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RECEIVED 22 January 2024

ACCEPTED 13 March 2024

PUBLISHED 03 April 2024

CITATION

Galanis C, Neuhaus L, Hananeia N, Turi Z,
Jedlicka P and Vlachos A (2024) Axon
morphology and intrinsic cellular properties
determine repetitive transcranial magnetic
stimulation threshold for plasticity.
Front. Cell. Neurosci. 18:1374555.
doi: 10.3389/fncel.2024.1374555

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Axon morphology and intrinsic cellular properties determine repetitive transcranial magnetic stimulation threshold for plasticity

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Introduction: Repetitive transcranial magnetic stimulation (rTMS) is a widely used therapeutic tool in neurology and psychiatry, but its cellular and molecular mechanisms are not fully understood. Standardizing stimulus parameters, specifically electric field strength, is crucial in experimental and clinical settings. It enables meaningful comparisons across studies and facilitates the translation of findings into clinical practice. However, the impact of biophysical properties inherent to the stimulated neurons and networks on the outcome of rTMS protocols remains not well understood. Consequently, achieving standardization of biological effects across different brain regions and subjects poses a significant challenge.

Methods: This study compared the effects of 10 Hz repetitive magnetic stimulation (rMS) in entorhino-hippocampal tissue cultures from mice and rats, providing insights into the impact of the same stimulation protocol on similar neuronal networks under standardized conditions.

Results: We observed the previously described plastic changes in excitatory and inhibitory synaptic strength of CA1 pyramidal neurons in both mouse and rat tissue cultures, but a higher stimulation intensity was required for the induction of rMS-induced synaptic plasticity in rat tissue cultures. Through systematic comparison of neuronal structural and functional properties and computational modeling, we found that morphological parameters of CA1 pyramidal neurons alone are insufficient to explain the observed differences between the groups. Although morphologies of mouse and rat CA1 neurons showed no significant differences, simulations confirmed that axon morphologies significantly influence individual cell activation thresholds. Notably, differences in intrinsic cellular properties were sufficient to account for the 10% higher intensity required for the induction of synaptic plasticity in the rat tissue cultures.

Conclusion: These findings demonstrate the critical importance of axon morphology and intrinsic cellular properties in predicting the plasticity effects of rTMS, carrying valuable implications for the development of computer models aimed at predicting and standardizing the biological effects of rTMS.

KEYWORDS

synaptic plasticity, morphology, axons, inhibition, excitation, whole-cell patch-clamp recordings, organotypic tissue cultures

Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive technique that modulates cortical excitability beyond the stimulation period (Chen et al., 1997; Huang et al., 2005; Suppa et al., 2016). Despite its increasing use for treating neuropsychiatric disorders such as major depression (Cocchi et al., 2018; Garnaat et al., 2018; Rehn et al., 2018; Voigt et al., 2019; Somaia et al., 2022), the cellular and molecular mechanisms of rTMS in human cortical networks remain not well-understood (Müller-Dahlhaus and Vlachos, 2013; Cirillo et al., 2017). Animal models, both *in vivo* and *in vitro*, have provided important insights into mechanisms by which rTMS modifies neuronal circuit excitability and plasticity (Vlachos et al., 2012; Tokay et al., 2014; Lenz et al., 2016; Hong et al., 2020; Romero et al., 2022; Eichler et al., 2023). It has been shown for example that rTMS affects the functional and structural properties of excitatory and inhibitory synapses (Tokay et al., 2009; Vlachos et al., 2012; Lenz et al., 2016), and that it facilitates the reorganisation of abnormal cortical circuits (Tang et al., 2021; Moretti et al., 2022). High frequency rTMS enhances plasticity in the primary motor cortex and mitigates cognitive deficits of aged mice (Ma et al., 2019; Cambiaghi et al., 2021). Conversely, low-frequency rTMS triggers plasticity in the dentate gyrus of the hippocampus with potential antidepressant-like effects (Cambiaghi et al., 2020). Recently, experimental evidence for an involvement of microglia, the brains resident immune cells in rTMS-induced synaptic plasticity was provided (Eichler et al., 2023).

Although rTMS has shown robust neurobiological effects in animal models, its efficacy in humans varies significantly (Goldsworthy et al., 2014; López-Alonso et al., 2014; Vallence et al., 2015; Guerra et al., 2020) due to challenges in dose standardization, among others (Peterchev et al., 2012; Turi et al., 2021). Considerable effort has been made to standardize the electric field strength across brain regions and subjects to improve reproducibility and better understand the effects of single pulse and rTMS across brain regions (Opitz et al., 2011; Thielscher et al., 2011; Saturnino et al., 2019). Meanwhile, it is becoming increasingly clear that computational models that predict the strength and orientation of TMS-induced electric field must be extended to biological effects, i.e., the electric fields must be coupled to biophysically realistic models (Aberra et al., 2018; Shirinpour et al., 2021). Indeed, these computational approaches provided important insight into the role of neuronal morphologies, specifically axons and myelination, which seem to play a critical role for single pulse TMS (Aberra et al., 2020). Yet, the majority of these models rely on partial reconstructions from acute brain slices or “artificial axons,” highlighting the challenge of achieving complete neuronal morphologies. Despite some efforts to model rTMS-induced changes in intracellular calcium levels as a proxy for predicting plasticity outcomes (Shirinpour et al., 2021), the current understanding of the dose–response relationship governing rTMS-induced synaptic plasticity remains limited. As a consequence, it is currently also not possible to compute and standardize synaptic plasticity induction across brain regions and subjects.

This study employed a cross-species analysis to elucidate the effects of 10 Hz repetitive magnetic stimulation (rMS) on CA1 pyramidal neurons in mouse and rat entorhino-hippocampal slice cultures (c.f., Vlachos et al., 2012; Lenz et al., 2016; Eichler et al., 2023), underscored by the well-documented connectivity and plasticity of the CA1 region. This approach allowed for an exploration

of highly standardized rMS conditions on synaptic plasticity induction in two similar yet distinct neuronal networks. We found that CA1 pyramidal neurons in rat slice cultures required a 10% stronger intensity (measured by maximum stimulator output, MSO) than those in mice for the induction of plasticity. Leveraging multiscale single-cell modeling with axon morphology reconstructions our findings indicate that neuronal morphology alone does not predict rTMS-plasticity thresholds. However, axon morphologies significantly affect individual cell activation thresholds across species. Differences in intrinsic cellular properties were sufficient to account for the 10% higher intensity required for the induction of synaptic plasticity in the rat tissue cultures. Thus, intrinsic cellular properties are crucial in determining the effects of rTMS on plasticity. These findings highlight the significance of considering morphology and intrinsic properties for computerized predictions of activation thresholds and standardization of rTMS-induced synaptic plasticity.

Materials and methods

Ethics statement

Mice and rats were maintained in a 12 h light/dark cycle with food and water *ad libitum*. Every effort to minimize the distress and pain of animals was made. All experimental procedures were performed according to the German animal welfare legislation, approved by the appropriate animal welfare committee and the animal welfare officer of the University of Freiburg.

Animals

Mice of the strain C57BL/6J and rats of the strain Wistar (CrI:WI) of both sexes were used in this study.

Experimental design

Organotypic tissue cultures were prepared from mice and rats of both sexes at postnatal day 3–5 and allowed to mature for 3 weeks in a humidified atmosphere prior to experimental assessment. Cultures were stimulated in a standard 35 mm petri dish with rMS (10 Hz, 900 pulses); sham-stimulated cultures underwent the same handling but without stimulation. Whole cell voltage-clamp and current-clamp recordings were conducted 2–4 h after stimulation. High density microelectrode array (HD-MEA) recordings and current-clamp recordings were also performed on non-stimulated slice cultures to determine species-specific differences. CA1 pyramidal neurons from non-stimulated tissue cultures were *post hoc* stained and reconstructed using NeuroLucida 360 (ver. 2019.1.3; MBF Bioscience). These reconstructions were utilized for multiscale single-cell modeling.

