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The Effects of Temporal Interference Stimulation on Long-Term Synaptic Plasticity in CA1 Hippocampal Neurons

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Abbreviations

AMPA	–	α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPA	–	α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
bAP	–	back-propagating action potential
BCM	–	Bienenstock-Cooper-Munro (theory)
CA1	–	<i>Cornu ammonis</i> area 1
CA3	–	<i>Cornu ammonis</i> area 3
DG	–	dentate gyrus
EC	–	entorhinal cortex
E/I	–	excitation/inhibition
EPSC	–	excitatory postsynaptic current
EPSP	–	excitatory postsynaptic potential
GABA	–	gamma-aminobutyric acid
HFS	–	high-frequency stimulation
I _h	–	hyperpolarization-activated cation current
LFS	–	low-frequency stimulation
LTD	–	long-term depression
LTP	–	long-term potentiation
MTL	–	medial temporal lobe
NMDA	–	N-Methyl-D-aspartate
NMDAR	–	N-Methyl-D-aspartate receptor
PV	–	parvalbumin-expressing interneurons
Pyr	–	pyramidal neurons
STDP	–	spike timing-dependent plasticity
SWRs	–	sharp wave-ripples
t-LTD	–	timing-dependent long-term depression
t-LTP	–	timing-dependent long-term potentiation
TI	–	temporal interference
tTIS	–	transcranial temporal interference stimulation

Introduction

Non-invasive brain stimulation is widely used to treat disorders such as stroke, traumatic brain injury, Alzheimer’s disease, Parkinson’s disease, and depression (Rektorová *et al.*, 2025). However, the most frequently used techniques are limited to superficial cortical areas. Transcranial temporal interference stimulation (tTIS) has emerged as a promising approach for non-invasive stimulation of deep brain structures, opening new possibilities for targeting regions such as the hippocampus in health and disease. To achieve deep and focal stimulation, tTIS applies multiple high-frequency electric fields that overlap and create a low-frequency modulation within the targeted regions while minimizing effects on superficial cortical areas (Grossman *et al.*, 2017).

The tTIS technique has attracted considerable interest due to its ability to target the hippocampus and the potential to modulate memory-related processes. Several studies have reported improved memory performance following hippocampal tTIS (Violante *et al.*, 2023; Missey *et al.*, 2025). At the same time, the mechanisms underlying these effects remain debated. The electric field strengths induced by tTIS in the human brain are relatively low and may be insufficient to directly elicit neuronal firing (Vieira *et al.*, 2024). Nonetheless, some studies report post-stimulation carry-over effects (Missey *et al.*, 2026), suggesting that long-term synaptic plasticity mechanisms may contribute to the observed behavioral changes. Multiple studies propose that low-intensity stimulation exerts its effects by modulating synaptic plasticity; however, the exact mechanisms by which stimulation influences plasticity remain underexplored (Kronberg *et al.*, 2020; F. Wang *et al.*, 2025).

Direct experimental measurements of synaptic changes during stimulation are difficult; therefore, computational modeling is frequently used to investigate neuronal processes at single-neuron and single-synapse resolution. Such models enable compartment-specific analysis, allow separation of dendritic and somatic effects, and support systematic exploration of stimulation parameters (Mirzakhali *et al.*, 2020; Esmaeilpour *et al.*, 2021). Therefore, computational modeling provides a strong framework for exploring synaptic plasticity mechanisms under tTIS.

The primary goal of this thesis is to investigate how temporal interference stimulation modulates synaptic plasticity in a computational model of a CA1 hippocampal pyramidal neuron under different synaptic input conditions. In order to reach this goal, the following objectives were defined:

1. Develop a CA1 neuron model implementing synaptic plasticity and tTIS, and determine the model parameters required to simulate low- and high-intensity stimulation.
2. Characterize the effects of tTIS on synaptic plasticity under artificial synaptic input, evaluating its effects on somatic and dendritic spiking, synaptic weight dynamics, and synaptic potentiation and depression.

3. Investigate how the modulation of synaptic plasticity by tTIS changes across a range of synaptic input frequencies and identify the input regimes most sensitive to stimulation.
4. Examine whether the observed tTIS effects generalize to biologically realistic synaptic inputs.

1. Literature review

1.1. Temporal interference stimulation

Temporal interference (TI) stimulation, or transcranial electrical temporal interference stimulation (tTIS), is a novel non-invasive deep-brain stimulation technique based on the interference of multiple high-frequency sinusoidal electric fields (Grossman *et al.*, 2017). These fields interact to produce a low-frequency envelope that stimulates the deep target structures while sparing the surrounding tissue (Grossman *et al.*, 2017).

The core principle behind TI—that the interference of multiple non-effective high-frequency currents produces a localized, effective low-frequency envelope—is not entirely new. Similar approaches, such as interferential current stimulation, interferential current therapy, or simply interferential therapy, have been known since the mid-1900s (Goats, 1990; Budde *et al.*, 2023). However, their use was largely limited to peripheral applications in physical therapy, including muscle stimulation, pain management, and promotion of healing-related processes (Goats, 1990). In 2017, Grossman and colleagues were the first to demonstrate that tTIS can safely and non-invasively stimulate deep-brain structures in mice (Grossman *et al.*, 2017), introducing the TI technique to the field of cognitive neuroscience.

This section provides an overview of the main principles of tTIS, the biochemical and neural mechanisms underlying tTIS focality, potential subthreshold tTIS applications, and their implications for synaptic plasticity.

1.1.1. Principles of temporal interference stimulation

The tTIS technique is built on two fundamental concepts in physics: the superposition principle and wave interference. The superposition principle states that when two or more waves meet, their effects are summed at each point in space and time. The superposition of waves creates interference, a pattern that emerges when waves overlap and combine. When two waves rise and fall in sync, they are said to be in phase. In this case, the interference is constructive: their peaks and troughs add together to create a wave with a larger amplitude (Goats, 1990). If the phase difference is equal to half a wavelength, the peak of one wave coincides with the trough of the other. The waves cancel each other out—this is known as destructive interference (Goats, 1990).

Waves with different frequencies also interfere. When the wave peaks align, they interfere constructively, increasing the amplitude of the resulting wave—and decreasing it as the waves fall out of sync and interfere destructively (Figure 1a) (Goats, 1990). This creates a beat pattern where the amplitude varies over time, a phenomenon known as amplitude modulation. The slowly varying amplitude pattern is commonly referred to as the low-frequency envelope. The rate at which the amplitude of the resulting wave changes over time is equal to the frequency difference between the two original waves, and is called the beat frequency or difference frequency, Δf (Goats, 1990).

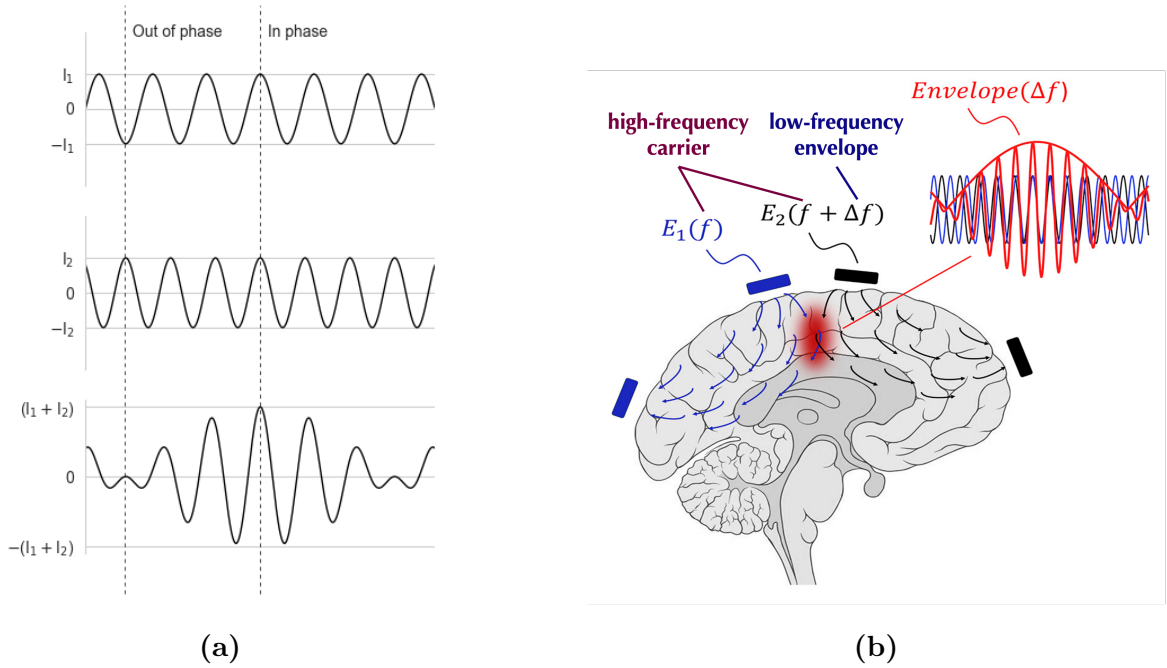


Figure 1. Wave superposition and interference as the basis of tTIS: interference between high-frequency waves generates a low-frequency amplitude-modulated envelope. (a) Amplitude modulation results from the interference of two sinusoidal waves with slightly different frequencies. In-phase oscillations lead to constructive interference (increased amplitude), while out-of-phase oscillations produce destructive interference (signal cancellation). Constructive and destructive interference cause the amplitude of the combined signal to vary over time, forming a low-frequency envelope at the difference frequency Δf . (b) tTIS employs the superposition principle to target deep brain regions. Two currents are applied via scalp electrodes at kHz frequencies f and $f + \Delta f$ and create electric fields E_1 and E_2 in the brain. These electric fields interfere at the target region to produce an amplitude-modulated envelope at the difference frequency Δf that is sufficiently low to modulate neuronal activity. Adapted from Grossman *et al.* (2017), CC BY 4.0.

In tTIS, interference is generated by applying two high-frequency sinusoidal currents via two pairs of scalp electrodes. The two sinusoids are typically in the kilohertz range, with a small difference in frequency between them: $f_1 = f$ and $f_2 = f + \Delta f$ (Figure 1b) (Grossman *et al.*, 2017). The high-frequency f is called the carrier frequency, and, when delivered alone, is substantially less effective at modulating neuronal activity than tTIS (Grossman *et al.*, 2017; Esmaeilpour *et al.*, 2021). In on-target regions, interference between the two frequencies f_1 and f_2 creates an amplitude-modulated envelope that oscillates at the difference frequency Δf (Grossman *et al.*, 2017). When the difference frequency falls within the range neurons respond to (e.g., 5 Hz), the envelope becomes the effective stimulus that modulates neural activity (Grossman *et al.*, 2017). This mechanism was validated by *in vivo* whole-cell patch-clamp recordings in the somatosensory cortex of anesthetized mice. Spiking under 10 Hz tTIS (2 kHz carrier, 10 Hz difference frequency) resembled responses to a pure 10 Hz sinusoid stimulation, whereas the carrier alone did not elicit spiking, showing that neurons follow the low-frequency envelope rather than the high-frequency carrier (Grossman *et al.*, 2017).

In contrast to conventional electrical stimulation techniques, tTIS does not aim to focus the electric fields directly at the stimulation site. Instead, the electric fields are distributed more broadly throughout the tissue. The mechanism of targeting in tTIS is not spatial but

temporal: stimulation occurs at the location where the applied high-frequency currents interfere in time to produce a low-frequency envelope (Grossman, 2018). Thus, the stimulation site (i.e., the peak envelope modulation) depends on the relative amplitude and orientation of the applied currents and can be flexibly steered by adjusting the relative current strengths, without physically repositioning the electrodes (Grossman *et al.*, 2017; Violante *et al.*, 2023). This allows targeting any brain site with a trade-off between the locus depth and stimulation width and strength (Grossman, 2018). In practice, computational models (e.g., the finite element method) are often used to optimize electrode placement and current ratios to achieve desired field interactions (S. Lee *et al.*, 2020; S. Lee, Park, Choi, Lim, *et al.*, 2022). Including additional electrode pairs and alternative envelope waveforms have been proposed as potential strategies to provide further control over tTIS focality (S. Lee, Park, Choi, Lee, & Im, 2022; Acerbo *et al.*, 2024; Botzanowski *et al.*, 2025).

1.1.2. Neural mechanisms of tTIS targeting

The exact biophysical mechanisms underlying tTIS targeting remain under debate. In their seminal work, Grossman and colleagues introduced the tTIS technique based on two related assumptions: (1) due to the low-pass filtering properties of neuronal membranes, neurons are largely unresponsive to the high-frequency carrier signals, and consequently, (2) stimulation occurs mainly at the spatial region where the two electric fields overlap to form a low-frequency envelope (Grossman *et al.*, 2017; Mirzakhilili *et al.*, 2020). Further studies have challenged these assumptions, suggesting that the neural responses to tTIS may not be fully explained by passive membrane filtering alone.

Active membrane dynamics may be necessary to extract the low-frequency envelope

It is generally assumed that neurons are less responsive to high-frequency stimulation due to the intrinsic electrical properties of their membranes. The membrane time constant τ , defined by the resistance and capacitance of the membrane, determines how rapidly the membrane can follow changes in voltage (Dayan & Abbott, 2005). Above a certain stimulation frequency threshold, the membrane potential simply fails to adjust, causing the neuron membrane to act as a low-pass filter (Dayan & Abbott, 2005).

In the context of tTIS, this assumption leads to a paradox: the same membrane properties that suppress responses to high-frequency carriers would, in principle, also attenuate the amplitude-modulated low frequencies (Dmochowski & Bikson, 2017; Cao & Grover, 2018), since the tTIS waveform is a sum of two high-frequency sinusoids and contains only high-frequency components (Mirzakhilili *et al.*, 2020). This suggests that passive membrane filtering alone cannot account for neuronal responses to tTIS. Mirzakhilili *et al.* (2020) proposed that ion-channel-mediated signal rectification is necessary to extract the low-frequency envelope. Using computational modeling, they demonstrated that rectification arises primarily from the nonlinear gating properties of fast sodium channels. During stimulation, fast Na^+ channels rapidly ac-

tivate and create a strong inward current before K^+ channels can counteract it, which skews the membrane potential toward depolarization and enables the neuron to follow the low-frequency envelope (Figure 2a) (Mirzakhilili *et al.*, 2020).

This underlying biophysical mechanism suggests that active, rather than passive, membrane properties are necessary to extract the low-frequency tTIS waveform, implying that pure high-frequency carrier stimulation cannot be assumed to be negligible. Given the temporal targeting of tTIS, large cortical areas are exposed to tonic high-frequency stimulation (Mirzakhilili *et al.*, 2020). Importantly, to achieve suprathreshold responses, comparable stimulation strengths are required for both tTIS and unmodulated kilohertz waveforms (Opančar *et al.*, 2025; Semenov *et al.*, 2025). Under strong high-frequency stimulation, neuronal dynamics become nonlinear and more complex: for example, neurons may show only an initial transient response or have sustained firing throughout the stimulation period (Mirzakhilili *et al.*, 2020). In peripheral nerves, stimulation at kilohertz frequencies can disrupt the ability of axons to propagate action potentials, a phenomenon known as conduction block (Kilgore & Bhadra, 2014). Computational modeling suggests that suprathreshold tTIS stimulation may also induce conduction block in non-targeted regions (Mirzakhilili *et al.*, 2020). However, conduction block has been observed primarily at high stimulation amplitudes *in vivo* (Budde *et al.*, 2023), suggesting that it is unlikely to occur under the low-amplitude conditions typical of human tTIS.

tTIS focality may arise due to network-level interactions

These observations complicate simple explanations of tTIS focality (Figure 2b, left). If comparable stimulation strength is required for both modulated and unmodulated high-frequency stimulation, it remains unclear how the localized neuronal responses emerge.

Caldas-Martinez *et al.* (2024) proposed that the focality of tTIS could stem from interactions at the network level. They observed that under suprathreshold stimulation in *ex vivo* mouse cortex, fast-spiking inhibitory parvalbumin-expressing (PV) interneurons were less likely than regular-spiking excitatory pyramidal (Pyr) neurons to exhibit TI stimulation (Figure 2b, right) (Caldas-Martinez *et al.*, 2024). A neuron is defined as exhibiting TI stimulation if, for some stimulation amplitude, it fires under a modulated sinusoid but not under a pure sinusoid.

To understand this difference, the authors examined firing responses in isolation. When synaptic transmission was blocked, both neuron types stopped exhibiting TI stimulation (Caldas-Martinez *et al.*, 2024), consistent with previous studies reporting similar activation thresholds for tTIS and pure kHz stimulation (Opančar *et al.*, 2025; Semenov *et al.*, 2025). However, the two cell types displayed distinct firing patterns: Pyr neurons responded similarly to modulated and unmodulated sinusoids, whereas PV interneurons increased their firing rates under pure sinusoidal stimulation (Figure 2b, middle) (Caldas-Martinez *et al.*, 2024). When embedded in a network, this increased PV activity enhances suppression of Pyr neuron firing in off-target areas. In on-target regions, firing rates for both PV and Pyr neurons remain similar, thus resulting in the observed tTIS focality (Caldas-Martinez *et al.*, 2024).

