive control we used the spleen homogenate of a 6-month-old wild-type rat (Spleen). (**D**) Correlation analysis of LTP magnitude and caspase-1 activation as measured by p20 level in each rat across all three experimental groups.

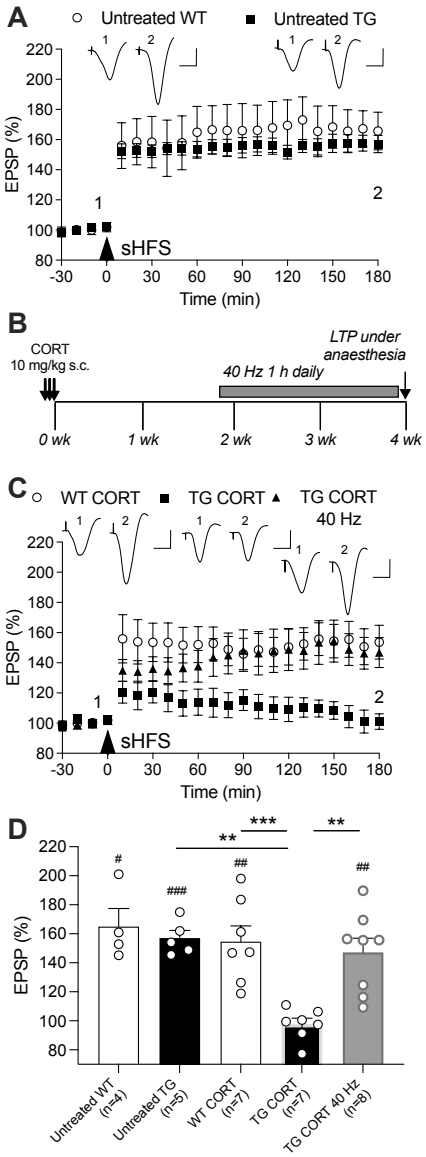

FIGURE 2. Reversal of corticosterone-exacerbated LTP deficit in APP transgenic rats by 40 Hz sensory stimulation. (**A**) Strong high-frequency stimulation (sHFS) triggered similar magnitude LTP in both wild-type (Untreated WT) and transgenic (Untreated TG) animals. (**B**) TG and wild-type WT rats received 3 daily s.c. injections of corticosterone (CORT) followed by two-week treatment with auditory and visual stimulation (1 h/day) synchronized at 40 Hz. Four weeks after CORT and ~24 h after the last sensory stimulation session, sHFS was applied under urethane anaesthesia. (**C**) While sHFS triggered robust LTP in WT rats pre-treated with CORT (WT CORT), the same pre-treatment strongly inhibited LTP in TG rats (TG CORT). Forty Hz sensory stimulation reversed the LTP deficit in TG animals (TG CORT 40 Hz). (**D**) The summary bar chart shows the magnitude of LTP during the last 10 min for data in **A** and **C**. Insets show representative field EPSP traces at the times indicated. Calibration bars: vertical, 1 mV; horizontal, 10 ms. Values are mean ± SEM. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with pre-HFS, paired t-test; $^{**}p < 0.01$, $^{***}p < 0.001$ one-way ANOVA followed by Bonferroni's multiple-comparison tests.

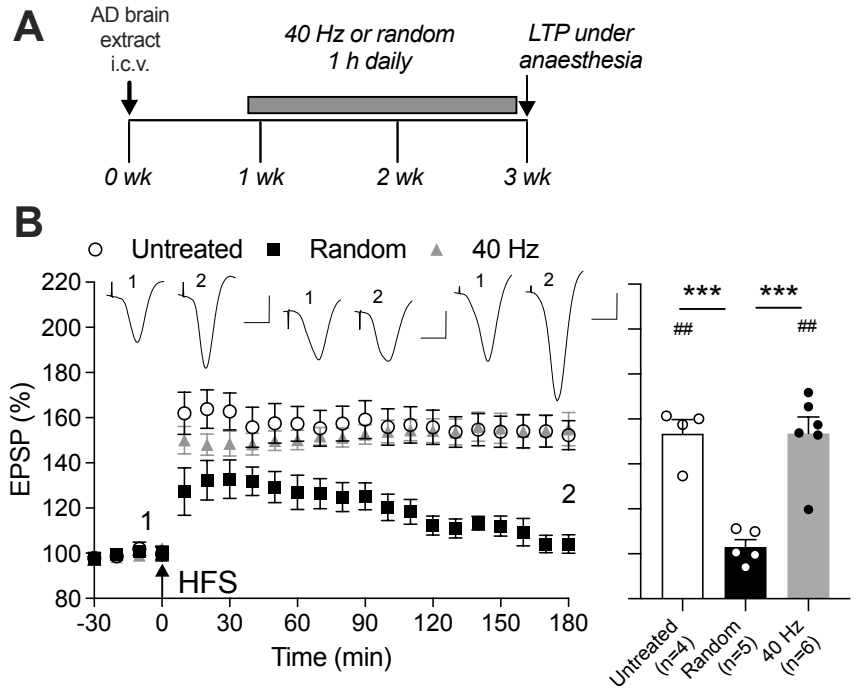

FIGURE 3. Forty Hz sensory stimulation reverses persistent LTP inhibition by synaptotoxic AD brain-derived Aß and tau in wild-type rats. **(A**) Schematic diagram of treatment regimen. (**B**) 40 Hz, in contrast to random (10-80 Hz), sensory stimulation reversed the persistent inhibition of HFS-induced LTP caused by a single i.c.v. injection of AD brain aqueous extract. Untreated animals served as an additional interleaved control. Left-hand panel shows the time course of LTP. Summary bar chart of LTP magnitude during the last 10 min is in the right-hand panel. Insets show representative field EPSP traces at the times indicated. Calibration bars: vertical, 1 mV; horizontal, 10 ms. Values are mean ± SEM. $^{\#\#}p < 0.01$ compared with pre-HFS, paired t-test; ***$p < 0.001$, one-way ANOVA followed by Bonferroni's multiple-comparison tests.