Preparation of organotypic tissue cultures

300 μ m thick organotypic tissue cultures containing the hippocampus and the entorhinal cortex were prepared at postnatal day 3–5 from mice and rats of either sex as described previously

(Vlachos et al., 2012; Galanis et al., 2021). The tissue cultures were maintained in an incubator at 35°C with 5% CO₂ for at least 18 days before any experimental assessment. Tissue culture medium was changed 3 times per week and consisted of 50% (v/v) MEM, 25% (v/v) basal medium eagle (BME), 25% (v/v) heat-inactivated normal horse serum, 25 mM HEPES, 0.15% (w/v) NaHCO₃, 0.65% (w/v) glucose, 0.1 mg/mL streptomycin, 100 U/mL penicillin, and 2 mM Glutamax (pH 7.3 with HCl or NaOH).

rMS *in vitro*

Tissue cultures were transferred in a standard 35 mm petri dish filled with standard extracellular solution (129 mM NaCl, 4 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 4.2 mM glucose, 10 mM HEPES, 0.1 mg/mL streptomycin, 100 U/mL penicillin, pH 7.4, preheated to 35°C; 365 mOsm with sucrose). A 70 mm figure-of-eight coil (D70 Air Film Coil, Magstim) connected to a Magstim Super Rapid2 Plus1 (Magstim) was placed 1 mm above the lid of the petri dish and the cultures were stimulated with a protocol consisting of 900 pulses at 10 Hz. Tissue cultures were orientated in a way that the induced electric field within the tissue was approximately parallel to the dendritic tree of CA1 pyramidal neurons. Species- and time-matched cultures were not stimulated, but otherwise identically treated served as the controls.

Whole-cell voltage-clamp recordings

Whole-cell voltage-clamp recordings of CA1 pyramidal cells were conducted as previously described (Vlachos et al., 2012; Lenz et al., 2016; Galanis et al., 2021). Recordings were conducted at 35°C. The bath solution contained 126 mM NaCl, 2.5 mM KCl, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, 2 mM CaCl₂, 2 mM MgCl₂, and 10 mM glucose and was saturated with 95% O₂/5% CO₂. Miniature α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated excitatory postsynaptic currents (mEPSCs) were recorded in the presence of 10 μ M D-APV and 0.5 μ M TTX in the bath solution while the patch pipettes contained 126 mM K-gluconate, 4 mM KCl, 4 mM ATP-Mg, 0.3 mM GTP-Na₂, 10 mM PO-creatine, 10 mM HEPES, and 0.1% (w/v) biocytin (pH 7.25 with KOH, 290 mOsm with sucrose). Miniature inhibitory postsynaptic currents (mIPSCs) were recorded in the presence of 0.5 μ M TTX, 10 μ M D-APV, and 10 μ M CNQX in the bath solution while the patch pipettes contained 125 mM CsCl, 5 mM NaCl, 2 mM MgCl₂, 2 mM Mg-ATP, 0.5 mM Na₂-GTP, 0.1 mM EGTA and 10 mM HEPES (pH = 7.33 with CsOH; 275 mOsm with sucrose). Neurons were recorded at a holding potential of -70 mV. Series resistance was monitored in 2–4 min intervals and recordings were discarded if the series resistance reached ≥ 30 M Ω and the leak current changed significantly.

Whole-cell current-clamp recordings

Whole-cell current-clamp recordings of CA1 pyramidal cells were conducted at 35°C. The bath solution contained 126 mM NaCl, 2.5 mM KCl, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, 2 mM CaCl₂, 2 mM MgCl₂, 10 mM glucose, 10 μ M D-APV, 10 μ M CNQX, and

10 μ M bicuculline methiodide and was saturated with 95% O₂/5% CO₂. Patch pipettes contained 126 mM K-gluconate, 4 mM KCl, 4 mM ATP-Mg, 0.3 mM GTP-Na₂, 10 mM PO-creatine, 10 mM HEPES, and 0.1% (w/v) biocytin (pH 7.25 with KOH, 290 mOsm with sucrose). Neurons were hyperpolarized with -100 pA and then depolarized up to +400 pA with 1-s-long 10 pA current injection steps. Recordings were discarded if the series resistance reached ≥ 15 M Ω .

High-density microelectrode array recordings

HD-MEA recordings of mouse and rat tissue cultures were conducted at 35°C. The bath solution was similar to the one used for voltage-clamp recordings without the addition of any drugs. Cultures were placed on an Accura HD-MEA chip (3Brain, Switzerland) and acclimatized for 2 min before recording. Each tissue culture was recorded for 10 min with a BioCAM Duplex (3Brain, Switzerland).

Neuronal filling, *post hoc* staining and imaging

CA1 pyramidal neurons were patched with pipettes containing 126 mM K-gluconate, 4 mM KCl, 4 mM ATP-Mg, 0.3 mM GTP-Na₂, 10 mM PO-creatine, 10 mM HEPES, and 1% (w/v) biocytin (pH 7.25 with KOH, 290 mOsm with sucrose). The neurons were kept in the whole-cell configuration for at least 10 min during which they were depolarized with 100 ms current injections of 200 pA at 5 Hz. Tissue cultures were fixed in a solution of 4% (w/v) PFA and 4% (w/v) sucrose in 0.01 M PBS for 1 h and further processed and images as previously described (Galanis et al., 2021).

Neuronal reconstructions

CA1 pyramidal cells were reconstructed using NeuroLucida 360 (ver. 2019.1.3; MBF Bioscience) as described previously (Shirinpour et al., 2021). Somata were reconstructed in 2D using the contour method in order to avoid overestimation of soma volume due to the somatic whole cell recording method used to fill the cells and the high signal intensity of these compartments.

Electric field modeling

Finite element method was used to create a three-dimensional mesh model consisting of two compartments, representing the bath solution and tissue cultures. The physical dimensions of the mesh model were based on the physical parameters of the *in vitro* settings, with a coil-to-Petri dish distance of 1 mm and the coil positioned above the culture. Electrical conductivities of 1.654 S/m and 0.275 S/m were assigned to the bath solution and culture, respectively. The rate of change of the coil current was set to 1.4 A/ms at 1% MSO and scaled up to higher stimulation intensities. Simulations of macroscopic electric fields were performed using SimNIBS (3.2.6) and MATLAB (2023a). A validated 70 mm MagStim figure-of-eight coil was utilized in all simulations (Thielscher and Kammer, 2004). The 99th percentile

of the E-field, which represents the robust maximum value, was extracted from the volume compartment of the tissue culture.

Single-cell modeling

Reconstructions were imported into the NeMo-TMS pipeline and endowed with a Jarsky model (Jarsky et al., 2005). When axons are “swapped,” the original axon is removed from the cell at the point of intersection with the soma or dendrite, and replaced with the axon of another cell that has been severed at the same point. Each cell is oriented with the apical dendrite pointing in the positive y direction, and axon orientations relative to this are preserved in the swapping process. For single-cell simulations, TMS is simulated as a uniform electric field of varying intensity, with the threshold defined as the smallest TMS amplitude that elicits a somatic action potential.

Experimental design and statistical analysis

Analyses were performed with the person analyzing the data blind to the experimental condition. For this project, we used one or two tissue cultures from each animal. Electrophysiological data were analyzed using pClamp 11.2 software suite (Molecular Devices), the Easy Electrophysiology 2.5.0.2 (Easy Electrophysiology Ltd.) and BrainWave (3Brain) software. Statistical comparisons were made using Mann–Whitney test (to compare two groups) two-way ANOVA and Kruskal–Wallis test as indicated in the figure captions and text (GraphPad Prism 7). *p* values of <0.05 were considered a significant difference. All values represent mean ± SEM.

Digital illustrations

Confocal image stacks were exported as 2D projections and stored as TIFF files. Figures were prepared using Photoshop graphics software (Adobe). Image brightness and contrast were adjusted.