A related mechanism was proposed by Esmaeilpour *et al.* (2021), who suggested that tTIS

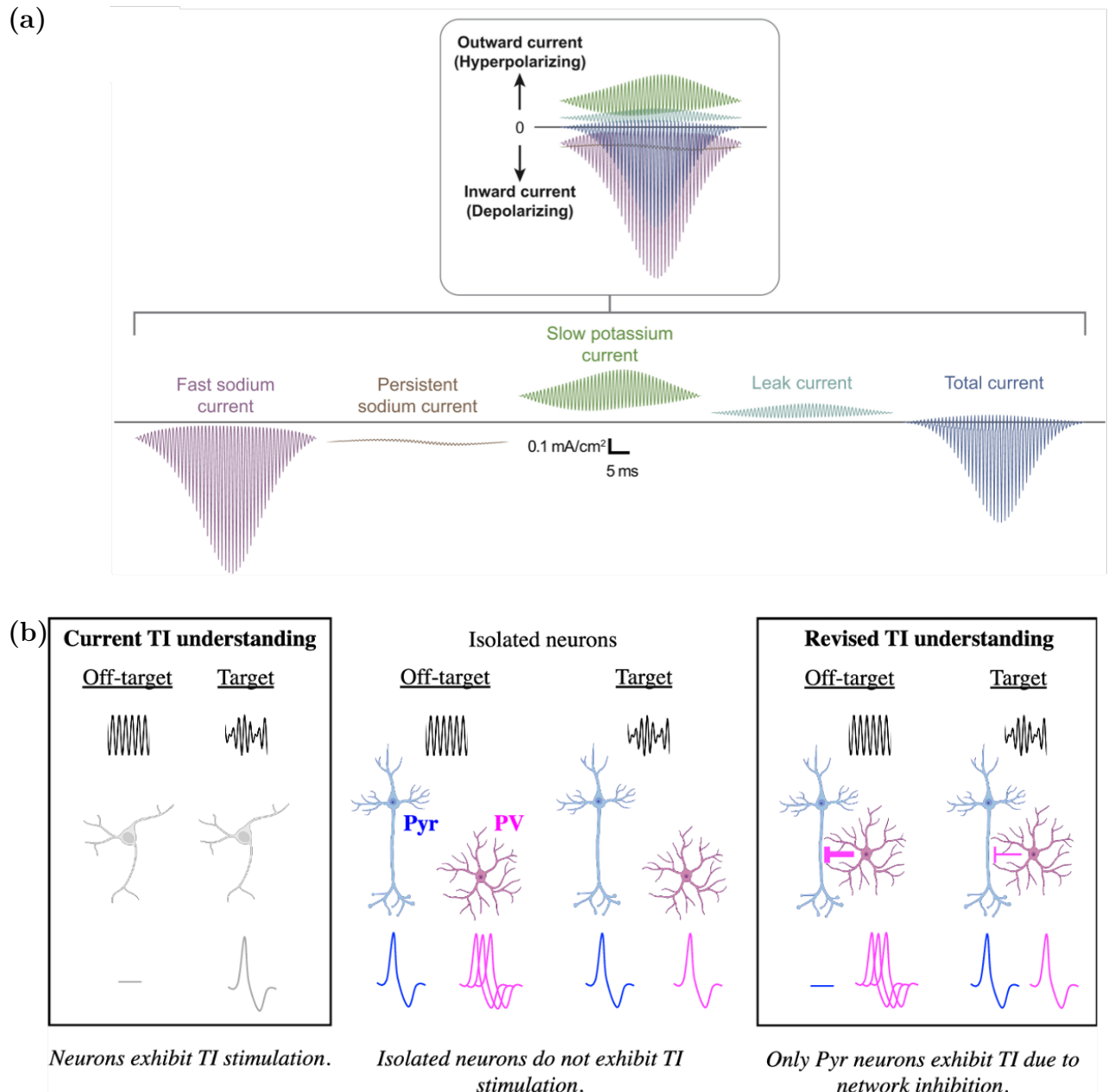


Figure 2. Biophysical and network-level neuronal responses to tTIS. (a) Computational modelling of ionic current dynamics during tTIS. The asymmetry between fast inward sodium current and slow outward potassium current creates a net depolarizing bias. This bias allows the neuronal membrane to rectify the high-frequency carrier and extract the low-frequency envelope. Adapted from Mirzakhali *et al.* (2020), CC BY-NC-ND. (b) Responses to tTIS may arise from network-level effects. (Left) The seminal TI paper (Grossman *et al.*, 2017) proposed that neurons are unresponsive to unmodulated high-frequency stimulation. (Middle) In isolation, inhibitory parvalbumin-expressing (PV) interneurons show an increased spiking rate in the off-target regions, whereas the firing rate of pyramidal (Pyr) neurons remains unchanged. (Right) In neuronal networks, enhanced PV interneuron activity in off-target regions suppresses Pyr neuron firing. This suppression is absent in the on-target regions, resulting in tTIS focality. Adapted from Caldas-Martinez *et al.* (2024), CC BY-NC-ND 4.0.

selectivity arises from different responses to TI and pure kilohertz waveforms at the network level. Using hippocampal slice experiments and computational modeling, they showed that amplitude-modulated electric fields induced modulation of gamma oscillations in deep regions, while unmodulated kHz stimulation resulted in steady increases in activity in superficial areas (Esmailpour *et al.*, 2021). Their model further suggested that selectivity arises from fast membrane dynamics and network adaptation mechanisms, particularly slow inhibition by gamma-aminobutyric acid (GABA) B-type receptors. In off-target regions exposed to unmodulated kHz stimulation, sustained activation of inhibitory GABA_B receptors progressively suppresses network activity. In contrast, in on-target regions exposed to amplitude-modulated fields, the periodically varying stimulation envelope reduces sustained inhibition and allows the network to remain responsive to the low-frequency modulation, resulting in continued modulation of oscillatory activity (Esmailpour *et al.*, 2021).

Taken together, Mirzakhali *et al.* (2020), Esmailpour *et al.* (2021), and Caldas-Martinez *et al.* (2024) explain tTIS mechanisms at two levels: nonlinear ion-channel dynamics enable individual neurons to respond to tTIS waveforms, whereas network-level interactions allow for spatially localized responses. However, these mechanisms were investigated under high-intensity stimulation conditions. The electric fields achieved during human tTIS are substantially weaker (Grossman, 2018); therefore, it remains unclear to what extent these findings translate to human stimulation.

1.1.3. Stimulation effects in different neuronal compartments

The effects of extracellular electrical stimulation are highly compartment-specific. Due to differences in morphology, membrane properties, ion channel distribution, and orientation relative to the electric field, neuronal compartments respond differently to externally applied electric fields (Bikson *et al.*, 2004; Radman *et al.*, 2009).

Multiple studies suggest that axons are more susceptible to extracellular stimulation than somas or dendrites, exhibiting two- to four-fold greater polarization in response to electric fields (Bikson *et al.*, 2004; Rahman *et al.*, 2013; Chakraborty *et al.*, 2017). Prior computational work has highlighted axons as the primary targets of tTIS as well (Mirzakhali *et al.*, 2020; Esmailpour *et al.*, 2021). Due to the high density of fast voltage-gated Na⁺ channels in the initial segment, the rectification of TI signal is particularly pronounced in axons (Fleidervish *et al.*, 2010; Mirzakhali *et al.*, 2020). Moreover, nonlinear voltage-gated channel dynamics play a role in amplifying the membrane response to extracellular fields (Chakraborty *et al.*, 2017), and further contribute to the resonant properties that enable axons to respond selectively to certain stimulation frequencies (Esmailpour *et al.*, 2021). Axons have small diameters and shorter time constants, allowing them to follow high-frequency stimuli that other neuron compartments filter out (Esmailpour *et al.*, 2021). Sensitivity to tTIS has been linked to how closely axonal time constants match the carrier frequency (Esmailpour *et al.*, 2021), as well as how closely intrinsic axonal resonant frequencies align with the low-frequency envelope (Mirzakhali *et al.*,

2020; Zhu & Yin, 2023).

Albeit to a lesser extent, the soma and dendrites can also be affected by external electric fields. However, published work on tTIS either does not address these compartments or suggests that they are less responsive to TI fields compared to axons (Mirzakhali *et al.*, 2020; Esmailpour *et al.*, 2021). At the same time, relatively few studies have examined somatic and dendritic responses to tTIS directly. Since the basic principles of membrane polarization observed under other forms of extracellular stimulation are expected to apply in the context of tTIS, the following discussion is primarily based on studies using uniform or low-frequency oscillating electric fields, with a focus on dendritic effects.

In the dendrites and the soma, the activation threshold is higher; thus, extracellular stimulation is unlikely to generate an action potential (Radivojevic *et al.*, 2016). This stimulation regime is considered subthreshold, as it alters membrane potential without reliably inducing spikes (Gerstner *et al.*, 2014). By comparison, suprathreshold stimulation directly elicits firing activity (Gerstner *et al.*, 2014). Even though the observed effects of suprathreshold stimulation are considerably stronger, subthreshold stimulation can still shape brain activity by influencing spike firing rate and timing through changes in neuronal excitability, signal integration, and synaptic efficacy (Bikson *et al.*, 2004; Abera *et al.*, 2025). While somatic polarization primarily affects excitability by shifting the membrane potential and altering the likelihood of action potential firing (Rahman *et al.*, 2013), dendritic polarization induces frequency-dependent responses (Aspart *et al.*, 2018; Toloza *et al.*, 2018) and shapes synaptic plasticity and the integration of synaptic inputs (Bikson *et al.*, 2004; Cavarretta *et al.*, 2014; Chakraborty *et al.*, 2017).

The response to extracellular stimulation is shaped by the morphology of the neuron and its dendritic tree (Radman *et al.*, 2009; Aspart *et al.*, 2018). In the presence of an external electric field, neurons polarize in a compartment-specific manner, especially in cell types with asymmetric dendritic morphology (Radman *et al.*, 2009). Symmetric cells, such as interneurons, are less sensitive to polarization effects compared to highly asymmetric pyramidal cells (Radman *et al.*, 2009), which contain a long apical dendrite extending toward the cortical surface and shorter basal dendrites branching near the soma. The effects of stimulation also depend on the orientation of the neuronal compartment relative to the direction of the applied electric field (Toloza *et al.*, 2018; Missey *et al.*, 2021; Jain *et al.*, 2023). The strongest polarization in the dendrites is observed when the electric field is aligned parallel to the somato-dendritic axis—the longitudinal axis running from the soma along the dendritic tree (Bikson *et al.*, 2004; Toloza *et al.*, 2018). When exposed to a uniform extracellular electric field oriented along this axis, the pyramidal neuron exhibits both hyperpolarizing and depolarizing effects, with basal and apical dendrites polarizing in opposite directions (Bikson *et al.*, 2004). As a result, stimulation can alter the action potential threshold and shift the spike initiation site along the apical dendrites (Bikson *et al.*, 2004). The biphasic polarization pattern arises from the voltage gradient along the neuron, as the current enters at one pole and exits at the other (Bikson *et al.*, 2004). Bikson *et al.* (2004) emphasized the primary contribution of passive, rather than

active, membrane properties to this symmetric polarization profile and suggested that it may reflect differences in time constants along the dendritic tree.

Active conductances in the dendrites can further amplify responses to extracellular stimulation and create resonance. A computational study by Aspart *et al.* (2018) demonstrated that, compared to the soma or basal dendrites, apical dendrites are particularly sensitive to low-frequency (10-20 Hz) fields and exhibit larger polarization responses. The authors attribute this property to the presence of hyperpolarization-activated cation current (I_h) (Aspart *et al.*, 2018). This current is mediated by HCN1 (h) channels, which are strongly enriched in the distal apical dendrites of pyramidal neurons (Buss *et al.*, 2024) and induce resonance in response to both current injection as well as electric field stimulation (Aspart *et al.*, 2018; Toloza *et al.*, 2018). The I_h current acts as a high-pass filter and, when combined with the low-pass filtering property of the neuronal membrane, produces an amplified resonant response at specific stimulation frequencies (11 ± 2 Hz) (Toloza *et al.*, 2018).

While axons appear to be the most sensitive targets of tTIS due to their fast membrane dynamics, dendritic and somatic polarization may still influence neuronal excitability and, importantly, shape synaptic processing. These effects are also strongly dependent on stimulation intensity and whether the applied electric fields operate in a subthreshold or suprathreshold regime (Radman *et al.*, 2009).

1.1.4. Subthreshold stimulation effects and implications for synaptic plasticity

Most tTIS studies in rodents employ suprathreshold stimulation intensities and report cortical electric field amplitudes up to 300–400 mV/mm (Grossman *et al.*, 2017; Rampersad *et al.*, 2019). In contrast, stimulation intensities in human tTIS typically range from 1 to 5 mA applied at the scalp electrodes (Violante *et al.*, 2023; Schaeffer *et al.*, 2025), which correspond to electric field strengths of approximately 0.2–1 mV/mm in the cortex (S. Lee *et al.*, 2020; Ness *et al.*, 2025). Thus, tTIS in humans likely operates in the subthreshold regime (Peng *et al.*, 2025).

Rather than eliciting spikes, subthreshold stimulation shapes neural activity by modulating membrane polarization, influencing cortical excitability, altering spike timing, and entraining ongoing neural oscillations (Radman *et al.*, 2007; Polanía *et al.*, 2018; Vogeti *et al.*, 2022). It can influence synaptic plasticity (Aberra *et al.*, 2025) and, over longer timescales, lead to network reorganization (Lu *et al.*, 2019). Plasticity-related mechanisms are thought to underlie the therapeutic effects of subthreshold stimulation across a range of neuropsychiatric and neurological disorders, including depression (J. C. Lee *et al.*, 2021), Alzheimer’s disease (Rowan *et al.*, 2014), and stroke (Kim *et al.*, 2021). Studies of tTIS therapeutic applications and their underlying mechanisms are currently limited. Given that tTIS in humans is predominantly subthreshold, it is hypothesized to engage similar plasticity-related mechanisms as other neurostimulation techniques (Peng *et al.*, 2025).

The current evidence remains inconclusive regarding the therapeutic efficacy of subthreshold tTIS in humans. Compared to other non-invasive stimulation techniques, tTIS is less effective

at modulating neural activity at comparable field strengths, and fails to reliably entrain neural oscillations (Esmailpour *et al.*, 2021; Vieira *et al.*, 2024) or increase electroencephalogram (EEG) band power (von Conta *et al.*, 2022; Iszak *et al.*, 2023). Neuronal membranes are less sensitive to high-frequency stimulation, which means that kHz frequencies used in tTIS require higher intensities for effective modulation (Esmailpour *et al.*, 2021). The effects of subthreshold tTIS depend on interactions with ongoing brain oscillations at the single-neuron level, and may disrupt rather than enhance neuronal entrainment (Vieira *et al.*, 2024). These effects closely resemble those observed with conventional transcranial alternating current stimulation (tACS) (Johnson *et al.*, 2020; M. R. Krause *et al.*, 2022) and could be overcome by higher stimulation amplitudes (Vieira *et al.*, 2024). However, increasing stimulation intensity to achieve sufficient modulation at the target region may also induce off-target effects (Esmailpour *et al.*, 2021).

Even though the ability of tTIS to entrain neural oscillations appears to be limited, subthreshold tTIS can alter spike timing (Vieira *et al.*, 2024) and reshape connectivity within neural networks (Zhu *et al.*, 2022; Violante *et al.*, 2023). Behavioral studies in humans further demonstrate that tTIS can affect episodic memory (Violante *et al.*, 2023; Missey *et al.*, 2025), navigation (Beanato *et al.*, 2024), reinforcement learning (Wessel *et al.*, 2023), as well as motor learning and performance (Wessel *et al.*, 2023; Vassiliadis *et al.*, 2024; Zheng *et al.*, 2024). The effects of tTIS are not unidirectional: depending on envelope frequency and target region, it can either disrupt or improve memory and learning (Y. Wang *et al.*, 2024; Missey *et al.*, 2025), and increase or decrease brain activity as measured by the blood-oxygen-level-dependent (BOLD) signal (Violante *et al.*, 2023; Wessel *et al.*, 2023; Vassiliadis *et al.*, 2024).

Despite the measurable behavioral effects, direct evidence linking tTIS to changes in synaptic plasticity in humans remains limited, with most studies providing only indirect support. For example, Violante *et al.* (2023) showed that tTIS stimulation in the hippocampus can enhance the accuracy of episodic memories and reported a higher accuracy at re-test after tTIS as compared to sham. Missey *et al.* (2026) were among the first to report a significant tTIS carry-over effect. The study focused on reducing epileptic biomarkers in the hippocampus, and reported significant improvements 24 hours post-stimulation compared to baseline (Missey *et al.*, 2026). These findings suggest that tTIS may engage mechanisms beyond entrainment during the stimulation, potentially inducing synaptic plasticity. An *in vivo* experiment in mice further suggested that the effects of tTIS may reflect changes in neurotransmitter metabolism, neuronal calcium signaling, and enhanced synaptic plasticity (Qi *et al.*, 2024). However, it remains unclear whether these findings directly translate to human tTIS, as the authors do not explicitly specify whether the applied stimulation was subthreshold.

tTIS has emerged as a promising non-invasive brain stimulation technique capable of targeting deep brain regions. Although the exact mechanisms underlying tTIS remain incompletely understood, prior studies suggest that its effects depend on membrane dynamics and interactions within neuronal networks. Computational and experimental work has consistently identified axons as the most sensitive targets of tTIS; however, dendritic and somatic polarization may still contribute to subthreshold modulation of neuronal excitability and synaptic

processing. Importantly, human studies using subthreshold stimulation intensities report altered cortical excitability as well as effects on memory and learning, suggesting that tTIS may influence processes underlying long-term synaptic plasticity.

1.2. Hippocampus

The hippocampus is a key brain structure that supports long-term declarative (explicit) memory, defined as the ability to consciously recall facts and events (Hasselmo, 2011). It is frequently used to study mechanisms of synaptic plasticity (Neves *et al.*, 2008), processes thought to underlie memory formation. The Schaffer collateral synapses in the hippocampus play a central role in hippocampal memory processing and are among the most well-studied synapses in the mammalian brain (Brown *et al.*, 2004).