Results

10 Hz repetitive magnetic stimulation induces plasticity of excitatory and inhibitory synapses in mouse CA1 pyramidal neurons

A 10 Hz stimulation protocol consisting of 900 pulses at 50% MSO was used to assess the effects of rMS on synaptic plasticity in brain tissue cultures prepared from mice of either sex (Figures 1A–C). Individual CA1 pyramidal neurons were patched and AMPA receptor-mediated mEPSCs were recorded 2–4 h after stimulation. In line with our previous work (c.f., Vlachos et al., 2012; Lenz et al., 2015, 2020; Eichler et al., 2023) a significant increase in mean mEPSC amplitude was observed as compared to age-/time-matched control cultures that were treated in the exact same way except for 10 Hz rMS (control; Figures 1D,E).

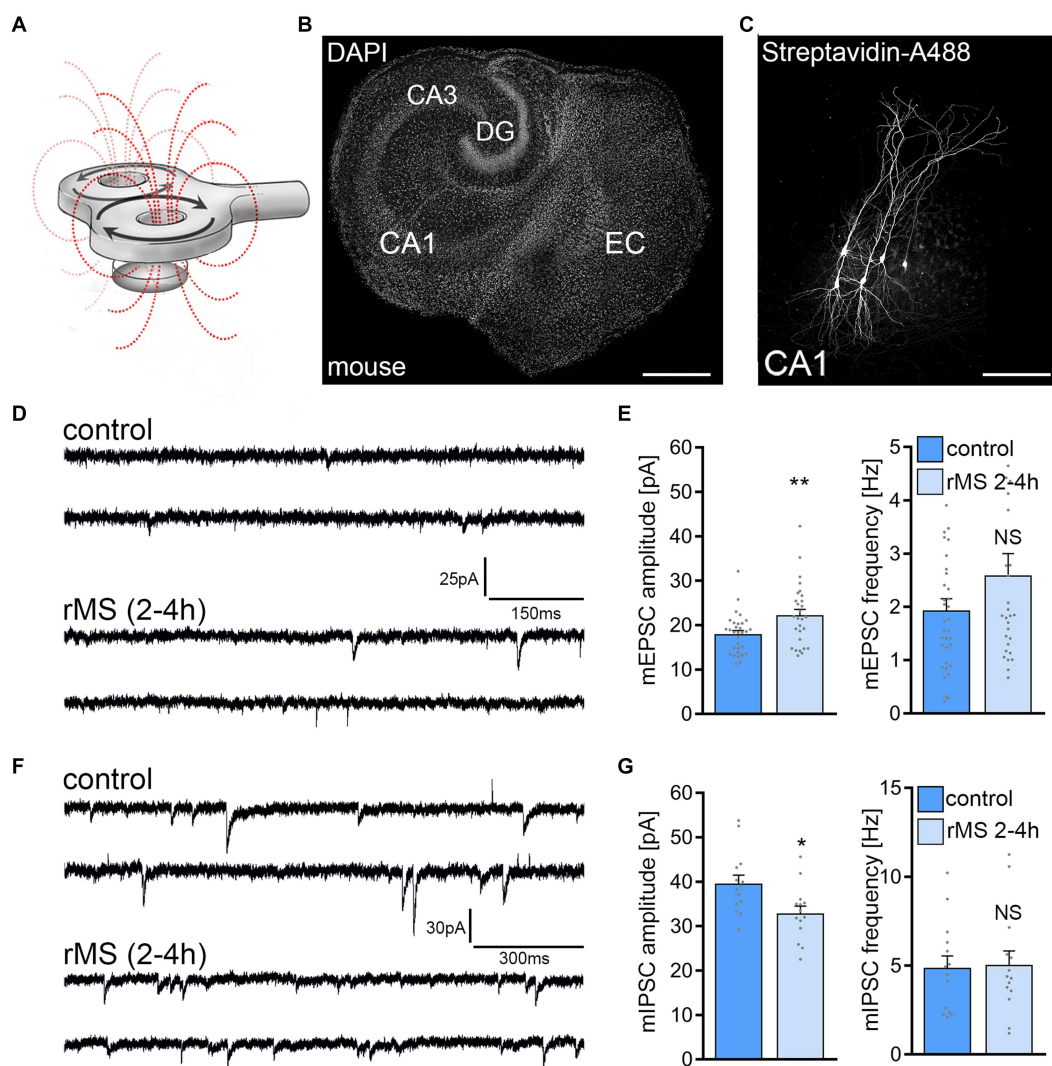
In a different set of cultures, we assessed 10 Hz rMS-induced changes in GABA receptor mediated mIPSCs onto CA1 pyramidal neurons using the experimental approach described above. A reduction in mean mIPSC amplitude was observed in these experiments as reported in our previous study (Figures 1E,G; c.f., Lenz et al., 2016). These results confirm the robust effects of 10 Hz rMS on mEPSC and mIPSC amplitudes of CA1 pyramidal neurons in mouse entorhino-hippocampal tissue cultures, which are consistent with a potentiation of excitatory synapses and a depression of inhibitory synapses.

10 Hz repetitive magnetic stimulation at 50% MSO does not affect synaptic strength in rat CA1 pyramidal neurons

The same 10 Hz protocol (10 Hz, 900 pulses, 50% MSO) was applied to tissue cultures prepared from rat brains (Figure 2), aiming to assess the RMS effects across two similar yet distinct neuronal networks. Age-matched rat entorhino-hippocampal cultures displayed a larger cross-section than mouse tissue cultures (Figure 2A), without any apparent morphological differences in CA1 pyramidal neurons (Figure 2B). Recordings of AMPA receptor-mediated mEPSCs from CA1 pyramidal neurons showed no statistically significant differences between control and 10 Hz rMS-stimulated preparations (Figures 2C,D). Inhibitory synaptic strength was also unaffected, as no significant differences in mean mIPSC amplitude and frequency were detected 2–4 h after stimulation (Figures 2E,F).

Macroscopic electric field simulations reveal distinct maximum electric fields generated in mouse and rat tissue cultures

The electric field (E-field) strength induced in the mouse and rat slice cultures was described using computational modeling (Saturnino et al., 2019). Three-dimensional mesh models were created with two compartments (i.e., bath solution and slice cultures) using the finite element method (Figure 3A). The physical dimensions of the mesh models were adapted from data obtained in mouse and rat brain tissue cultures (Figure 3B). Macroscopic modeling of the E-field revealed that stimulation at 50% MSO induces a stronger electric field in the mouse (20.4 V/m) when compared to the rat tissue culture (19.3 V/m). Based on the modeling we determined that 53% MSO stimulation of rat tissue cultures would result in an E-field that is comparable to what we estimated in the mouse tissue cultures stimulated with 50% MSO (Figure 3C). Accordingly, another set of rat tissue cultures was stimulated with 53% MSO (10 Hz, 900 pulses) and AMPA receptor-mediated mEPSCs were recorded from CA1 pyramidal neurons 2–4 h after stimulation. No significant differences in mean mEPSC amplitude and frequency were observed in these experiments (Figure 3D). We conclude that simulation-based standardization of electric fields may not suffice to achieve comparable biological effects in mouse and rat CA1 pyramidal neurons, i.e., in neurons embedded in networks with comparable architectures and properties.



Baseline network activity is not significantly different between mouse and rat tissue cultures

To test for differences in spontaneous network activity between mouse and rat entorhino-hippocampal slice cultures basal firing rates and field potential rates were recorded in a different set of 3-week-old mouse and rat tissue cultures using HD-MEA recordings (Figures 4A,B). No significant differences between mouse and rat tissue cultures were observed in firing and field potential (FP) rates in these experiments (Figures 4C–F). We conclude that baseline network activity is not responsible for

the inability of rMS to induce plasticity in rat CA1 pyramidal neurons.

No significant differences in structural properties of cultured mouse and rat CA1 pyramidal neurons

To investigate whether differences in CA1 pyramidal neuron size and complexity could explain the variation in rMS outcome, we reconstructed biocytin-filled and streptavidin-A488 stained CA1 pyramidal neurons from both rat and mouse hippocampal tissue

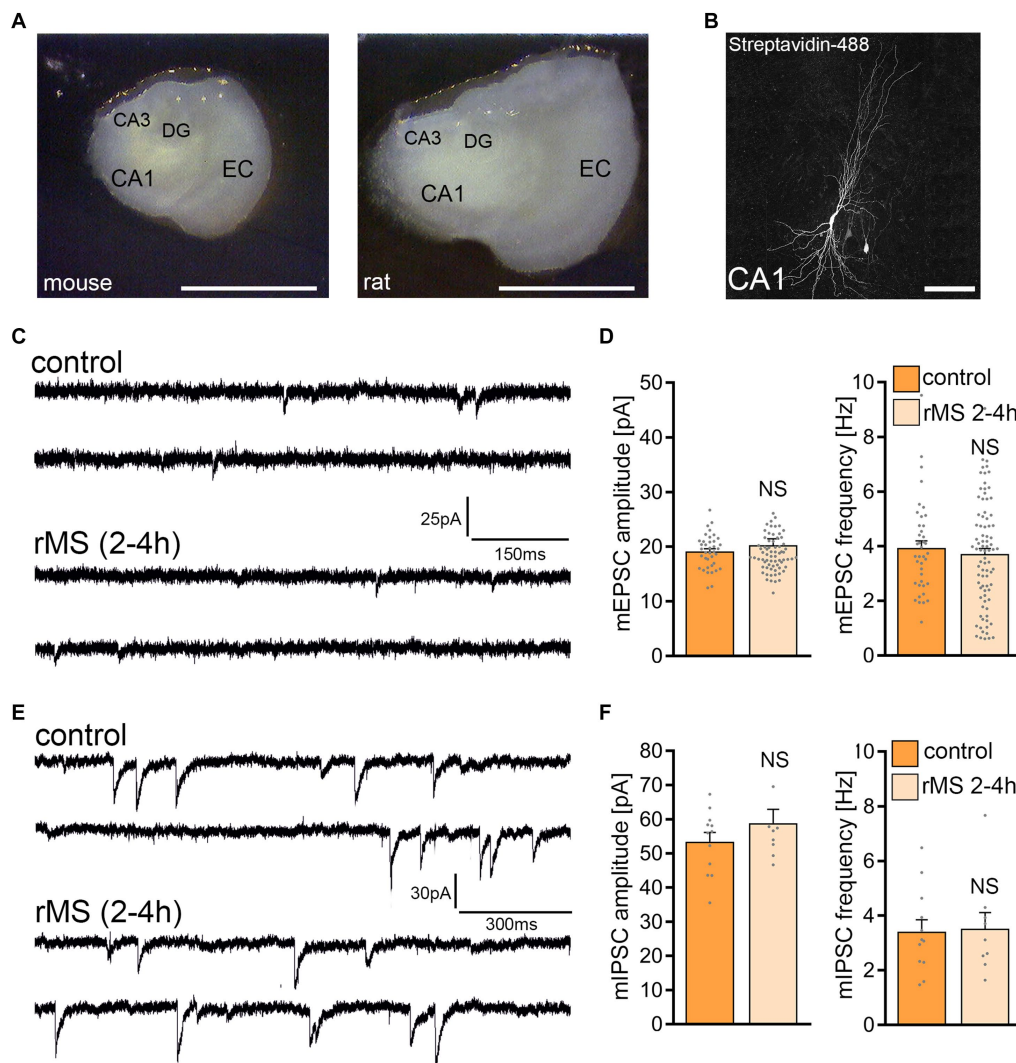


FIGURE 2

10 Hz repetitive magnetic stimulation (rMS) at 50% maximum stimulator output fails to induce synaptic plasticity in rat CA1 pyramidal neurons. (A) Overview images of a mouse and rat organotypic tissue culture. DG, Dentate gyrus; EC, entorhinal cortex; CA1 and CA3, *Cornu Ammonis* areas 1 and 3. Scale bar, 1500 μm . (B) Patched rat CA1 pyramidal neuron filled with biocytin and identified *post hoc* with streptavidin-A488. Scale bar, 50 μm . (C,D) Sample traces and group data of AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) recorded from rat CA1 pyramidal neurons in sham-control and rMS-stimulated cultures 2–4 h after stimulation (control, $n = 38$ cells; rMS, $n = 71$ cells; Mann–Whitney test). (E,F) Sample traces and group data of GABA receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) recorded from rat CA1 pyramidal neurons in sham-control and rMS-stimulated cultures 2–4 h after stimulation (control, $n = 12$ cells; rMS, $n = 9$ cells; Mann–Whitney test). Data are mean \pm SEM. NS, Not significant.

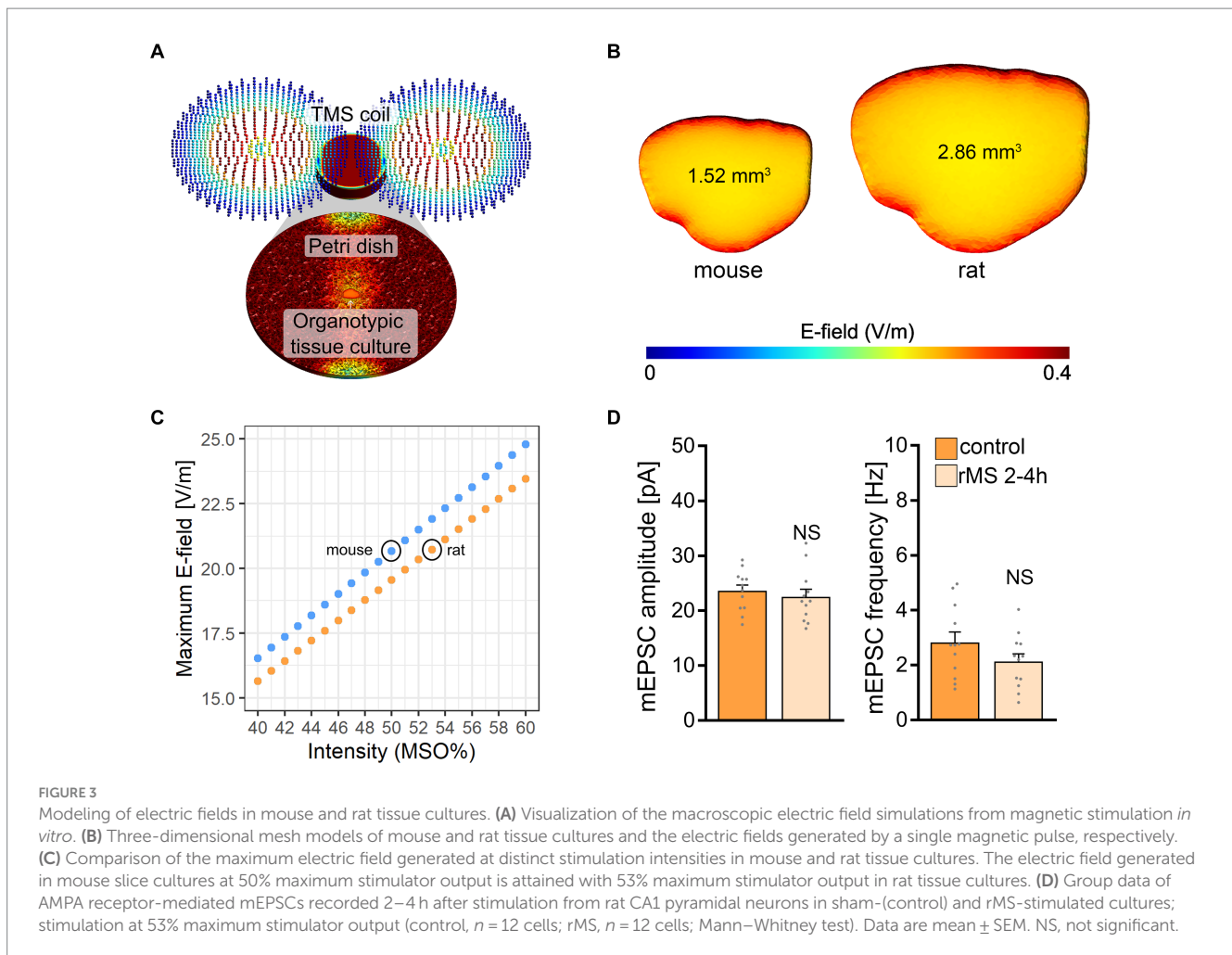
cultures and analyzed their dendrites and axons (Figure 5). This was motivated by the observation that the brain sizes of mice and rats, as well as their tissue cultures, differ.