While the hippocampus is also known for its role in spatial coding, the present work focuses on synaptic plasticity mechanisms along the Schaffer collateral pathway. Therefore, this section covers hippocampal neuroanatomy and circuitry, the role of Schaffer collateral synapses and endogenous hippocampal oscillations in memory-related processes, and the effects of hippocampal stimulation.

1.2.1. Neuroanatomy and circuitry

In humans and other primates, the hippocampus is located bilaterally within the medial temporal lobe (MTL) (Knierim, 2015). The MTL is divided into two anatomically and functionally distinct regions: the hippocampal formation and the parahippocampal region (Eichenbaum, 2004). The hippocampal formation is a curved, S-shaped structure (Figure 3A) composed of the hippocampus proper (*Cornu ammonis* (CA) subfields CA1-CA3), the dentate gyrus (DG), the subiculum, and transitional areas such as the parasubiculum and presubiculum (Eichenbaum, 2004). It serves as the primary site of memory processing within the MTL.

In contrast, the parahippocampal region serves as an input/output hub that connects the hippocampal formation with cortical and subcortical areas (Morris *et al.*, 2025). This region is further subdivided into the perirhinal, parahippocampal¹ and entorhinal (EC) cortices (Morris *et al.*, 2025).

The hippocampal formation is one of a few convergent brain regions that receives highly processed multimodal sensory information from a variety of cortical and subcortical sources (Eichenbaum, 2004). The primary gateway for information entering the hippocampal formation is the entorhinal cortex (Morris *et al.*, 2025). It receives converging input from the neocortex and subcortical regions via the parahippocampal and perirhinal cortices, respectively, as well as additional input from the dorsal presubiculum (postsubiculum) (Hasselmo, 2011). This integrated information is then relayed to the CA1 region of the hippocampus either directly or indirectly (Morris *et al.*, 2025).

In the indirect pathway, information propagates to the CA1 through three unidirectional

¹Parahippocampal cortex in primates is homologous to the postrhinal cortex in rodents.

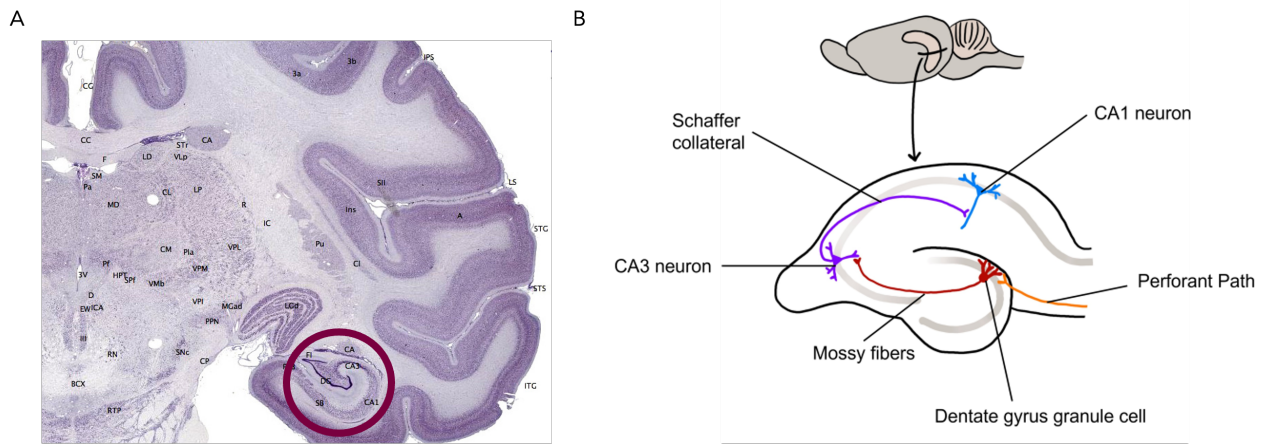


Figure 3. The location of the hippocampal formation and its internal circuitry. (A) Coronal section of the macaque monkey brain (Nissl stain) showing the hippocampal formation (circled). Image courtesy of brainmaps.org, CC BY 3.0. (B) Trisynaptic pathway of the rodent hippocampus. Adapted from Hedges (2022), CC BY-NC-SA 4.0.

excitatory connections (Figure 3B) (Morris *et al.*, 2025). The first synapse is formed by projections from layer II neurons of the entorhinal cortex to the granule cells of the dentate gyrus. These axons constitute part of the perforant pathway, which represents the major cortical input to the hippocampal formation. The granule cells of the dentate gyrus then project to the pyramidal cells in the CA3 region of the hippocampus via the mossy fiber pathway. Finally, axons of the CA3 cells give rise to the Schaffer collateral pathway and form excitatory synapses on the proximal regions of the dendrites of the CA1 pyramidal neurons. Since the indirect pathway follows three successive synaptic connections (EC to DG, DG to CA3, CA3 to CA1), it is often referred to as the trisynaptic pathway (Hasselmo, 2011; Morris *et al.*, 2025).

The CA1 area serves as the primary output node of the hippocampus, transmitting the processed DG/CA3 information to the rest of the cerebrum (Morris *et al.*, 2025).

1.2.2. Trisynaptic pathway and information flow

The dentate gyrus is the first step along the trisynaptic pathway and is typically presumed to preprocess the incoming information and prepare it for subsequent processing in the CA3 region (Morris *et al.*, 2025). Some theoretical studies argue that the dentate gyrus decorrelates EC inputs, a process known as pattern separation (Knierim & Neunuebel, 2016; Senzai, 2019).

A complementary process, pattern completion, could then be attributed to the CA3 region (Knierim & Neunuebel, 2016; Senzai, 2019). Pattern completion refers to the ability to retrieve a complete memory pattern when presented with incomplete, decorrelated inputs (Knierim & Neunuebel, 2016). This function could be supported by the sparsely interconnected local CA3 circuitry (Rebola *et al.*, 2017). The recurrent connections in the CA3 region are proposed to function as an attractor network, which could support the rapid storage and retrieval of associative memories (Rolls, 2007).

The nature of computations downstream of the DG-CA3 circuit is less understood (Morris *et al.*, 2025). CA1 projects to multiple brain regions where the information is distributed and embedded in large-scale recurrent networks (Morris *et al.*, 2025); therefore, isolating specific

computational roles becomes more challenging. Theoretical models suggest that area CA1 uses the EC inputs to attach additional context to otherwise uninformative patterns of CA3 attractors, thereby enabling the encoding and recall of information-rich memories when large numbers of similar memories are stored (Kaifosh & Losonczy, 2016).

The synaptic interactions between CA3 and CA1 pyramidal neurons are widely implicated in memory formation. N-methyl-D-aspartate receptor (NMDAR)-dependent long-term synaptic plasticity is considered to be a key neural mechanism underlying memory formation and consolidation, and has been extensively characterized at the Schaffer collateral synapses in the hippocampus (Morris *et al.*, 2025). Activity-dependent plasticity at Schaffer collateral synapses participates in spatial memory acquisition (Tsien *et al.*, 1996), correlates with associative learning (Gruart *et al.*, 2006), and is proposed to aid memory retrieval (Carr & Frank, 2012; Rolls, 2013). During novel memory formation, synaptic patterns at the Schaffer collateral projections evolve over timescales of days (Karlsson & Frank, 2008), suggesting that connection strength may be progressively stabilized to consolidate memory representations for subsequent replay (Carr & Frank, 2012).

1.2.3. Hippocampal oscillations

The hippocampus is a highly oscillatory system (Hasselmo, 2011). The frequency dynamics within the hippocampus are essential for coding components of both space and time, which together form a basis for episodic memories (Hasselmo, 2011). These neuronal dynamics reflect synchronized rhythmic activity of neuronal populations and can be measured as local field potentials oscillating in the theta and gamma frequency ranges, or as sharp wave-ripples (Colgin, 2016).

Theta rhythm is the most prominent oscillation of the hippocampus and has been recorded throughout most regions of the hippocampal formation (Amilhon *et al.*, 2020). Theta rhythms are slow oscillations in the 4–8 Hz frequency range (Lega *et al.*, 2011), and are thought to emerge from interactions between the medial septum and entorhinal cortex, as well as within intrinsic hippocampal networks (Amilhon *et al.*, 2020; Nuñez & Buño, 2021). They are predominantly observed during wakefulness and rapid eye movement sleep, and are important for memory formation, locomotion, spatial navigation, and emotional processing (Korotkova *et al.*, 2018; Amilhon *et al.*, 2020). Some hippocampal neurons exhibit strong coupling to theta oscillations and preferentially fire during specific phases of the rhythm (Guth *et al.*, 2025). The timing of firing relative to the theta phase can influence synaptic plasticity; for example, stimulation delivered near the peak of the local theta rhythm preferentially induces synaptic potentiation, whereas stimulation at the trough leads to depression (Hyman *et al.*, 2003; Stella & Treves, 2011).

Gamma oscillations are faster hippocampal rhythms in the 30–120 Hz frequency range (Tamura *et al.*, 2017). In the hippocampus, gamma oscillations are modulated by the theta rhythm and tend to increase in amplitude during specific phases of the theta cycle, a phenomenon known as theta-gamma coupling (Tamura *et al.*, 2017). Gamma oscillations are

thought to support information processing along different hippocampal pathways (Colgin *et al.*, 2009). Slow gamma frequencies ($\sim 25\text{--}45$ Hz) are linked to inputs from CA3 and are suggested to support retrieval of previously stored information, whereas fast gamma frequencies ($\sim 65\text{--}100$ Hz) arise from entorhinal cortex inputs and are associated with memory encoding (F. Wang *et al.*, 2025). Theta-gamma coupling is therefore thought to temporally organize neuronal activity during memory processing (Ursino & Pirazzini, 2024).

Sharp wave-ripples (SWRs) are brief, high-frequency (100–250 Hz), and large-amplitude oscillations that represent highly synchronized neuronal activity (Buzsáki, 2015). They are most prominent during quiet wakefulness and non-rapid eye movement sleep (Joo & Frank, 2018). Due to their highly synchronized nature, SWRs are thought to support synaptic plasticity mechanisms and stabilization of memories (Buzsáki, 2015; Joo & Frank, 2018). SWRs are associated with replay of prior experiences and contribute to memory consolidation and retrieval by mediating information transfer between the hippocampus and neocortex during periods of reduced sensory input (Buzsáki, 2015; Yang *et al.*, 2024).

Hippocampal oscillations are necessary for various aspects of memory processing and are closely associated with synaptic plasticity (Hadler *et al.*, 2024). Therefore, disruption of endogenous hippocampal rhythms, either by external stimulation or disease, can lead to impairments in cognitive functions mediated by the hippocampal formation (Kloc *et al.*, 2020).

1.2.4. Stimulation of the hippocampus

Hippocampal function is impaired in a range of neurological and neuropsychiatric disorders that disrupt endogenous oscillatory activity, alter neuronal excitability, induce neuronal death, or involve a combination of these mechanisms (Luppi *et al.*, 2022; Koketsu *et al.*, 2024; Lepeu *et al.*, 2024). Consequently, brain stimulation has been explored as a potential approach to alleviate these symptoms and improve cognitive function (Mankin & Fried, 2020). Historically, direct hippocampal stimulation in humans was limited to invasive approaches using intracranial electrodes implanted for clinical purposes (Mankin & Fried, 2020). Among these approaches, deep brain stimulation was explored for its potential to reduce memory deficits in Alzheimer’s disease, epilepsy, and traumatic brain injury (Mankin & Fried, 2020; Mehak *et al.*, 2022).

The primary aim of hippocampal stimulation is to synchronize endogenous oscillations to a specific (typically theta) frequency in order to improve memory processes (Hanslmayr *et al.*, 2019; Mankin & Fried, 2020). Stimulation of the hippocampus and hippocampus-adjacent structures increases hippocampal theta activity and enhances network connectivity (Solomon *et al.*, 2018; Kragel *et al.*, 2025). Hippocampal regions located near white matter and within strongly functionally connected networks appear to be more sensitive to stimulation and exhibit the largest increases in theta power (Solomon *et al.*, 2018; Mohan *et al.*, 2020). Frequency-dependent effects have also been reported, with high-frequency stimulation associated with excitation and low-frequency stimulation with inhibition of neuronal activity (Mohan *et al.*, 2020).

Hippocampal stimulation leads to opposing effects, enhancing memory in some cases (Jun

et al., 2019; Badillo Goicoechea *et al.*, 2026) and impairing it in others (Lacruz *et al.*, 2010; Jacobs *et al.*, 2016), or having no effect (Suthana *et al.*, 2012). The stimulation outcome appears to depend on neural factors such as task-related brain activity (Jun *et al.*, 2020) or the timing of stimulation relative to different phases of memory processing (Merkow *et al.*, 2017; Badillo Goicoechea *et al.*, 2026). For example, memory impairment is more pronounced when stimulation is applied between encoding and recall rather than during either stage (Merkow *et al.*, 2017), while stimulation delivered before encoding is associated with improved memory performance (Badillo Goicoechea *et al.*, 2026).

Overall, current hippocampal stimulation approaches remain largely limited to invasive techniques and produce variable effects depending on stimulation parameters and ongoing neural activity. Despite promising progress in this field, the mechanisms underlying stimulation-induced memory changes remain poorly understood, hindering effective application of these approaches to human disease (Mankin & Fried, 2020).

The internal hippocampal circuitry and endogenous oscillations play central roles in memory encoding, consolidation, and retrieval within the medial temporal lobe. Therefore, hippocampal stimulation has emerged as a promising approach for alleviating memory deficits associated with neurological and psychiatric disorders. However, the mechanisms and effects of deep brain stimulation remain incompletely understood, making hippocampal neuromodulation an active and rapidly evolving field of research.

1.3. Synaptic plasticity

Neural circuits are non-static and continuously undergo extensive remodeling and reorganization in response to behavioral contexts and experience (Ho *et al.*, 2011). This phenomenon of experience-driven changes in synaptic connectivity is called synaptic plasticity (Ho *et al.*, 2011), and is frequently described within the framework of Hebbian theory, famously summarised as “Neurons that fire together, wire together” (Hebb, 2005). The most well-studied and thus representative forms of synaptic plasticity are the long-term potentiation (LTP) and long-term depression (LTD), observed in the CA1 region of the hippocampus (Citri & Malenka, 2007). LTP has several properties that make it a suitable mechanism of memory formation: first, it is observed in each main pathway of the information flow in the hippocampus; second, it is rapidly induced; and third, once generated, it is maintained for hours or even days (Squire & Kandel, 2003).

This section focuses on the biochemical mechanisms of long-term synaptic plasticity in the Schaffer collateral pathway and on spike-timing-dependent plasticity (STDP), a process closely linked to LTP and LTD. It then briefly explores the effects of brain stimulation on synaptic plasticity.

1.3.1. Long-term potentiation and depression

LTP has two stages: induction, a rapid sequence of molecular events following synaptic stimulation, and maintenance, long-term processes in the dendritic spine that sustain enhanced synaptic efficacy (Squire & Kandel, 2003). LTP is primarily expressed postsynaptically; however, some supporting presynaptic mechanisms also exist (MacDougall & Fine, 2014). In the Schaffer collateral pathway, LTP induction relies on the postsynaptic activation of two types of glutamatergic receptors: α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (AMPA) and N-methyl-D-aspartate (NMDA) receptor (NMDAR) (Arrigoni & Greene, 2004).

AMPA and NMDA receptors are found co-localized in the postsynaptic density of a single dendritic spine (Citri & Malenka, 2007; Kandel *et al.*, 2021). Following a presynaptic spike and glutamate release, AMPARs are activated first (Citri & Malenka, 2007; Ho *et al.*, 2011). When open, they are permeable to monovalent Na^+ and K^+ cations and generate most of the excitatory postsynaptic current (EPSC) near the resting membrane potential (Citri & Malenka, 2007; Ho *et al.*, 2011). AMPAR-mediated currents have rapid rise and decay kinetics, providing the initial depolarization required to fire a postsynaptic spike (Kandel *et al.*, 2021).

The second type of glutamatergic receptors—NMDARs—have two unique properties that directly contribute to the induction of LTP. Firstly, their opening depends on both presynaptic glutamate release and postsynaptic depolarization (Citri & Malenka, 2007; Ho *et al.*, 2011). Unlike AMPARs, NMDARs provide little contribution to the initial postsynaptic response (Citri & Malenka, 2007). These receptors display strong voltage dependence and, at negative membrane potentials, are blocked by extracellular magnesium (Citri & Malenka, 2007; Ho *et al.*, 2011). Cell depolarization leads to dissociation of the magnesium block and channel opening (Citri & Malenka, 2007; Ho *et al.*, 2011). Due to the kinetics of ligand-gating, the NMDAR-mediated currents rise and decay at a slower time course than AMPAR currents, contributing to the late and slow phase of the EPSC (Kandel *et al.*, 2021).

Another property that differentiates NMDA and AMPA receptors is NMDAR permeability to Ca^{2+} ions (Kandel *et al.*, 2021). The increased intracellular Ca^{2+} concentration is necessary for triggering LTP (Citri & Malenka, 2007; Lisman *et al.*, 2012). Calcium influx activates intracellular signaling cascades that translate electrical activity into long-term synaptic changes (Kandel *et al.*, 2021) and promotes AMPAR trafficking to the synapse (Hangen *et al.*, 2018). Increased AMPA receptor conductance leads to a larger excitatory postsynaptic potential (EPSP) (Ho *et al.*, 2011), the defining feature of enhanced synaptic strength.