No significant differences were observed between the two groups in apical and basal dendritic length (Figures 5C,D). Sholl and diameter/volume analyses (Figures 5E–G) did not show any statistical significance between CA1 dendrites and their complexity of rat and mouse CA1 pyramidal neurons in entorhino-hippocampal tissue cultures. Similarly, no significant differences were observed when CA1 axons were reconstructed and compared in mouse and rat tissue cultures (Figures 5I–L). We conclude, that structural properties of CA1 pyramidal neurons are not statistically different and cannot explain why the rat tissue cultures do not respond to 10 Hz rMS even when the E-field is closely matched based on E-field simulations.

Realistic multiscale single-cell modeling predicts no major differences in rMS-induced depolarization of mouse and rat CA1 pyramidal neurons

We assessed the impact of rMS on CA1 pyramidal neurons through a multiscale computational model that connects the physical input parameters of rMS to dendritic and axonal morphologies (Figure 6). This approach was necessary because our morphological analysis might not have encompassed distinctions pertinent to the neuronal activation induced by rMS.

When examining the dendritic architecture of CA1 neurons in mice and rats, and employing a standardized artificial axon across all cells (c.f., Aberra et al., 2018; Shirinpour et al., 2021;



Eichler et al., 2023), our simulations revealed no significant difference in the depolarization threshold elicited by rMS (Figures 6A,B). Subsequently, we investigated whether axonal morphologies might underlie the observed variability in our experimental outcomes. An additional series of simulations was conducted, this time integrating the authentic axonal morphologies of these neurons. Again, no significant differences in the depolarization thresholds were observed between the two groups (Figure 6C).

A noteworthy insight emerged from these simulations, confirming previous observations (e.g., Aberra et al., 2018, 2020): the axon's influence is pivotal in establishing the rMS-induced depolarization threshold (Table 1). We followed up on this observation, by establishing connections between the axons responsible for the lowest and highest rMS depolarization thresholds across all mouse and rat cells. Indeed, an almost 2-fold difference in the depolarization thresholds was observed in these simulations across all reconstructed neurons (Figure 6D). Yet, despite these simulation results, the dissimilarity in rMS-triggered plasticity between mouse and rat tissue cultures remained unresolved, eluding a complete explanation based solely on the interactions of dendritic and axonal morphologies.

Active and passive membrane properties reveal differences in excitability between mouse and rat CA1 pyramidal neurons

Next, active and passive membrane properties were recorded from CA1 pyramidal neurons and analyzed. Indeed, this set of experiments identified significant differences in the passive and active properties between mouse and rat CA1 pyramidal neurons (Figure 7).

While no significant differences in membrane capacitances were noted between the two species (mouse: 90.7 ± 5.863 pF and rat: 101.7 ± 6.204 pF; Mann–Whitney test; $p = 0.37$; data not shown), the input resistance of mouse CA1 pyramidal neurons was significantly higher as compared to rat CA1 pyramidal neurons (mouse: 156.8 ± 11.65 MOhm and rat: 67.25 ± 4.909 MOhm; Mann–Whitney test; $p < 0.001$; $U = 279$), while the cells of both mice and rats were resting at comparable membrane potentials (Figures 7A–C). Consistently, the current–voltage (I/V) curves demonstrated that depolarizing mouse CA1 pyramidal neurons required less current compared to those in rat slice cultures.

Looking at the active membrane properties (Figures 7D–F) a similar trend was observed with the most striking differences

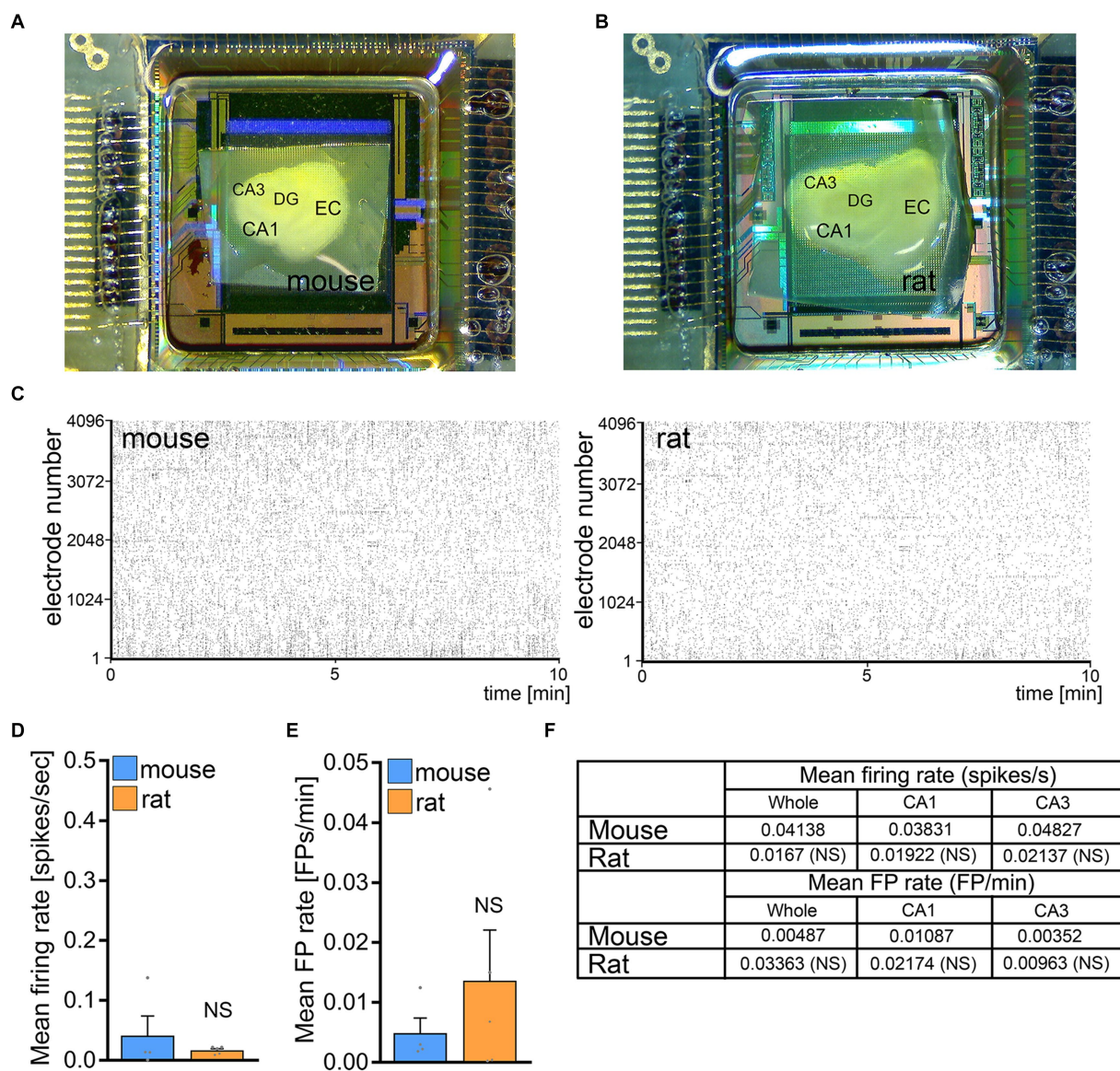


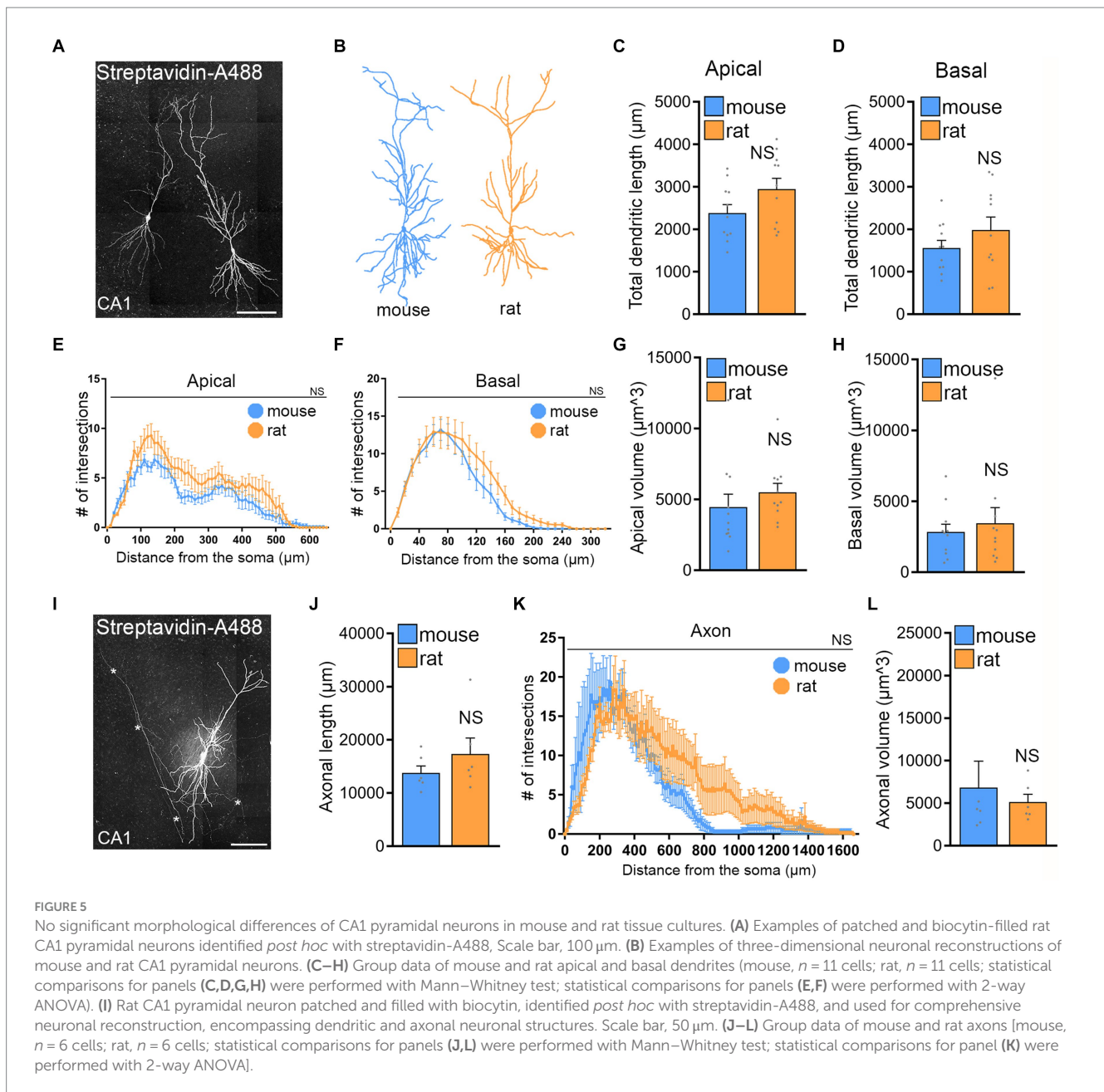
FIGURE 4

No significant differences in baseline network activity in mouse and rat tissue cultures. (A,B) Overview images of mouse and rat tissue culture on high-density microelectrode array chips. DG, dentate gyrus; EC, entorhinal cortex; CA1 and CA3, *Cornu Ammonis* areas 1 and 3. (C) Raster plots of spikes during a 10 min recording period in mouse and rat tissue cultures. (D–F) Group data of mean firing rate and mean field potential rate from mouse and rat tissue cultures (mouse, $n = 4$ cultures; rat, $n = 5$ cultures; Mann–Whitney test). Data are mean \pm SEM. NS, not significant.

being in the action potential induction threshold (mouse: -31.81 ± 0.877 mV; rat: -28.47 ± 0.744 mV; Mann–Whitney test; $p = 0.0021$; $U = 794$) and the first spike latency (mouse: 419.8 ± 56.03 ms; rat: 715 ± 77.36 ms; Mann–Whitney test; $p = 0.0074$; $U = 15$; data not shown). Figure 7F, shows that current injections produced stronger responses in mouse CA1 pyramidal neurons than in rat neurons, i.e., higher action potential frequencies at a lower current injection. These results indicated that mouse CA1 pyramidal neurons are more excitable than rat neurons, suggesting that higher stimulation intensities may be needed to induce rMS-induced plasticity in rat tissue cultures.

60% MSO induces rMS-mediated plasticity in rat organotypic tissue cultures

Subsequently, we tested whether a 10Hz stimulation protocol applied at a higher intensity would induce plasticity in rat CA1 pyramidal neurons. Indeed, when rat tissue cultures were stimulated with 10Hz rMS at 60% MSO a robust increase in the mean mEPSC amplitude was detected (Figure 8A), similar to what we observe in the mouse cultures stimulated at 50% MSO (cf., Figures 1E). In addition, a significant reduction in mean mIPSC amplitude was evident 2–4h after rMS stimulation at 60% MSO in a different set of rat tissue cultures (Figure 8B; c.f., Figure 1G). These results demonstrate that rat



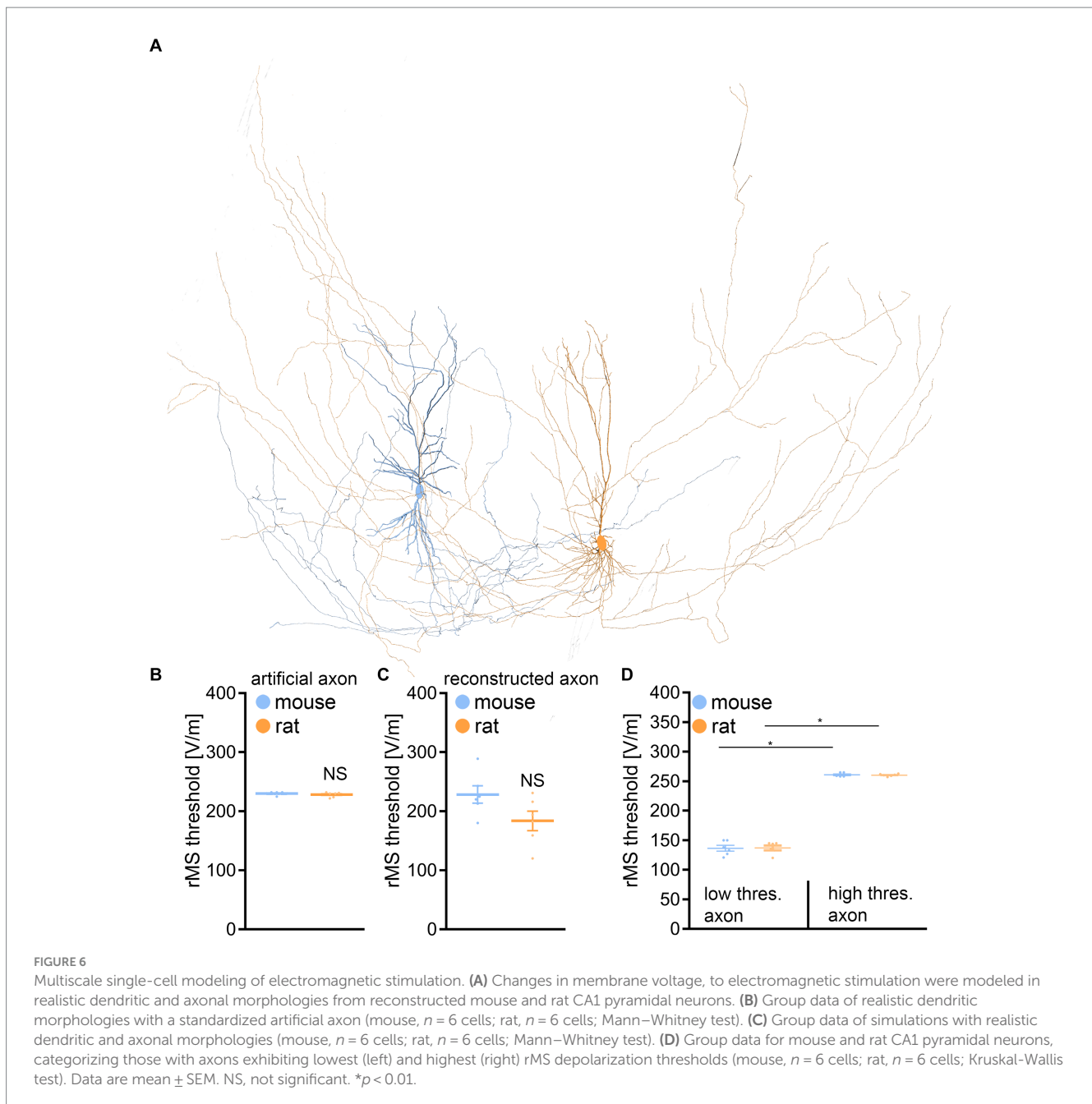
CA1 pyramidal neurons do express rMS-induced plasticity, but require a higher stimulation intensity for rMS-induced potentiation of excitatory synapses and depression of inhibition to occur.