The direction of synaptic change—whether the synapse is potentiated or depressed—can be determined by the magnitude and time course of the postsynaptic Ca^{2+} rise (Graupner, 2010). This theory, known as the calcium control hypothesis, states that potentiation requires a large, rapid increase in intracellular Ca^{2+} , while depression is caused by a smaller, prolonged postsynaptic Ca^{2+} rise (Lisman, 1989). These dynamics are mediated by NMDARs, with smaller contributions from other calcium sources, such as voltage-gated Ca^{2+} channels and non-NMDA glutamate receptors (Lisman, 1989). Lower calcium concentrations lead to AMPAR

dephosphorylation and internalization (Beattie *et al.*, 2000; Creamer, 2020), resulting in a reduced EPSP and, thus, LTD.

1.3.2. Spike-timing-dependent plasticity

Interest in synaptic plasticity and its role in memory emerged in 1973, when Tim Bliss and Terje Lømo discovered that tetanic stimulation (high-frequency, high-intensity synaptic stimulation) enhances synaptic strength in excitatory hippocampal synapses for several hours post-stimulation (Bliss & Lømo, 1973; Nicoll *et al.*, 1988). However, the sustained high-frequency presynaptic activity needed to induce LTP *in vitro* is unlikely to occur under physiological conditions. In the mid-1990s, theoretical scientists recognized that the temporal order of spikes was important for transmitting and storing information within neural circuits, leading to the development of the concept of spike-timing-dependent plasticity (Markram *et al.*, 2012).

STDP is a temporally asymmetric form of Hebbian learning characterized by the relative timing between the spikes of pre- and postsynaptic neurons (Sjöström & Gerstner, 2010). In many types of synapses, a presynaptic spike within a narrow time window before the postsynaptic spike drives the synapse towards LTP (pre-post LTP), whereas a presynaptic spike after the postsynaptic spike induces LTD (post-pre LTD) at the same synapse (Sjöström & Gerstner, 2010). Due to their temporal nature, these types of plasticity are referred to as timing-dependent long-term potentiation (t-LTP) and depression (t-LTD) (Inglebert & Debanne, 2021).

Mechanistically, STDP can be explained by the calcium control hypothesis (Shouval, 2010). After the postsynaptic neuron fires, a back-propagating action potential (bAP) carries information about the spike into the dendrites and provides the depolarization necessary to relieve the NMDAR Mg^{2+} block (Shouval, 2010). If the bAP is preceded by a presynaptic spike, extracellular glutamate is already bound to NMDARs at the synapse. Therefore, the additional depolarization produced by the bAP leads to a large and rapid Ca^{2+} influx through NMDARs (Nevian & Sakmann, 2004), triggering t-LTP. In contrast, when pre- and postsynaptic activities are less precisely aligned in time, prolonged Ca^{2+} dynamics favor t-LTD rather than potentiation (Shouval, 2010). This can occur when the postsynaptic neuron fires before the presynaptic neuron (post-pre LTD), or when presynaptic activity arrives outside the narrow potentiation window (pre-post LTD) (Dan & Poo, 2006).

Some experimental protocols involving subthreshold postsynaptic depolarization demonstrate that postsynaptic somatic spiking is not required for STDP (Ngezahayo *et al.*, 2000; Sjöström *et al.*, 2004; Sjöström & Gerstner, 2010). If adequate postsynaptic depolarization is present locally at the dendrite, plasticity can be induced in the absence of somatic firing or when bAP is insufficient (Kampa *et al.*, 2007). The depolarization may arise from spatially restricted dendritic spikes, which generate sufficient intracellular Ca^{2+} increases to trigger potentiation at individual synapses (Golding *et al.*, 2002).

STDP mechanisms are thought to mediate information processing and memory storage in hippocampal circuits, including Schaffer collateral synapses (Cepeda-Prado *et al.*, 2021).

Both CA3 and CA1 neurons encode information using temporally structured firing patterns (Middleton & McHugh, 2016), making the precise timing of pre- and postsynaptic spikes critical for determining synaptic change. Although CA1 firing is typically sparse (Ahmed & Mehta, 2009), synaptic plasticity is still robust (Frank *et al.*, 2004), suggesting that mechanisms such as dendritic spikes and local nonlinearities contribute to plasticity induction.

1.3.3. Stimulation shapes synaptic plasticity

The described synaptic plasticity mechanisms are thought to underlie a vast array of cognitive functions, including memory, learning, spatial navigation, adaptability, and habit formation. Impaired synaptic plasticity is implicated in multiple neurological and psychiatric disorders, including epilepsy (Postnikova *et al.*, 2021), schizophrenia (Forsyth & Lewis, 2017), and depression (Appelbaum *et al.*, 2022), making it an active, albeit indirect, target of brain stimulation (Wolf & Abi-Dargham, 2022). Prior works have consistently reported persistent post-stimulation carry-over effects, such as reduced cortical excitability in people with epilepsy (Missey *et al.*, 2026), improvements in memory (Grover *et al.*, 2022), and changes in endogenous brain oscillations (Kragel *et al.*, 2025), suggesting that neurostimulation in humans may induce long-term synaptic modifications.

The stimulation efficacy and outcomes depend on multiple parameters, including stimulation frequency (Froc & Racine, 2005), waveform shape (Gilbert *et al.*, 2023), timing (Huerta & Lisman, 1996; Kragel *et al.*, 2025), duration (Andrews *et al.*, 2013), intensity (Mohan *et al.*, 2020; Zhang *et al.*, 2025), and the targeted brain region (Mohan *et al.*, 2020). Stimulation interacts with ongoing brain activity; consequently, its effects can be highly variable between individuals due to differences in cognitive and behavioral state (McInnes *et al.*, 2025).

Classical electrical stimulation studies demonstrate that low-frequency stimulation (LFS; typically around 1 Hz) induces synaptic depression, while high-frequency stimulation (HFS; 50–100 Hz) promotes potentiation (Froc & Racine, 2005). This frequency-dependence is explained by intracellular calcium dynamics, as described by the calcium control hypothesis (Luscher & Malenka, 2012). Briefly, HFS causes strong temporal summation of EPSPs in the postsynaptic neuron, generating sufficient depolarisation to relieve the Mg^{2+} block of NMDARs and trigger a large persistent Ca^{2+} influx associated with LTP. LFS, on the other hand, produces smaller postsynaptic depolarizations that activate NMDARs weakly and repeatedly, leading to weak and prolonged Ca^{2+} elevations that favor LTD (Luscher & Malenka, 2012). Intermediate stimulation frequencies (5–10 Hz), which closely resemble endogenous theta oscillations, produce more variable plasticity outcomes, with studies reporting both potentiating and depressing effects (Sammari *et al.*, 2022).

The direction of stimulation-induced plasticity is further dependent on the previous state of the postsynaptic neuron. The Bienenstock-Cooper-Munro (BCM) theory proposes that the threshold between potentiation and depression dynamically shifts according to the recent past activity of the postsynaptic neuron (Cooper & Bear, 2012). Namely, weakly active neurons increase their sensitivity to synaptic inputs by reducing this threshold, while highly active

neurons become less sensitive and raise it (Cooper & Bear, 2012). As a result, stimulation frequencies that induce potentiation in one neuron may instead induce depression in another.

Stimulation may further influence synaptic outcomes through interactions with STDP mechanisms, namely by producing shifts in spike timing relative to endogenous oscillations (Radman *et al.*, 2007; M. R. Krause *et al.*, 2022; Wischnewski *et al.*, 2024). Hippocampal coding is strongly dependent on the temporal precision of neuronal spikes (Dragoi, 2020); therefore, even slight changes in spike timing may affect synaptic plasticity. Stimulation has also been shown to alter neuronal input-output relationships, modulate the probability of neuronal firing (Lafon *et al.*, 2017), and shape local dendritic dynamics (Huang *et al.*, 2024; Rathour & Kaphzan, 2024). Pharmacological blockade of NMDARs prevents stimulation-induced changes in cortical excitability (Wischnewski *et al.*, 2018), further supporting the notion that stimulation interacts with synaptic plasticity mechanisms. These effects are frequently associated with entrainment of endogenous oscillations; however, persistent plasticity-related aftereffects have also been observed in the absence of sustained neuronal entrainment (Vossen *et al.*, 2015; Vogeti *et al.*, 2022).

In short, external stimulation interacts with ongoing neuronal activity and shapes synaptic plasticity by altering spike timing and local dendritic dynamics. Such mechanisms are particularly relevant for low-intensity subthreshold stimulation approaches, including tTIS.

Synaptic plasticity in the hippocampus emerges through timing-dependent interactions between pre- and postsynaptic neurons and is shaped by postsynaptic depolarization and intracellular Ca^{2+} dynamics. These mechanisms are highly sensitive to external stimulation. Exogenous electric fields interact with past and present neuronal activity to influence synaptic plasticity outcomes. Therefore, low-intensity stimulation techniques, including tTIS, may shape memory processes through subtle modulation of neuronal dynamics and temporal coding.

2. Methods

This study focused on the computational modeling of hippocampal synaptic plasticity under temporal interference stimulation. No live animals or cell lines were involved. All simulations were carried out using the NEURON simulation environment (v8.2.2) (Carnevale & Hines, 2006). Statistical analysis of the simulation data was performed in Python v3.11.6 using custom scripts. The code is available upon request.

2.1. CA1 neuron model

For all simulations, a compartmental model based on a 3D reconstruction of a CA1 pyramidal neuron from the hippocampus of an adult rat was used (cell ID: c62564, available from www.neuromorpho.org) (Ishizuka *et al.*, 1995; Tecuatl *et al.*, 2024). The morphology included a soma, an axon, 61 basal dendrites, 53 apical dendrites, and 42 membrane sections forming the main apical trunk (Figure 4). Both the morphology and the biophysical membrane properties were previously published (ModelDB accession number 87535) (M. Migliore *et al.*, 2005) and have been used in prior computational modeling studies (Cavarretta *et al.*, 2014; Missey *et al.*, 2026).

The model employed standard uniform passive membrane properties with membrane time constant $\tau_m = 28$ ms and axial resistivity $R_a = 150 \Omega \cdot \text{cm}$. Active conductances included Na^+ , DR- and A-type K^+ conductances K_{DR} and K_{A} , and non-specific cation current I_h . The ion channel distribution and kinetics were based on experimental data from CA1 neurons and were empirically validated in prior studies (M. Migliore *et al.*, 2005). Na^+ and K_{DR} channels were uniformly distributed throughout the entire neuron. The distribution of K_{A} and I_h increased linearly with distance from the soma (for further details, see M. Migliore *et al.* (2005)).

All simulations were performed at the temperature of 35 °C, matching the conditions under which the ion channel kinetics were originally tuned (M. Migliore *et al.*, 2005). The resting membrane potential was initialized at -65 mV. Unless specified otherwise, a fixed time step of 25 μs was used.

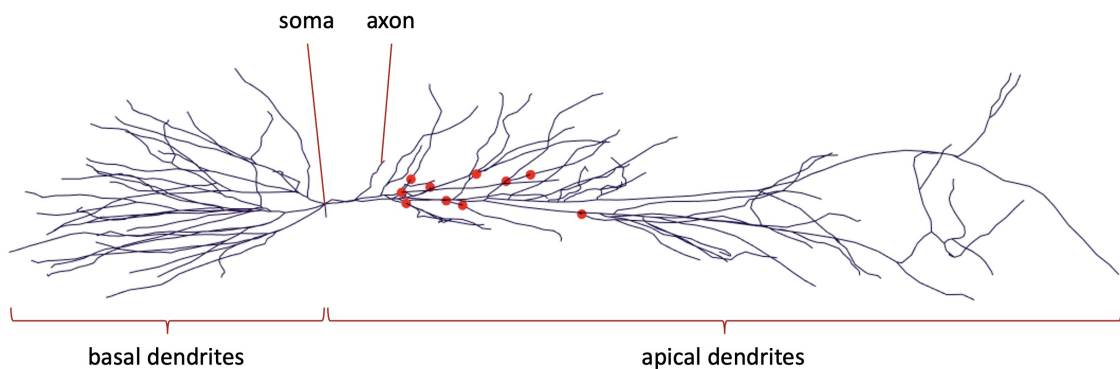


Figure 4. Morphology of the CA1 neuron from the rat hippocampus (cell ID: c62564). The red points indicate 10 randomly selected synapse locations on the proximal apical dendrites, modeling CA3 inputs via Schaffer collaterals.

Modelling synaptic plasticity

This study focuses on synaptic plasticity along the Schaffer collateral pathway. The CA3 inputs to the CA1 model neuron were implemented as 10 excitatory synapses targeting the proximal apical dendrites (100-300 μm from the soma) (Figure 4). Synaptic locations were randomly and independently sampled across 20 synaptic configurations; therefore, a total of 200 synapses were analyzed. The synapses were implemented as previously described by Gasparini *et al.* (2004) and Mazzara & Migliore (2023). Briefly, the synapses comprised an AMPA and an NMDA component. The AMPA component followed a double-exponential conductance profile with rise and decay time constants of 0.5 and 1 ms, respectively, and a reversal potential of 0 mV (Gasparini *et al.*, 2004; Mazzara & Migliore, 2023). The NMDA component was modeled using a kinetic scheme fit to experimental data, with an external Mg^{2+} concentration of 1 mM and a reversal potential of 0 mV (Mazzara & Migliore, 2023).

The evolution of synaptic weights followed a previously published STDP rule (Bianchi *et al.*, 2013; Mazzara & Migliore, 2023), consistent with experimental findings in CA1 pyramidal neurons. Briefly, the peak synaptic conductance was updated in an additive manner and was determined by presynaptic and postsynaptic spike times (t_{pre} and t_{post} , respectively). Presynaptic spike times were defined by input events, while postsynaptic spike times were detected based on a membrane potential threshold crossing at the dendrite; as in Mazzara & Migliore (2023), the plasticity rule was activated when the local dendritic membrane potential crossed the threshold of -20 mV. The synaptic weight dynamics were governed by an internal state variable $A(t)$ representing the degree of potentiation, as described by:

$$g_{\text{peak}}(t + \Delta t) = g_{\text{peak}}^0 + A(t)$$

$$A(t + \Delta t) = A(t) \cdot \left(1 - d \cdot \frac{e^{((t_{\text{pre}} - t_{\text{post}}) - M)^2}}{2V^2} \right) \text{ for } t_{\text{post}} - t_{\text{pre}} < 0, \text{ and}$$

$$A(t + \Delta t) = A(t) \cdot \left(g_{\text{peak}}^{\text{max}} - g_{\text{peak}}^0 - A(t) \right) \cdot p \cdot e^{-\frac{t_{\text{post}} - t_{\text{pre}}}{\tau}} \text{ for } t_{\text{post}} - t_{\text{pre}} > 0$$

where the constants $M = -24$ ms, $V = 6.32$ ms, $\tau = 2$ ms, $d = 0.3$, and $p = 1$ were chosen in accordance with experimental findings and were used without modification from the original studies (Bianchi *et al.*, 2013; Mazzara & Migliore, 2023). The variable g_{peak}^0 indicates the initial peak conductance (i.e., the estimated subthreshold synaptic conductance; see Chapter 2.2) and $g_{\text{peak}}^{\text{max}} = 2 \cdot g_{\text{peak}}^0$ denotes its upper limit. The parameter A describes the degree of potentiation and takes on values between 0 and $g_{\text{peak}}^{\text{max}}$. It was initialised at $A = 0.1 \cdot g_{\text{peak}}^{\text{max}}$ for all simulations (initial weight $w_0 = 0.1$). The reported synaptic weights correspond to the normalized degree of potentiation, i.e.,

$$w(t) = \frac{A(t)}{g_{\text{peak}}^{\text{max}}}$$

Modeling temporal interference stimulation

The tTIS was modeled as a spatially uniform, time-varying extracellular electric field composed of two high-frequency components:

$$E(t) = E_0 \cdot \sin(2\pi \cdot t \cdot f) + E_0 \cdot \sin(2\pi \cdot t \cdot (f + \Delta f) + \Phi)$$

where E_0 is the amplitude of the electric field, f is the carrier frequency, and Δf is the beat frequency. The parameter Φ marks the phase difference between the two TI waveforms and was fixed at $\Phi = 10$ radians in all simulations.

The external voltage applied to each compartment, $V_i^{\text{ext}}(t)$, was set to be proportional to its distance d_i to the (zero) iso-potential plane intersecting the soma (Figure 5):

$$V_i^{\text{ext}}(t) = -E(t) \cdot d_i$$

Unless specified otherwise, the iso-potential plane was oriented perpendicular to the somato-dendritic axis, resulting in an electric field aligned parallel to the somato-dendritic axis.

2.2. Model calibration

Prior to simulations of synaptic plasticity under tTIS, the model was configured to operate under biologically realistic conditions. This included selecting a stimulation amplitude at which stimulation alone did not elicit action potentials and adjusting synaptic conductances such that minimal synaptic input did not evoke neuronal firing.

Maximum subthreshold tTIS amplitude

Maximum subthreshold tTIS amplitude (further: subthreshold amplitude) was defined as the maximum tTIS electric field amplitude that did not elicit action potential firing in the absence of synaptic input (Figure 6). When estimating this parameter, synaptic inputs were omitted, and no synapses were included in the model. The subthreshold amplitude was estimated for iso-potential plane orientations parallel and perpendicular to the somato-dendritic axis, carrier frequencies of 1000 Hz, 2000 Hz, 5000 Hz, and 9000 Hz, and beat frequencies ranging from 1 to 130 Hz. For the 5000 Hz and 9000 Hz carrier frequencies, simulations were performed with a fixed time step of $2.5 \mu\text{s}$ to ensure sufficient temporal resolution.