Discussion

In this study, we explored the factors influencing the threshold for 10Hz rTMS-induced synaptic plasticity. Using mouse and rat entorhino-hippocampal slice cultures, we investigated neuronal structure, excitability, and network activity. In mouse CA1 pyramidal neurons, we confirmed the well-known potentiation of excitatory synapses and depression of inhibitory synapses, highlighting robust rTMS-induced synaptic plasticity under controlled conditions. However, despite similar neuronal morphology and network activity

in rat CA1 pyramidal neurons, standardizing electric fields through prospective modeling did not produce the same biological effect. Adjusting the stimulation protocol to account for rat neurons' lower excitability led to comparable synaptic changes. These results emphasize that electric field standardization alone cannot predict rTMS effects, necessitating realistic compartmental models of cellular properties in different brain regions for accurate predictions.

Over the past decade, the utilization of rTMS has experienced a significant surge in both research and clinical domains (Dayan et al., 2013; Paulus et al., 2013; Suppa et al., 2016; Blumberger et al., 2018; Lefaucheur et al., 2020; Lorentzen et al., 2022). Consequently, extensive efforts have been dedicated to identify the crucial parameters that influence the effects of rTMS on brain tissue (Deng et al., 2013; Lefaucheur et al., 2020; Zmeykina et al., 2020; Turi et al., 2021). Among these parameters, the induced electric field has been



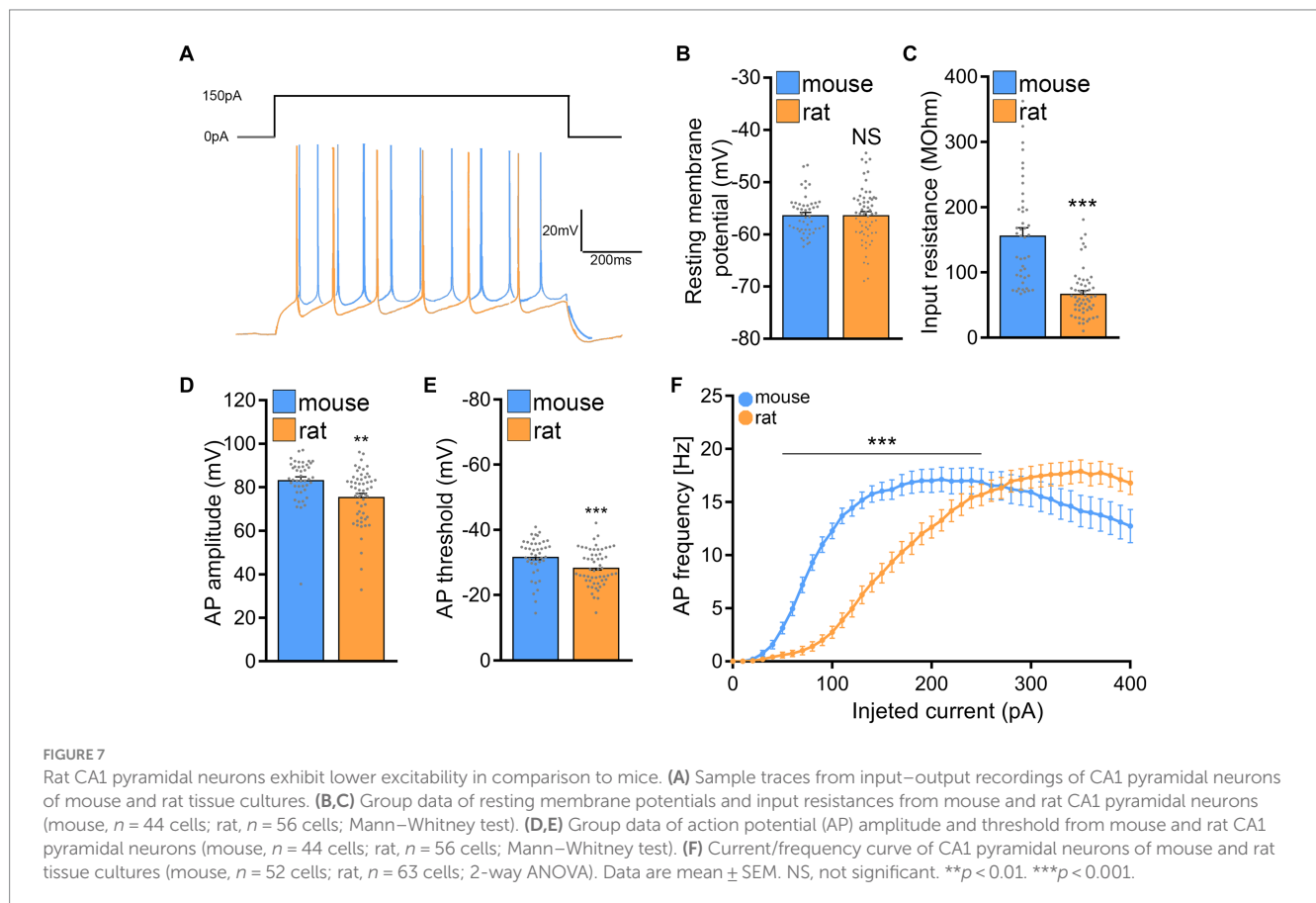
identified as a critical factor directly influencing the effects of rTMS on cortical tissue (Liu et al., 2018). While advancements in computational tools have enabled the calculation of rTMS-induced electric field (Thielscher et al., 2015), these models have primarily relied on mesoscopic structural parameters of the targeted stimulation area, i.e., head and brain geometries. In recent years, there has been a growing adoption of multiscale modeling approaches to investigate the impact of TMS on individual neurons (Kamitani et al., 2001; Aberra et al., 2018, 2020; Shirinpour et al., 2021). Notably, these neuronal models are being integrated into mesoscopic brain models, enabling exploration of the effects of cortical folding and the precise positioning of neurons, such as distinguishing between the gyral crown and gyral groove, in individual subjects (Salvador et al., 2011;

Seo and Jun, 2019; Aberra et al., 2020; Turi et al., 2022). While these models represent a significant advancement toward standardization and precision medicine in the field, it is increasingly evident that solely modeling electric fields and their interactions with individual neuronal morphologies (derived from animal models) may not be sufficient to predict and standardize the biological effects of rTMS across various brain regions and individuals (Turi et al., 2022). The findings from this cross-species study present experimental evidence, underscoring the insufficiency of meticulous experimental standardization and electric field modeling in guaranteeing robust biological effects of rTMS. Notably, computational modeling showed weaker induced electric fields in rat tissue cultures despite their size difference compared to mouse tissue cultures. Even when efforts were

TABLE 1 rMS-depolarization thresholds for individual cells with different axons attached.