Subthreshold synaptic conductance

The optimal synaptic conductance was independently estimated for each synaptic configuration using a long-term potentiation (LTP) induction protocol (Figure 6). Specifically, a standard LTP induction protocol was applied by delivering 100 Hz stimulation to all 10 synapses

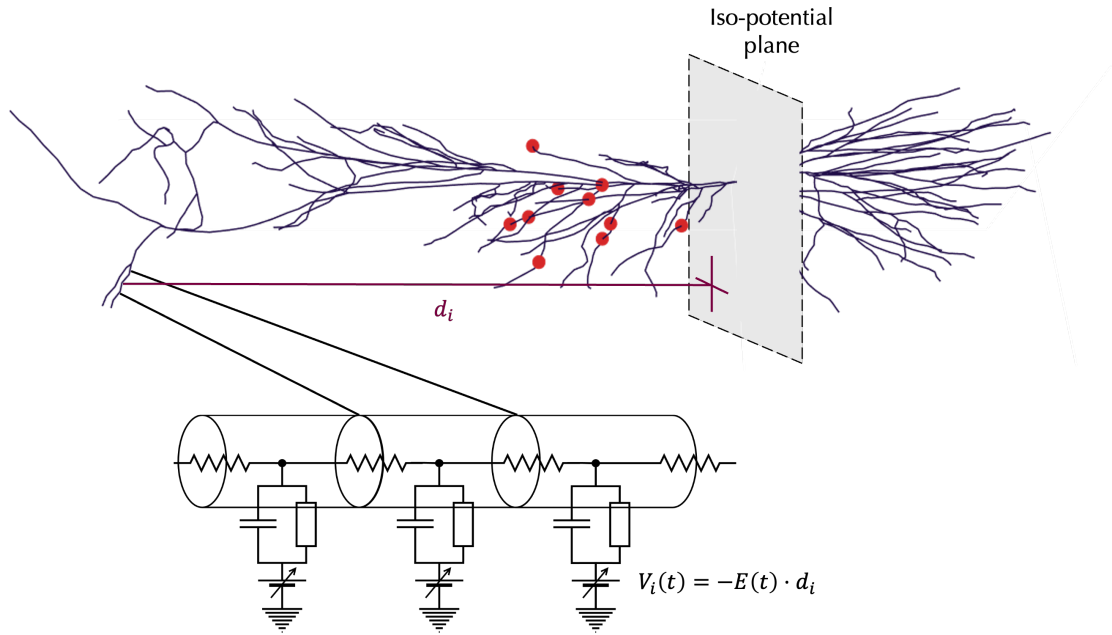


Figure 5. Compartmental CA1 neuron model and its interaction with the applied electric field. The neuron morphology is discretized into compartments, each represented by an equivalent electrical circuit. In each compartment, a spatially uniform electric field induces an extracellular potential $V_i^{\text{ext}}(t)$ proportional to its signed distance d_i from the iso-potential plane intersecting the soma. Synaptic inputs (red) are located on the proximal apical dendrites.

for 1 second. The complete simulation lasted for 3 seconds. A single test stimulus was delivered before (at $t = 0.1$ s) and after (at $t = 2.5$ s) the induction to assess excitability. The subthreshold synaptic conductance was defined as the highest synaptic conductance that did not result in somatic action potentials in response to the test stimuli (pre- and post-induction), but was sufficient to evoke local dendritic spiking during the high-frequency stimulation. The estimated subthreshold synaptic conductance was used in all subsequent simulations.

2.3. Modeling synaptic plasticity under tTIS

An overview of the simulation workflow is illustrated in Figure 6. The effects of tTIS on synaptic plasticity were explored under subthreshold stimulation amplitudes and synaptic conductances across different input regimes and stimulation intensities. In this study, we focused on 5 Hz tTIS using a 1000 Hz carrier. The 1000 Hz carrier was chosen because the neuron model exhibited the greatest sensitivity at this frequency among all tested carriers and because it has been investigated in previous studies (Grossman *et al.*, 2017; Mirzakhilili *et al.*, 2020; Thiele, Rufener, *et al.*, 2024; Missey *et al.*, 2026). Similarly, the 5 Hz beat frequency was selected due to its relevance in both computational and experimental work (Esmailpour *et al.*, 2021; Missey *et al.*, 2025). The iso-potential field orientation was set parallel to the somato-dendritic axis and remained fixed across simulations. For each set of stimulation parameters, simulations were repeated 20 times using different synaptic configurations. The conductance of all synapses was set to the precomputed, configuration-specific subthreshold value. Stimulation strength was defined as a percentage of the subthreshold amplitude specific to each carrier and beat frequency and varied across simulations (see Results for details). In all simulations, stimulation

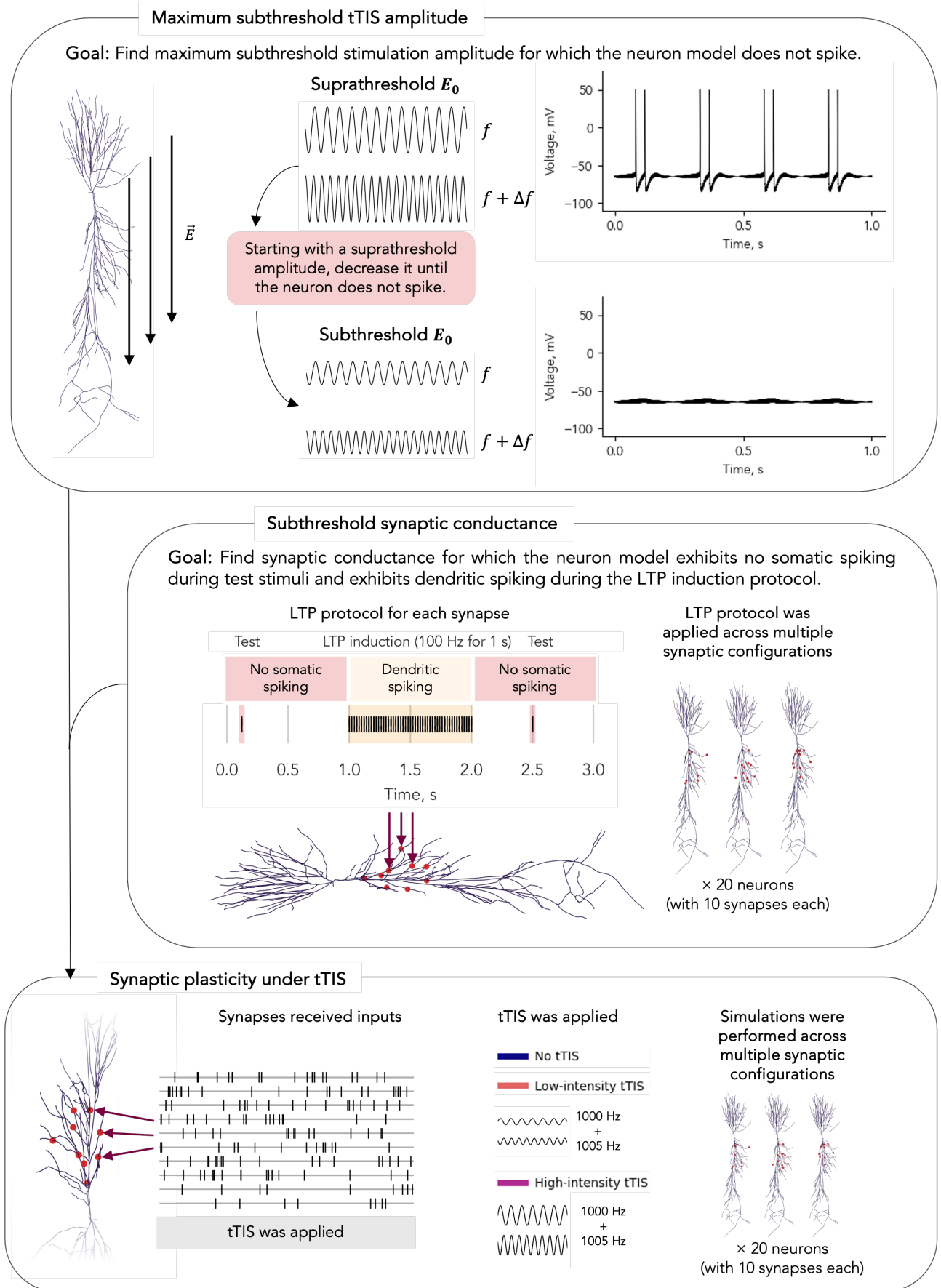


Figure 6. Overview of the simulation workflow. Maximum subthreshold tTIS amplitude and synaptic configuration-specific subthreshold synaptic conductance were determined independently prior to plasticity simulations.

was applied from the start and maintained throughout the simulation period.

To model CA3 input along the Schaffer collateral pathway, we considered two types of synaptic input. The first consisted of independently generated Poisson-distributed spike trains, with each synapse receiving its own input. The Poisson rates ranged from 1 to 25 Hz, and the specific rate used in each simulation is reported in the Results. Each simulation lasted 10 seconds.

The second input regime used *in vivo* recordings of action potentials from 10 CA3 pyramidal cells during open-field exploration, published in a previous study (R. Migliore *et al.*, 2016). Each spike train was randomly assigned to one of the 10 synapses. The recordings were approximately 10 minutes long and were used without modification.

2.4. Statistical analysis

All statistical analyses of the simulation data were performed in Python v3.11.6. The full list of software packages used in this thesis is provided in Appendix A.

The data were not assumed to follow a normal distribution, therefore, the following non-parametric statistical tests were used throughout the study to compare the significance between no stimulation and stimulation conditions:

1. **Chi-square test** was applied to assess differences in the fractions of potentiated, depressed, and stable synapses.
2. **Wilcoxon signed-rank test** was applied to evaluate the significance of synaptic weight changes using paired measurements across synaptic configurations.
3. **Mann-Whitney U test** was applied to compare firing rates across synaptic configurations. Although the measurements were paired across configurations, the Mann-Whitney U test was selected because a large number of zero-valued observations and ties prevented reliable application of the Wilcoxon signed-rank test.

3. Results

In this work, we investigate how tTIS influences synaptic plasticity using a computational model of a CA1 pyramidal neuron. We first determined the subthreshold stimulation amplitude and optimal synaptic conductance specific to the model neuron. We then characterized the effects of tTIS under artificial Poisson-distributed synaptic input, focusing on neuronal activity and synaptic plasticity across different stimulation intensities and synaptic input frequencies. Finally, we assessed whether these findings generalized to more naturalistic synaptic activity patterns using *in vivo* recordings from CA3 neurons as synaptic input to the model.

3.1. Subthreshold tTIS amplitude depends on field orientation, carrier frequency, and beat frequency

As a first step, we estimated subthreshold tTIS amplitude, defined as the maximum stimulation intensity that did not generate somatic spikes. Specifically, we evaluated subthreshold intensities across a range of tTIS carriers (1000, 2000, 5000, and 9000 Hz) and beat frequencies (1–130 Hz) used in prior studies (Missey *et al.*, 2026).

We evaluated subthreshold tTIS strength for two electric field orientations: fields aligned parallel and perpendicular to the somato-dendritic axis (Figure 7A). Across all tested carrier and beat frequencies, perpendicular fields required higher amplitudes to elicit action potentials (Figure 7A), indicating that the model neuron was more sensitive to stimulation when the fields were oriented parallel to its somato-dendritic axis. Higher carrier frequencies required higher stimulation amplitudes to generate spiking (Figure 7A). Subthreshold amplitude also varied with beat frequency, although this effect was less pronounced than the differences observed across carriers (Figure 7A).

To examine neuronal responses near the subthreshold stimulation amplitude, we applied 4 Hz tTIS with a 1000 Hz carrier and the parallel field orientation, using three stimulation strengths: 90%, 100%, and 110% of the subthreshold amplitude (Figure 7B). Stimulation at or below subthreshold amplitude intensity induced membrane potential fluctuations at the beat frequency. At higher intensity, stimulation evoked rhythmic spiking that aligned with the peaks of the amplitude-modulated envelope.

In the remainder of this study, we focused on analyzing the effects of 5 Hz tTIS with a 1000 Hz carrier. We restricted the analysis to the parallel field orientation because it allowed for greater sensitivity to stimulation. Under this orientation, 5 Hz tTIS with a 1000 Hz carrier evoked spiking in the model neuron at an intensity of 148.8 V/m. We later specify the stimulation strength as a percentage of this subthreshold amplitude. The intensity values corresponding to these percentages are listed in Supplementary Table B1. Estimated intensities for other electric field orientations, beat and carrier frequencies are provided in Supplementary Tables B2 and B3.

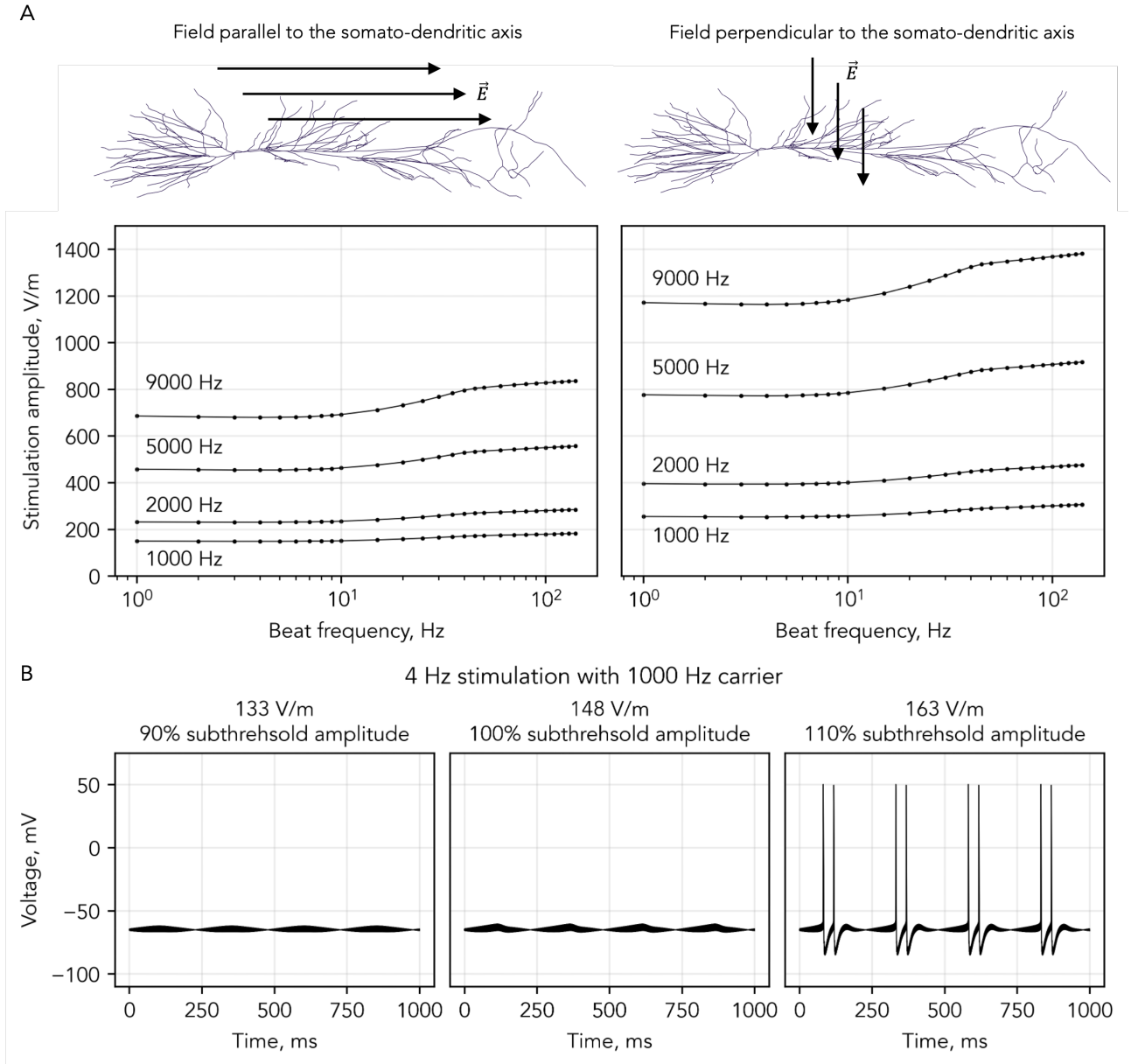


Figure 7. Subthreshold amplitude depends on field orientation, carrier, and beat frequency. (A) Estimated subthreshold tTIS amplitude for 1000, 2000, 5000, and 9000 Hz carrier frequencies as a function of beat frequency, for electric fields oriented either parallel or perpendicular to the somato-dendritic axis. (B) Example somatic voltage traces for 4 Hz TI stimulation with a 1000 Hz carrier with increasing stimulation strengths at 90%, 100%, and 110% of the subthreshold amplitude.

3.2. Estimating subthreshold conductance

Another important step in tuning the neuron model was selecting an appropriate synaptic conductance. This parameter strongly influenced the behavior of the model: if the conductance was too high, the neuron could spike with minimal input; if too low, stimulation could fail to elicit dendritic spiking, preventing synaptic potentiation. We found a narrow conductance regime in which plasticity occurred without persistent somatic spiking.

To estimate the subthreshold synaptic conductance, we applied an LTP induction protocol by delivering 100 Hz stimulation for 1 s (Figure 8A). Test stimuli were applied before and after induction. The test stimuli consisted of a single synaptic input delivered simultaneously to all

synapses. We defined the subthreshold conductance as the highest conductance that did not elicit action potentials in response to a test stimulus, either before or after induction, but was sufficient to evoke dendritic spikes during the LTP protocol. Consistent with previous work (Golding *et al.*, 2002), LTP could be induced without somatic spiking. After the induction protocol, the somatic EPSP amplitude increased in response to the same test stimulus, indicating successful potentiation under subthreshold conditions (Figure 8A).

We further analyzed the behavior of the model under different conductance values, specifically 90%, 100%, and 110% of the estimated subthreshold conductance value (Figure 8B). The neuron was highly sensitive to this parameter. At 90%, no somatic spikes were observed, and synaptic weights remained unchanged, indicating a lack of dendritic spiking activity. At 110%, the neuron exhibited somatic spiking during the LTP protocol, and synaptic weights increased. However, by our definition, this conductance value was too high, as the neuron continued to spike in response to the test stimulus after LTP induction. At the estimated optimal conductance (100%), the neuron also exhibited spiking during the LTP protocol, along with synaptic weight changes. As required by our definition, no somatic spikes were present either before or after the induction protocol.

Subthreshold synaptic conductance was estimated independently for each configuration of synaptic locations by applying the described LTP induction method (Figure 8C). The average somatic EPSP increased after the induction, indicating successful potentiation across configurations. The resulting configuration-specific conductance values were used in all subsequent analyses.

3.3. tTIS effects on synaptic plasticity under 5 Hz Poisson synaptic input

We explored the effects of 5 Hz tTIS with a 1000 Hz carrier on our model neuron under artificial Poisson-distributed synaptic inputs with a mean rate of 5 Hz (Figure 9A). To probe subthreshold stimulation effects, we varied the tTIS intensity from 10% to 90% of the subthreshold amplitude. To obtain the baseline for synaptic plasticity under 5 Hz synaptic input, simulations were also performed without external stimulation. The synaptic inputs were identical across conditions.

tTIS effects on somatic firing and synaptic plasticity

We first investigated whether the neuron model showed increased somatic and dendritic spiking under tTIS. For illustration purposes, example dendritic and somatic voltage traces are shown in Figure 9B. Stimulation with intensities higher than 40% and 60% of the subthreshold amplitude was associated with increased dendritic and somatic firing rates, respectively (Mann-Whitney U test, $p < 0.05$) (Figure 9C). At 50% intensity, the neuron model showed significantly increased dendritic but not somatic firing rates. The somatic firing rate increased by approximately 1 Hz on average (No tTIS: 0.18 ± 0.42 Hz; 50% tTIS: 0.93 ± 1.36 Hz; mean $\pm$ SD), accompanied by a similar increase in the dendritic firing rate (No tTIS: 1.17 ± 1.91 Hz;

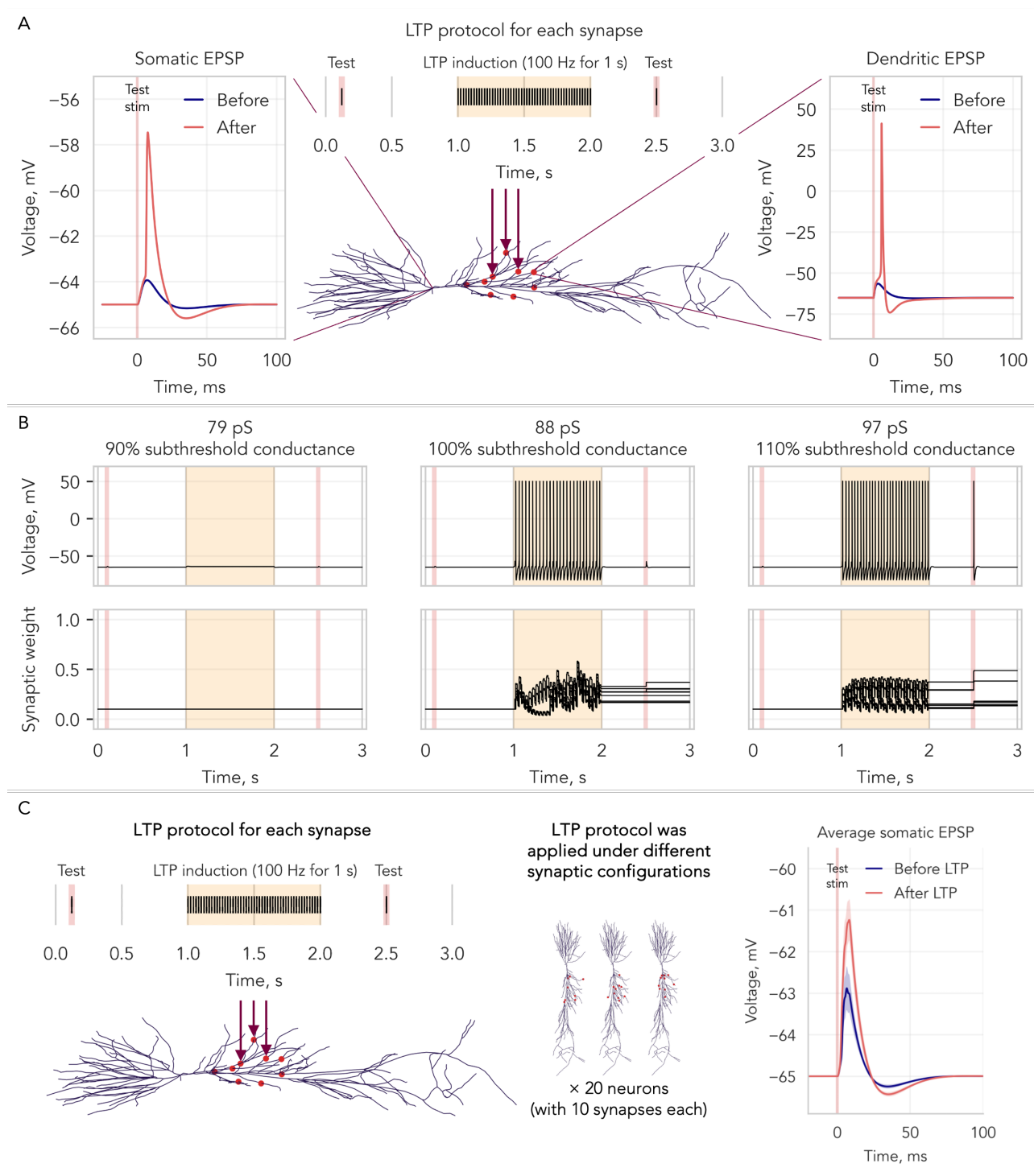


Figure 8. Estimating subthreshold conductance. (A) Subthreshold conductance was estimated using an LTP induction protocol. A standard LTP protocol (100 Hz, 1 s) was applied to each synapse, with test stimuli delivered before and after induction. Both somatic and dendritic EPSPs increased following LTP, confirming effective potentiation under subthreshold conditions. (B) Example somatic voltage traces and corresponding synaptic weight dynamics during LTP induction for synaptic conductances at 90%, 100%, and 110% of the subthreshold value. Subthreshold conductance (100%) produced stable potentiation without eliciting action potentials. (C) The LTP protocol was applied independently across synaptic configurations. Following induction, the average somatic EPSP increased across configurations.

50% tTIS: 2.01 ± 2.51 Hz).

We then validated whether tTIS can induce significant modulation of synaptic plasticity without inducing excessive somatic spiking. For clarity, example synaptic weight dynamics of potentiated, stable, and depressed synapses are shown in Figure 9D. Stable synapses are defined as synapses that did not undergo synaptic weight updates throughout the stimulation period, i.e., their final synaptic weight was equal to the initial weight of 0.1.

Increased dendritic firing rate at 50% intensity was accompanied by modulation of synaptic plasticity, indicated by a decrease in the fraction of stable synapses (chi-square test, $p < 0.01$) (Figure 9E). At lower intensities, synaptic weights remained largely unchanged (Supplementary Figure C1). Therefore, 50% intensity was selected as a representative low-intensity tTIS, as it was the lowest stimulation strength that produced measurable plasticity effects while avoiding strong somatic spiking. Similarly, 90% intensity was selected to represent high-intensity stimulation, as it consistently increased both somatic (3.08 ± 2.67 Hz) and dendritic (4.07 ± 3.46 Hz) firing rates, while also significantly increasing the number of synapses undergoing plasticity.

For completeness, we briefly assessed the effects of the pure carrier at field strengths comparable to the 90% stimulation intensity. We observed no significant effects of the carrier frequency on dendritic or somatic firing, nor any significant modulation of synaptic plasticity (Supplementary Figure D1).

tTIS effects on synaptic dynamics

We further analyzed how tTIS influences the temporal evolution of synaptic weights during ongoing Poisson synaptic input (Figure 10A). For this analysis, we calculated the average synaptic weight change $\Delta w(t)$ at each time point t :

$$\Delta w(t) = \frac{1}{N} \sum_{i=1}^N |w_i(t) - w_0|$$

where $w_i(t)$ is the synaptic weight of synapse i at time t , w_0 is the initial synaptic weight ($w_0 = 0.1$), and N is the total number of analysed synapses.

In both conditions, tTIS increased the average synaptic weight change Δw over time compared to the No tTIS condition (Figure 10B). This effect was intensity-dependent, with high-intensity stimulation producing the largest increase in synaptic weight throughout the simulation. The influence of high-intensity tTIS was particularly pronounced during the early phase of the simulation, where it produced stronger early synaptic weight changes compared to both low-intensity tTIS and No tTIS conditions (Supplementary Table E1, Supplementary Figure E1). The later phase of the dynamics remained relatively similar across conditions.

In addition, stimulation resulted in higher synaptic weight variability (average standard deviation under No tTIS: 0.05; 50% tTIS: 0.07; 90% tTIS: 0.10). Despite this increased variability, synaptic weight dynamics under low-intensity stimulation remained highly correlated with the baseline No tTIS condition (Pearson correlation coefficient between No tTIS and 50%

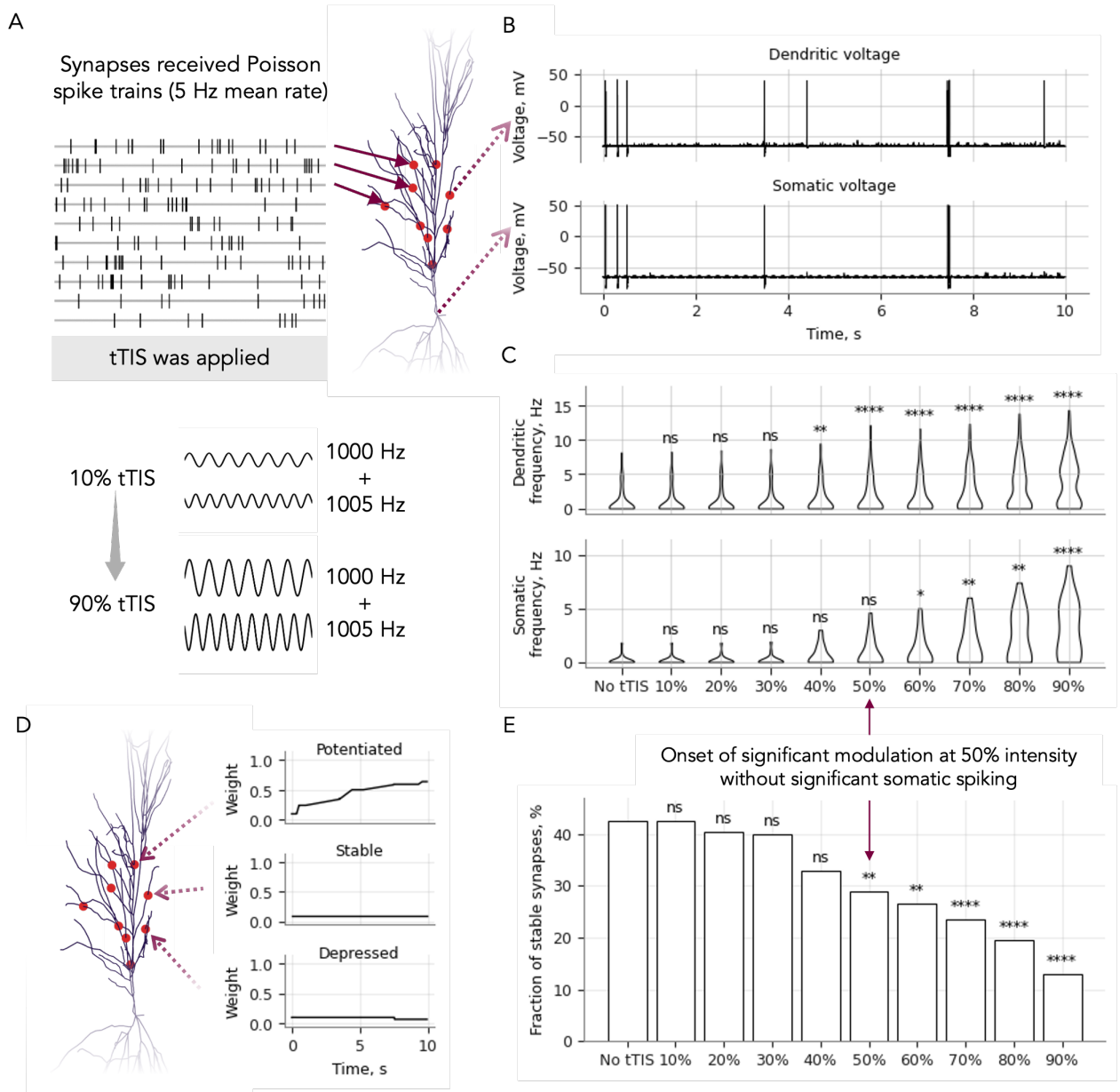


Figure 9. Low-intensity tTIS modulates synaptic plasticity during 5 Hz Poisson input in the absence of strong somatic firing. (A) Experimental protocol. Synapses received Poisson spike trains with a mean firing rate of 5 Hz. Simultaneously, 5 Hz tTIS with a 1000 Hz carrier was applied. Stimulation intensity varied between 10% and 90% of the subthreshold tTIS amplitude. To obtain the baseline for synaptic plasticity under 5 Hz synaptic input, simulations were also performed without external stimulation (No tTIS). Each simulation was performed for 10 seconds. Solid arrows indicate synaptic inputs; dashed arrows indicate the synapse and soma where voltage was recorded. (B) Example voltage traces in the dendrite and soma during tTIS at 50% intensity. (C) Dendritic and somatic firing frequencies, comparing No tTIS and tTIS at different intensities (Mann-Whitney U test). (D) Example synaptic weight traces for potentiated (LTP), stable, and depressed (LTD) synapses under 50% intensity tTIS. (E) Fraction of stable synapses that did not undergo synaptic plasticity by the end of the simulation period, under different stimulation intensities (chi-square test). All significance markers denote comparisons to the No tTIS condition (*not significant*, *ns*: $p \geq 0.05$; *: $p < 0.05$; **: $p < 10^{-2}$; ***: $p < 10^{-3}$; ****: $p < 10^{-4}$).

tTIS: 0.70); high-intensity stimulation introduced deviations from baseline dynamics (Pearson correlation coefficient between No tTIS and 90% tTIS: 0.42).

We then evaluated whether tTIS shifted the distributions of the final synaptic weights w at the end of the simulation period. To reduce the influence of short-term fluctuations, the final synaptic weight was calculated as the average synaptic weight during the last 500 ms of the simulation:

$$w_i = \frac{1}{M} \sum_{t=T-0.5}^T w_i(t)$$

where w_i is the final weight of synapse i , $w_i(t)$ is the synaptic weight of synapse i at time t , T is the total simulation duration in seconds (10 s), and M is the number of sampled time points within the final 500 ms interval.

tTIS shifted synaptic weights toward larger overall deviations from the initial weight, with a more pronounced effect observed at higher stimulation intensities (Figure 10C). The number of strongly potentiated synapses ($w > 0.8$) remained similar between conditions (No tTIS: 26; 50% tTIS: 28; 90% tTIS: 32). Instead, tTIS primarily increased the number of synapses undergoing low- to medium-level potentiation ($0.1 < w < 0.8$). Synaptic weights within this range were slightly higher under stimulation conditions (No tTIS: 0.32 ± 0.19 ; 50% tTIS: 0.35 ± 0.2 ; 90% tTIS: 0.36 ± 0.19 ; mean $\pm$ SD). High-intensity stimulation also increased the number of synapses within this range, with 98 synapses compared to 64 synapses under No tTIS and 72 synapses under 50% tTIS. This pattern suggests that tTIS recruits a larger population of synapses into moderate potentiation states rather than selectively strengthening a small subset of synapses to maximal weights.

We then separately analyzed synaptic weight behavior for synapses undergoing depression and potentiation across all stimulation conditions (Figure 10D and 10E). Based on their final synaptic weights w_i , all synapses were categorized into three classes:

$$\text{Class}(w_i) = \begin{cases} \text{LTD}, & w_i < 0.1 - \varepsilon \\ \text{Stable}, & |w_i - 0.1| \leq \varepsilon \\ \text{LTP}, & w_i > 0.1 + \varepsilon \end{cases}$$

where $\varepsilon = 10^{-4}$ defines the tolerance around the final synaptic weight.

A subset of synapses exhibited consistent plasticity outcomes across all stimulation conditions: 11 synapses underwent LTD, and 83 underwent LTP. These synapses were robustly biased toward depression or potentiation regardless of stimulation. For these synapses, we calculated the average weight over time:

$$w(t) = \frac{1}{N} \sum_{i=1}^N w_i(t)$$

where $w_i(t)$ is the synaptic weight of synapse i at time t , and N is the number of synapses.