	Axon 1	Axon 2	Axon 3	Axon 4	Axon 5	Axon 6	Axon 7	Axon 8	Axon 9	Axon 10	Axon 11	Axon 12
Mouse cell 1	225	224	265	184	267	212	183	232	194	218	162	150
Mouse cell 2	224	220	261	182	255	212	183	231	193	216	160	138
Mouse cell 3	223	218	259	180	249	212	182	230	193	215	159	133
Mouse cell 4	222	216	258	180	244	212	182	229	192	214	159	127
Mouse cell 5	222	217	258	180	242	212	182	229	191	214	157	121
Mouse cell 6	226	226	265	186	271	213	184	233	194	219	161	150
Rat cell 1	223	220	260	181	248	212	183	230	193	215	159	133
Rat cell 2	224	222	263	183	260	212	183	231	194	217	161	145
Rat cell 3	224	219	261	183	258	212	183	232	194	217	161	145
Rat cell 4	224	221	262	183	257	212	183	231	194	217	161	144
Rat cell 5	223	216	259	181	253	212	182	230	193	215	159	135
Rat cell 6	220	215	257	179	236	212	182	229	192	213	158	120

Values represent rMS-depolarization threshold in V/m.



made to match electric fields, the plasticity effects in rat cultures could not be reproduced.

In this context, it is crucial to highlight that our experiments revealed no statistically significant morphological differences between the cultured CA1 pyramidal neurons of mice and rats. The comprehensive analysis of both apical and basal dendrites demonstrated comparable total dendritic length, complexity, and

overall volume in both rat and mouse pyramidal neurons of organotypic tissue cultures. These results align with previously published data that compared mouse and rat hippocampal CA1 neurons in acute slice preparations (Routh et al., 2009). However, it is worth noting that the total volume of these cells, apart from the observed morphological features, was found to be higher in rat slices. Though differences between acute brain slices and tissue cultures

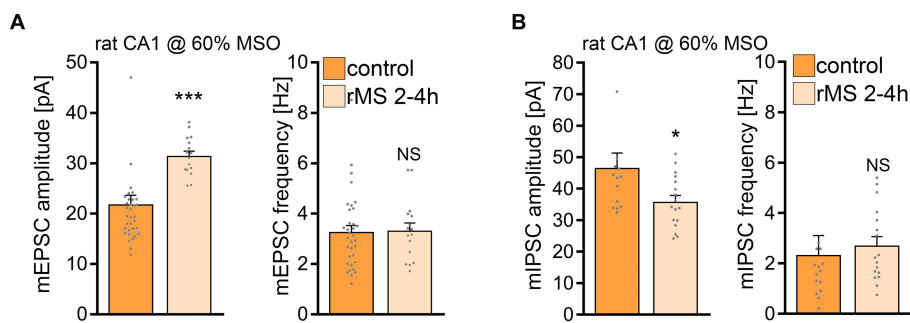


FIGURE 8

10 Hz repetitive magnetic stimulation (rMS) at 60% MSO induces synaptic plasticity in rat CA1 pyramidal neurons. (A) Group data of AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) recorded from rat CA1 pyramidal neurons from sham-(control) and rMS-stimulated cultures (control, $n = 34$ cells; rMS, $n = 16$ cells; Mann–Whitney test). (B) Sample traces and group data of miniature inhibitory postsynaptic currents (mIPSCs) recorded from rat CA1 pyramidal neurons from sham- (control) and rMS- stimulated cultures (control, $n = 14$ cells; rMS, $n = 17$ cells; Mann–Whitney test. One data point outside of axis limits in mIPSC amplitude and frequency respectively). Data are mean \pm SEM. NS, not significant. * $p < 0.05$. *** $p < 0.001$.

could contribute to the observed discrepancy, and reliable volume-reconstructions of patched somata were not feasible in our study, it is crucial to highlight the key advantage of tissue cultures. Using 3-week-old tissue cultures enabled us to investigate neurons within brain tissue that had not undergone acute slicing immediately before experimental assessment. This allowed us to study undamaged pyramidal neurons and enabled us to generate detailed morphological reconstructions, encompassing both dendrites and axons. Specifically, complete reconstructions of axons are of utmost importance for precise evaluation of rTMS outcomes, considering their substantial interaction with the electric field (Siebner et al., 2022). Previous studies, including our own work, often relied on artificial or simplified axon morphologies (Aberra et al., 2018, 2020; Shirinpour et al., 2021; Eichler et al., 2023). Importantly, our investigation revealed no significant differences in axons of cultured CA1 neurons between mice and rats. This finding suggests that the observed inability of rat CA1 neurons to exhibit synaptic plasticity cannot be trivially attributed to differences in axon morphology.

Nevertheless, our simulations identified axons that are twice as effective at depolarizing neurons, irrespective of soma and dendrite shapes. This emphasizes the need for a systematic assessment of various axonal morphologies in rTMS-induced synaptic plasticity, also considering factors like myelination and the role of oligodendrocytes. We propose the possibility of “super-responder cells” within complex cortical networks—cells highly responsive to rTMS at specific stimulation intensities. This notion finds support in the observation that not all neurons of the present and our previous studies (c.f., Vlachos et al., 2012; Lenz et al., 2016, 2020; Eichler et al., 2023) displayed elevated mEPSC amplitudes or decreased mIPSC within the 2–4 h following stimulation.

The results of the present study suggest that understanding the differing effects of rTMS on mouse and rat CA1 pyramidal neurons requires considering their intrinsic cellular properties. Consistent with prior research on rat and mouse slices (Routh et al., 2009), our study shows that rat CA1 pyramidal neurons have a higher action potential threshold compared to mice, making them less excitable. Notably, we found that rat CA1 neurons have lower input resistance than mouse neurons, further highlighting reduced excitability in rat

neurons. However, it is worth noting that a study by Routh and colleagues in 2009 reported similar input resistance between the two species (Routh et al., 2009) potentially due to differences in acute slices prepared from adult animals and organotypic tissue cultures.

Do morphological and biophysical properties alone predict rTMS outcomes adequately? Additional factors, like neuromodulators such as dopamine, serotonin, and noradrenaline, influence cortical excitability, impacting how neurons respond to rTMS and altering plasticity threshold, magnitude, and direction (Greenberg et al., 2000; Nitsche et al., 2006; Martorana et al., 2009; Nitsche et al., 2010; le Grand et al., 2011; Kuo et al., 2017). Furthermore, neuromodulators can impact the capacity of neurons to express plasticity without affecting excitability and other baseline functional and structural properties, a phenomenon known as metaplasticity (Abraham and Bear, 1996; Seol et al., 2007). It is important to also note that non-neuronal cells can significantly influence the capacity of neurons to express synaptic plasticity (Stellwagen et al., 2005; Henneberger et al., 2010; Allen, 2014; Andoh and Koyama, 2021; Sancho et al., 2021; Kleidonas et al., 2023). Our prior work has provided evidence that cytokines derived from microglia play a crucial role in facilitating rTMS-induced plasticity (Eichler et al., 2023). Finally, the impact of network activity on the outcome of rTMS must be considered. These factors collectively underscore the multifaceted nature of the processes involved in influencing and modulating the outcomes of rTMS-induced plasticity. Organotypic slice cultures serve as valuable tools for investigating these and other aspects of rTMS-induced plasticity, highlighting the necessity for rigorously validated computer models that link the induced electric fields with biophysically realistic neurons and networks. These models hold the potential to predict the biological outcomes of rTMS, offering valuable insights into its effects and guiding the adaptation of stimulation protocols to achieve consistent desired effects across different brain regions and individuals.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Animal welfare officer of the University of Freiburg. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CG: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. LN: Formal analysis, Investigation, Writing – original draft. NH: Data curation, Formal analysis, Investigation, Software, Writing – original draft. ZT: Data curation, Formal analysis, Investigation, Software, Writing – original draft. PJ: Data curation, Methodology, Software, Supervision, Writing – review & editing. AV: Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The work was supported by National Institutes of Health, USA (NIH;

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1R01NS109498) and by the Federal Ministry of Education and Research, Germany (BMBF, 01GQ2205A).

Acknowledgments

We thank Susanna Glaser and Emina Deumic for skillful assistance in tissue culturing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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