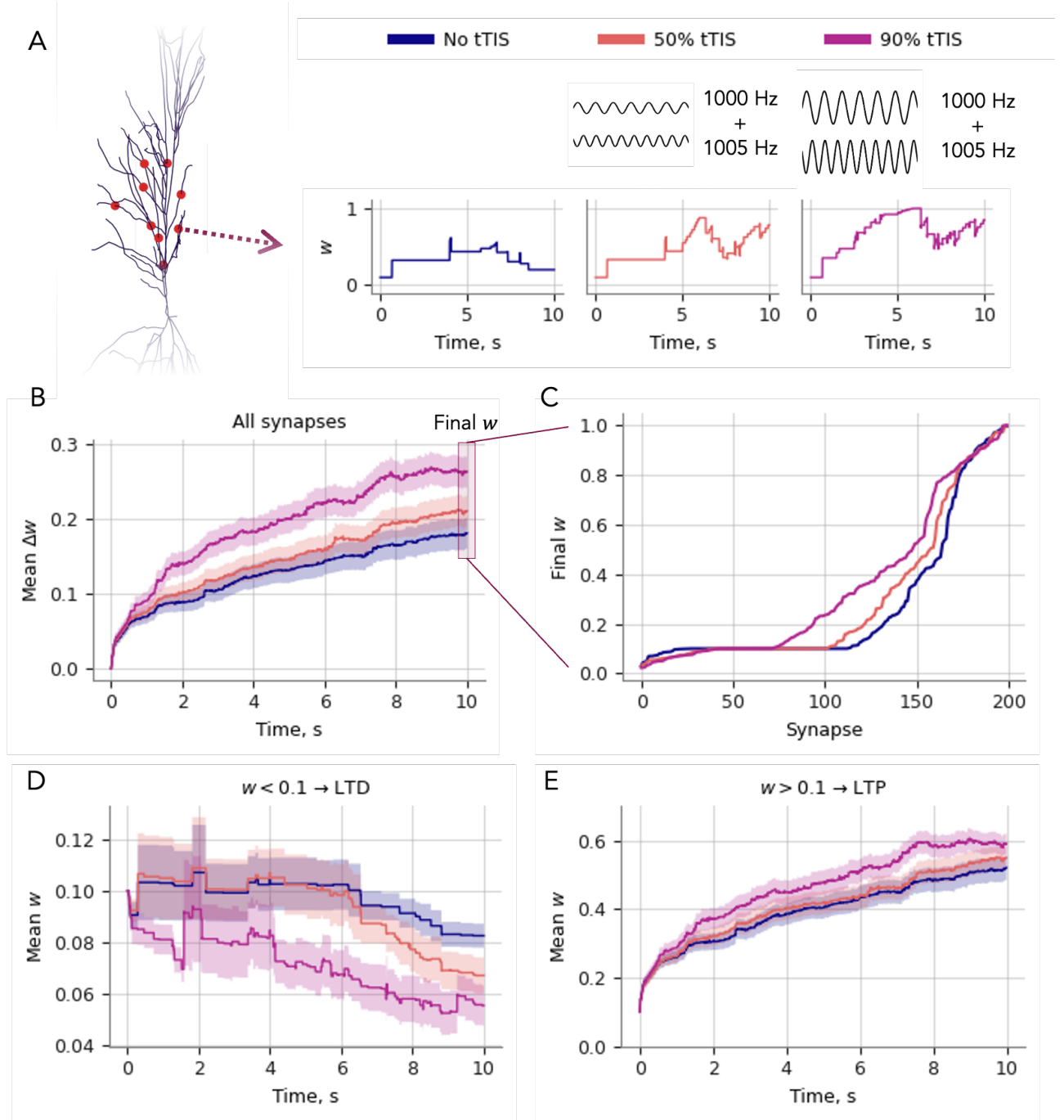


Figure 10. tTIS redistributes synaptic weights under 5 Hz Poisson input. (A) Example synaptic weight trace for a single synapse undergoing LTP. Traces are shown for No tTIS and tTIS at low (50%) and high (90%) intensity. (B) The dynamics of the average change in weight ($\Delta w_t = |w_t - w_0|$ for each time point t , $w_0 = 0.1$) for all synapses throughout the simulation period (10 s). Marked area—final synaptic weights. (C) Final synaptic weight distribution in different stimulation conditions. The synapses were ordered increasingly based on their weight. (D) Average synaptic weight dynamics for synapses that underwent synaptic depression (LTD) under all conditions ($n = 11$ synapses). (E) Average synaptic weight dynamics for synapses that underwent synaptic potentiation (LTP) under all conditions ($n = 83$ synapses).

Among synapses undergoing LTD, high-intensity stimulation produced a stronger progressive decrease in synaptic weights compared to both low-intensity and No tTIS conditions (Figure 10D). In contrast, synapses undergoing LTP exhibited similar temporal dynamics across conditions, although tTIS produced slightly larger weight increases (Figure 10E).

tTIS effects on potentiation and depression

Since only around half of the synapses maintained their plasticity direction across stimulation conditions, we further investigated how frequently synapses switched between LTD, stable, and LTP states under stimulation, as well as how tTIS affected the final synaptic weights of synapses that preserved their plasticity direction (Figure 11).

Sankey diagrams were used to visualize transitions between potentiated, stable, and depressed synapses across No tTIS and tTIS conditions (Figure 11A). Although high-intensity tTIS shifted some LTD synapses into potentiation, overall transitions between LTD and LTP states remained relatively rare under both low- and high-intensity stimulation. Instead, tTIS primarily recruited additional synapses into plasticity without a strong depressive or potentiating bias, as indicated by the transitions from the stable state toward both LTP and LTD.

For synapses that maintained their plasticity direction across conditions, tTIS significantly increased the magnitude of both potentiation and depression (Figure 11B). Synapses undergoing LTD exhibited lower final weights under stimulation, whereas synapses undergoing LTP reached higher final weights compared to the No tTIS condition. These effects were intensity-dependent, with larger weight differences observed under high-intensity stimulation. Nonetheless, even low-intensity tTIS produced significant, coordinated changes in final synaptic weights.

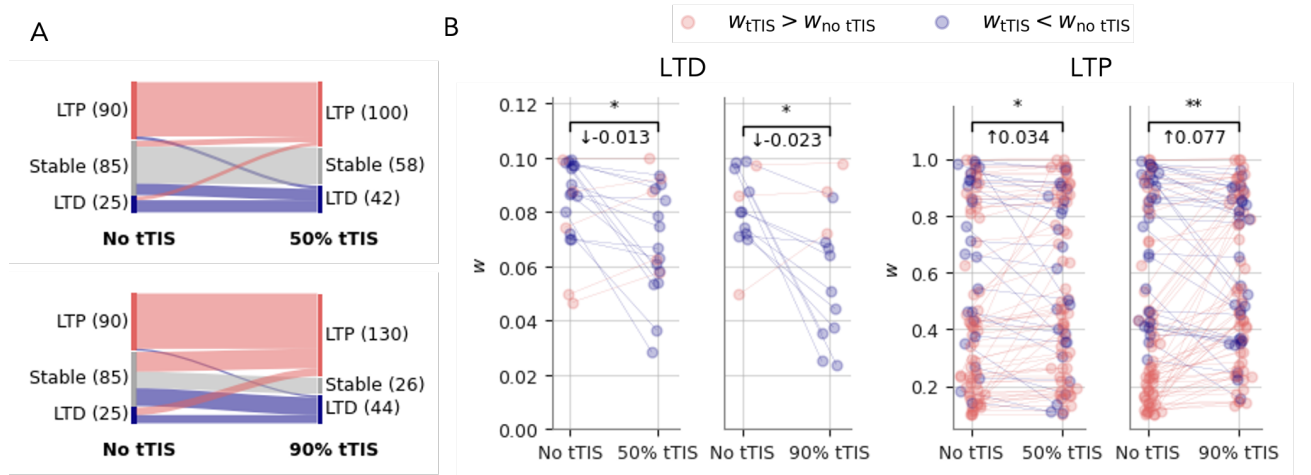


Figure 11. tTIS increases synaptic recruitment and the magnitude of synaptic weight change. (A) Sankey diagrams visualizing the number of potentiated (LTP), stable, and depressed (LTD) synapses and their transitions between No tTIS and tTIS at low (50%) and high (90%) intensity. (B) Differences in LTD and LTP magnitudes between No tTIS and tTIS under different stimulation intensities. Only synapses that were consistently classified as depressed or potentiated across both No tTIS and tTIS conditions are shown. Red and blue points indicate synapses with higher and lower final synaptic weights under tTIS compared to No tTIS, respectively. The numeric values indicate the average final weight difference between No tTIS and tTIS (Wilcoxon signed-rank test; *: $p < 0.05$; **: $p < 10^{-2}$).

Overall, tTIS could modulate synaptic plasticity at stimulation intensities that did not induce excessive somatic spiking and primarily increased dendritic firing frequency. At both low and high intensities, tTIS increased the number of synapses undergoing synaptic weight changes, recruiting additional synapses into both potentiation and depression. Synapses that were already plastic under No tTIS generally maintained their plasticity direction under stimulation while exhibiting larger synaptic weight changes.

3.4. tTIS effects on synaptic plasticity across synaptic input frequencies

As a next step, we varied the average rate of the artificial Poisson-distributed stimuli to explore whether the effects of tTIS depend on the synaptic input frequency (Figure 12A). The mean Poisson rate ranged between 1 and 25 Hz, encompassing physiologically relevant firing regimes of Schaffer collateral inputs to CA1 neurons during exploration and learning (Frerking *et al.*, 2005), as well as higher frequencies mimicking elevated neuronal activity. Synaptic plasticity was explored under low-intensity (50% subthreshold amplitude) and high-intensity (90% subthreshold amplitude) tTIS, as well as without external stimulation used to obtain a synaptic plasticity baseline. 5 Hz tTIS stimulation with a 1000 Hz carrier was applied.

To illustrate how synaptic weight dynamics vary across input regimes, example synaptic weight traces are shown for low (5 Hz), intermediate (10 Hz), and high (15 Hz) input firing rates under the explored stimulation intensities (Figure 12A). At low and intermediate input frequencies, tTIS increased the magnitude of synaptic weight changes compared to the No tTIS condition. In contrast, at high input frequencies, synaptic weight trajectories became more variable, and the effect of tTIS was reduced.

Consistent with these observations, tTIS increased the fraction of synapses undergoing plasticity, particularly at lower and intermediate input frequencies (Figure 12B, Stable). As input frequency increased, most synapses exhibited plastic changes even in the absence of stimulation.

Previously, we observed that tTIS increased the number of both depressed and potentiated synapses under 5 Hz Poisson-distributed input (Figure 11A). For synapses undergoing depression, this effect persisted at input frequencies up to approximately 10 Hz under both low- and high-intensity stimulation (Figure 12B, LTD). At higher input frequencies, the fraction of synapses undergoing LTD ranged from 5 to 15 % and was comparable across No tTIS and tTIS conditions. In contrast, the number of synapses undergoing LTP increased with input frequency and was highest under high-intensity stimulation (Figure 12B, LTP).

To quantify the tTIS effects across the full range of input frequencies f , we computed the average difference in synaptic weight change, $\Delta w_{\text{diff}}(f)$, between the tTIS and No tTIS conditions:

$$\begin{aligned}\Delta w_{\text{diff}}(f) &= \frac{1}{N} \sum_{i=1}^N (\Delta w_{i,\text{tTIS}}(f) - \Delta w_{i,\text{No tTIS}}(f)) \\ &= \frac{1}{N} \sum_{i=1}^N (|w_{i,\text{tTIS}}(f) - w_0| - |w_{i,\text{No tTIS}}(f) - w_0|)\end{aligned}$$

where $w_{i,\text{tTIS}}(f)$ and $w_{i,\text{No tTIS}}(f)$ are the final weights for the synapse i under tTIS and No tTIS conditions, respectively, when the synapse received Poisson-distributed stimuli with the mean firing rate of f Hz (the final weight is computed as described previously); w_0 is the initial synaptic weight ($w_0 = 0.1$), and N is the number of synapses. This measure captures how strongly tTIS modulates overall synaptic plasticity compared to the No tTIS condition. For example, positive values of $\Delta w_{\text{diff}}(f)$ indicate that, on average, tTIS increases the magnitude of synaptic weight changes compared to the no stimulation baseline. However, because $\Delta w_{\text{diff}}(f)$ is computed from absolute deviations from the initial synaptic weight w_0 , it does not capture the directionality of plasticity. For this reason, the metric was also computed separately for synapses undergoing potentiation and depression, including only synapses that maintained the same plasticity direction under both tTIS and No tTIS conditions.

Both high- and low-intensity stimulation increased $\Delta w_{\text{diff}}(f)$ in a frequency-dependent manner, with the strongest effects observed at low and intermediate input frequencies (approximately 1–11 Hz) (Figure 12C, All synapses). Although the effects of low-intensity stimulation were modest, they remained statistically significant, suggesting that even low-intensity tTIS introduced coordinated changes in synaptic plasticity. At higher input frequencies, synaptic weight trajectories became more variable across all conditions (e.g., Figure 12A, 15 Hz), and the differences between tTIS and No tTIS conditions were reduced.

A similar frequency-dependent pattern was observed when LTD and LTP synapses were analyzed separately (Figure 12C). Consistent with the observations under 5 Hz input (Figure 11B), tTIS increased the magnitude of both potentiation and depression. For synapses undergoing LTD, tTIS significantly enhanced synaptic depression only within a narrow range of low input frequencies (5–6 Hz for low-intensity stimulation and 5–8 Hz for high-intensity stimulation) (Figure 12C, LTD). At higher frequencies, the differences between stimulation conditions became highly variable, likely due to the low number of synapses consistently undergoing LTD across both No tTIS and tTIS conditions.

Synapses undergoing LTP exhibited the strongest stimulation-induced weight increases at low input frequencies as well (Figure 12C, LTP). However, the effective frequency range was broader than that of LTD synapses, extending from 1–5 Hz under low-intensity stimulation, and 1–7 Hz under high-intensity stimulation. At higher input frequencies, the differences between the No tTIS and tTIS conditions approached zero, indicating that synaptic inputs alone produced comparable levels of potentiation regardless of stimulation.

To better visualize how tTIS affects the distribution of synaptic weights across different input frequencies, we plotted a heatmap showing the difference between the rank-ordered final synaptic weights under tTIS and No tTIS conditions, with input frequencies along the rows and ordered synaptic weights along the columns (Figure 13). An example of the calculation procedure for 3 Hz synaptic input under high-intensity tTIS is shown in Figure 13A. Mathematically, this can be defined as follows:

$$\Delta w(f, i) = w_{\text{tTIS}}^{(i)}(f) - w_{\text{No tTIS}}^{(i)}(f)$$

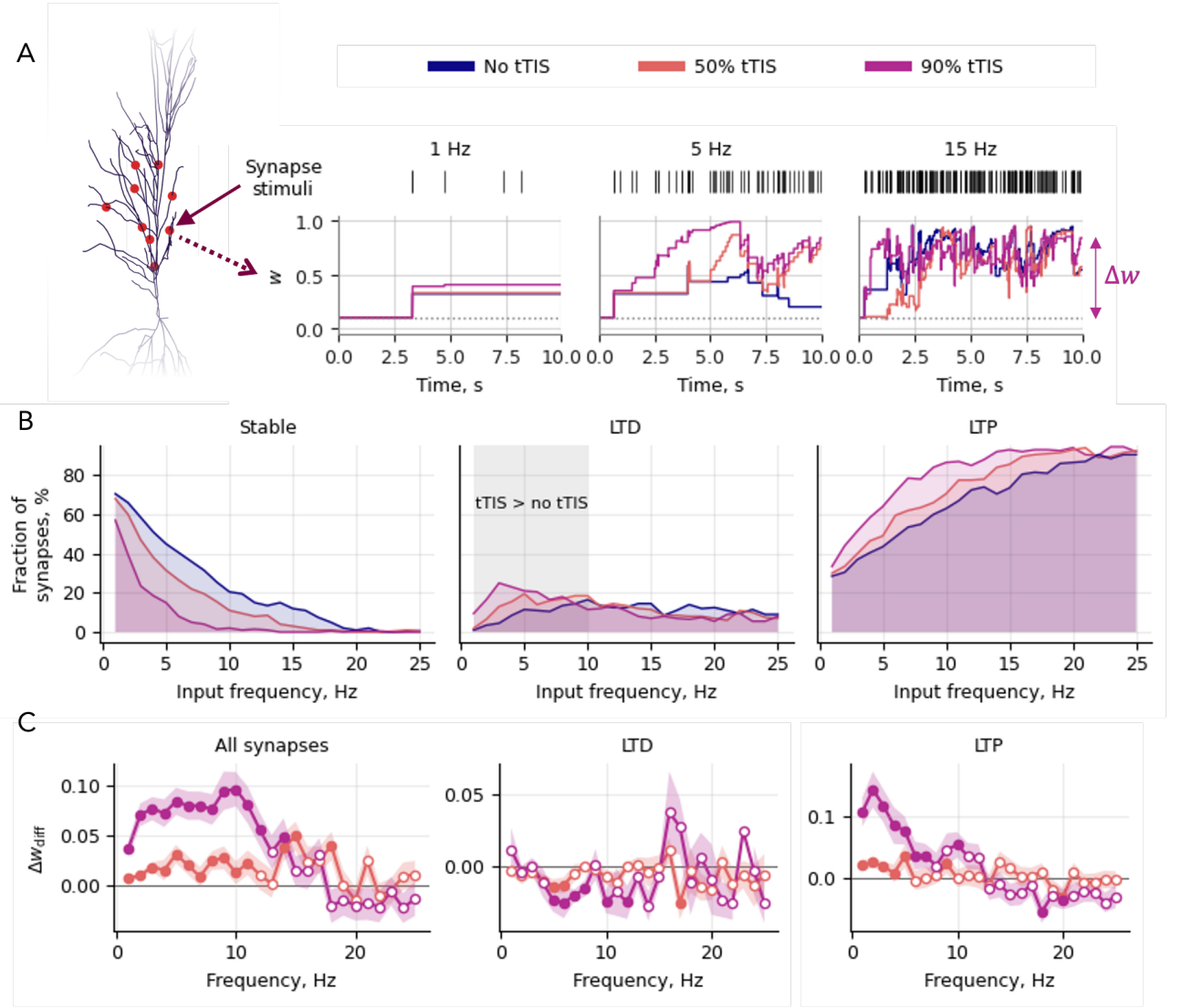


Figure 12. The effects of tTIS depend on synaptic input frequency. (A) Experimental protocol. Synapses received Poisson spike trains with firing rates ranging from 1 to 25 Hz. Simultaneously, 5 Hz tTIS with a 1000 Hz carrier was applied at low (50%) and high (90%) intensity. To obtain the synaptic plasticity baseline under different input frequencies, simulations were also performed without external stimulation (No tTIS). (B) Fraction of stable, depressed (LTD), and potentiated (LTP) synapses across synaptic input frequencies. (C) Average difference in synaptic weight change Δw_{diff} between No tTIS and tTIS conditions for all, depressed, and potentiated synapses. Filled points indicate significant differences between the stimulation and no-stimulation conditions (Wilcoxon signed-rank test, $p < 0.05$).

where $w_{\text{tTIS}}^{(i)}(f)$ and $w_{\text{No tTIS}}^{(i)}(f)$ are the i -th rank-ordered final synaptic weights under tTIS and No tTIS conditions, respectively, for Poisson-distributed input with mean firing rate f . Positive and negative values of $\Delta w(f, i)$ indicate that tTIS shifted synaptic weights toward stronger potentiation or stronger depression, respectively, compared to the No tTIS condition. For each input frequency, synaptic weights were sorted independently according to their final values under each stimulation condition; consequently, synapse positions are not preserved across frequencies. Additional examples for 15 Hz and 20 Hz input frequencies, as well as low-intensity stimulation, are provided in Supplementary Figures F1 and F2.

At low input frequencies, the synaptic weight distributions closely matched those observed under 5 Hz input (Figure 10C, Figure 13B). Specifically, for synaptic input frequencies up to

10 Hz, tTIS consistently increased the number and synaptic weights of moderately potentiated synapses: the largest weight differences were observed among synapses with intermediate final weights rather than the most strongly potentiated synapses (Figure 13B). This effect was more pronounced under high-intensity tTIS. Synapses undergoing depression also exhibited slightly lower final weights under stimulation.

The effects of stimulation diminished at higher synaptic input frequencies, where most synapses underwent plasticity even in the absence of external stimulation. For some of the highest tested input frequencies (>20 Hz), synapses in the upper range of the distribution were less strongly potentiated under tTIS than under No tTIS.

These results suggest that tTIS modulates synaptic plasticity in a frequency- and intensity-dependent manner. At low and intermediate input frequencies, tTIS enhanced both the magnitude of synaptic weight changes and the fraction of synapses undergoing plasticity. At higher input frequencies, synapses were driven into plasticity by input alone, and the effects of stimulation diminished.

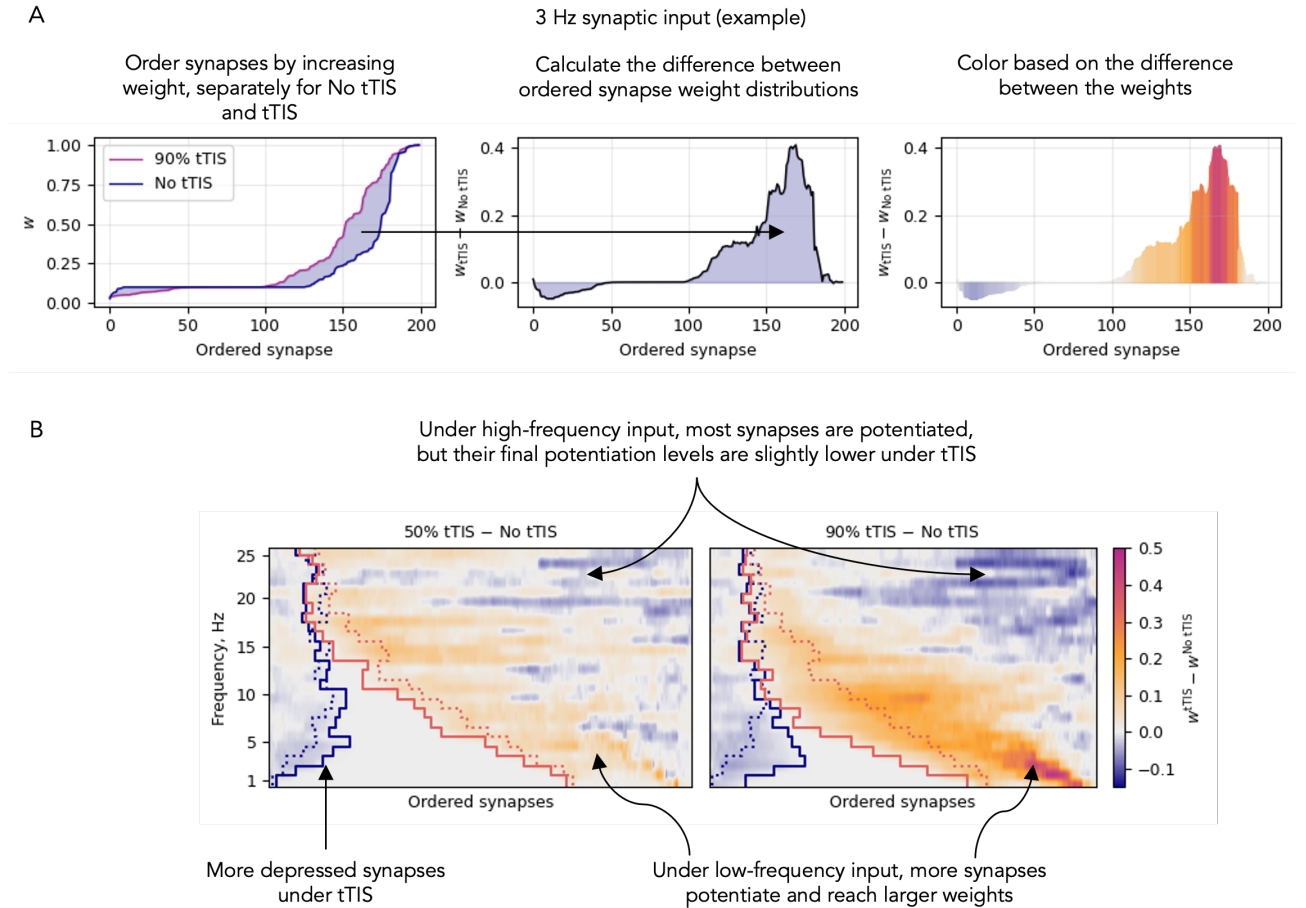


Figure 13. Final synaptic weight distribution differences between tTIS and baseline conditions. (A) Example computation of the difference between rank-ordered final synaptic weights under tTIS and No tTIS conditions for 3 Hz synaptic input under high-intensity tTIS. (B) Heatmap of differences between rank-ordered final synaptic weights under tTIS and No tTIS across input frequencies. Solid lines denote potentiated (red) and depressed (blue) synapses under tTIS; dashed lines denote the same categories under No tTIS.

3.5. tTIS effects on synaptic plasticity under *in vivo* input

Lastly, we validated whether the observed tTIS effects on synaptic plasticity generalize to biologically realistic synaptic inputs. In this protocol, artificial Poisson-distributed synaptic inputs were replaced with spike trains recorded from CA3 pyramidal neurons during open-field exploration *in vivo* (Figure 14A). Unlike Poisson-distributed input, the *in vivo* spike trains contained temporally structured activity patterns and variable firing rates characteristic of hippocampal exploration behavior. Despite these differences, tTIS produced qualitatively similar effects on synaptic plasticity.

Under *in vivo* inputs, both low- and high-intensity stimulation increased the somatic firing frequency throughout the simulation period (Figure 14C). Although significant, these increases were modest (No tTIS: 0 ± 0 ; 50% tTIS: 0.05 ± 0.07 ; 90% tTIS: 0.27 ± 0.25 ; mean $\pm$ SD).

The average synaptic weight dynamics followed patterns similar to those observed under 5 Hz Poisson synaptic input (Figure 10B, Figure 14C). Under *in vivo* input, tTIS increased the magnitude of synaptic weight changes over time, with the strongest effects observed under high-intensity stimulation (Supplementary Table G1). The *in vivo* simulations were substantially longer than the Poisson input simulations (600 s vs 10 s) and the temporal evolution of synaptic weights occurred over extended time scales (Supplementary Table G1, Supplementary Figure G1).

Analysis of synaptic state transitions further demonstrated that stimulation recruited additional synapses into both potentiation and depression while preserving the overall balance between LTD and LTP states (Figure 14D). Compared to No tTIS, high-intensity stimulation increased the number of synapses undergoing plasticity, whereas low-intensity stimulation produced more modest effects.

To assess how tTIS affected the overall excitability of the model neuron, we delivered a single test stimulus simultaneously to all synapses and evaluated whether stimulation altered the probability of evoked neuronal firing. We did not observe any differences in firing responses: the neuronal firing pattern across different synaptic configurations remained identical under No tTIS and both low- and high-intensity tTIS conditions (Supplementary Figure H1).

Overall, these findings suggest that the modulatory effects of tTIS on synaptic plasticity persist under temporally structured *in vivo* hippocampal inputs.

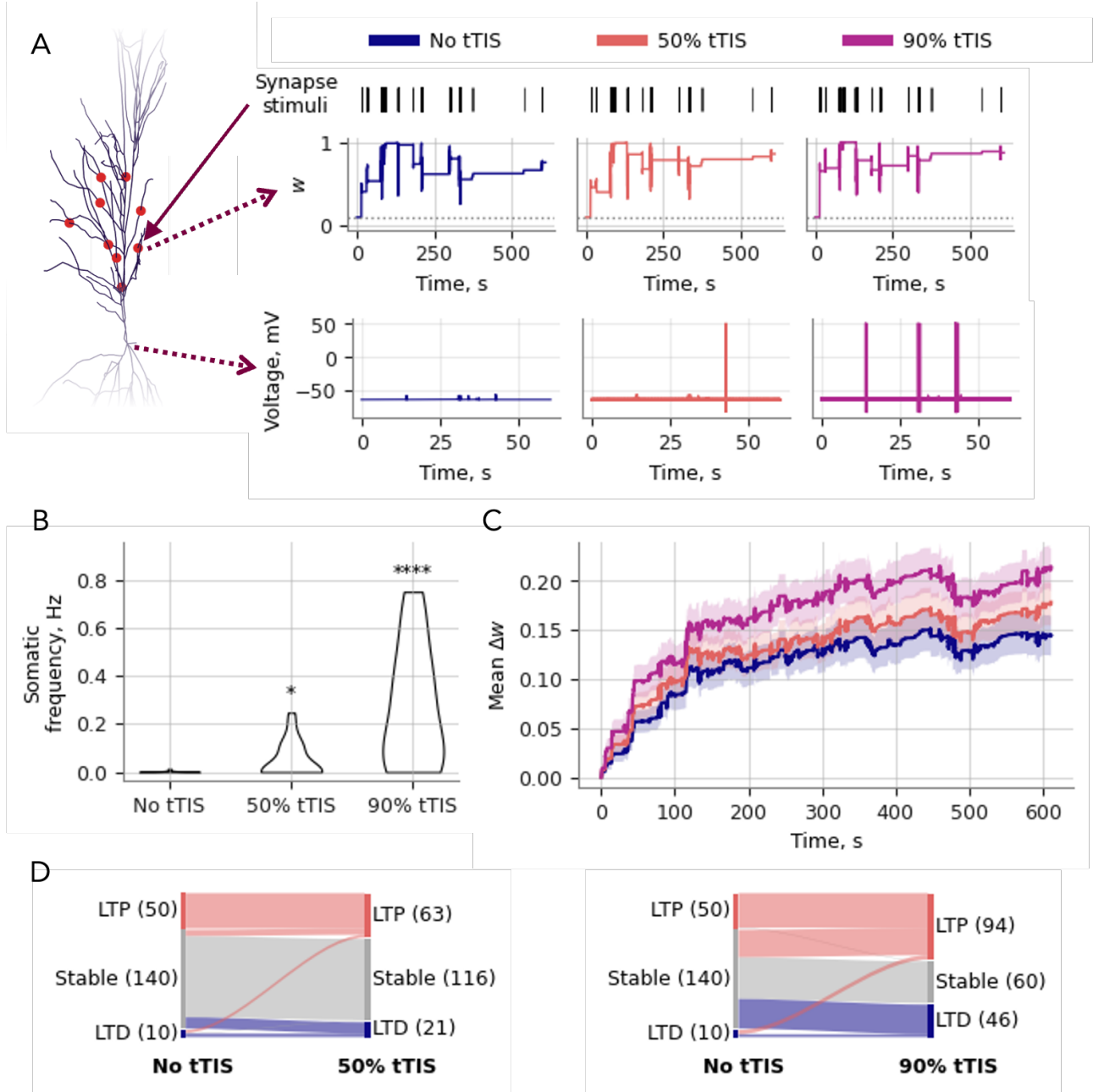


Figure 14. Observed tTIS effects are retained under *in vivo* synaptic inputs. (A) Experimental protocol. Synapses received synaptic inputs from *in vivo* recordings. Simultaneously, 5 Hz tTIS with a 1000 Hz carrier was applied at low (50%) and high (90%) intensity. To obtain the synaptic plasticity baseline under different input frequencies, simulations were also performed without external stimulation (No tTIS). Examples of synaptic inputs, synaptic weight traces, and somatic voltage traces under all stimulation conditions are shown. (B) Somatic firing frequency, comparing No tTIS and tTIS at different intensities (Mann-Whitney U test; *: $p < 0.05$; ****: $p < 10^{-4}$). (C) Average dynamics of synaptic weight change ($\Delta w_t = w_t - w_0$, for each time point t). (D) Sankey diagrams visualizing the number of potentiated (LTP), stable, and depressed (LTD) synapses and their direction switches between No tTIS and tTIS at low (50%) and high (90%) intensity.

4. Discussion

We used a computational model of a CA1 hippocampal neuron to explore how 5 Hz tTIS delivered using a 1000 Hz carrier affects synaptic plasticity at Schaffer collateral synapses under an STDP-based plasticity framework. The model suggests that tTIS could increase dendritic firing, resulting in larger synaptic weight changes and a greater fraction of synapses undergoing plasticity. These effects were more pronounced under low and intermediate synaptic input frequencies and were also observed under *in vivo*-like synaptic inputs. Stimulation increased the number of synapses undergoing both potentiation and depression, did not impose a directional bias, and preserved overall synaptic dynamics. Despite significant changes in synaptic weight distributions, tTIS did not alter neuronal excitability under the resulting synaptic weights. Overall, these findings suggest that 5 Hz tTIS modulates ongoing synaptic plasticity dynamics by increasing the recruitment of synapses into plasticity rather than by inducing potentiation or depression.

4.1. tTIS increases synaptic sensitivity

Our findings suggest that tTIS modulates plasticity primarily by increasing the likelihood that synapses undergo plastic changes, indicated by both more frequent weight updates and a larger fraction of synapses undergoing plasticity. The tTIS waveform induces membrane potential oscillations that bias the membrane potential toward depolarization (Mirzakhali *et al.*, 2020), thus increasing the probability of dendritic spikes under low-intensity input, shifting their timing, and modulating local calcium dynamics. These mechanisms are central to STDP and determine whether synapses cross the thresholds required for potentiation or depression. In this way, small shifts in membrane voltage may lead to increased sensitivity to synaptic inputs and stronger coincidence detection under tTIS.

The strongest effects of tTIS on recruiting synapses into plasticity and altering their weights occurred under low- to medium-frequency synaptic inputs, suggesting that tTIS preferentially strengthens the influence of weak synaptic drive. Under sparse synaptic inputs, spike timing is governed by local dendritic calcium spikes that are highly sensitive to polarizations induced by external electric fields (Huang *et al.*, 2024). As synaptic input and neuronal excitability increase, the primary influence on spike timing shifts to somatic spikes rather than dendritic dynamics (Huang *et al.*, 2024). This suggests that tTIS primarily modulates plasticity through dendritic depolarization, with reduced effects under strong synaptic input, where synaptic activity alone may be sufficient to drive plasticity. Consistent with this, modulation of synaptic plasticity emerged alongside elevated dendritic, but not somatic, firing rates. This aligns with prior work suggesting that tTIS operates in a subthreshold regime and does not strongly alter somatic spiking *in vivo* (Vieira *et al.*, 2024).

The most sensitive indicator of tTIS influence on plasticity was the decreased number of stable synapses. For some synapses, membrane potential oscillations induced by tTIS may have triggered additional spikes that would not otherwise have occurred, recruiting those synapses

into plasticity. This suggests that tTIS increases the likelihood that marginal synapses cross the plasticity thresholds. Prior work reported similar effects under direct current stimulation (DCS) and proposed that stimulation increases the probability of potentiation for weakly activated synapses, thus enhancing Hebbian associativity (Kronberg *et al.*, 2020). As memory traces are thought to emerge from collective synaptic modifications across synaptic populations (Benna & Fusi, 2016), increasing the number of synapses undergoing plasticity may allow weaker inputs to contribute to the memory encoding.

4.2. tTIS does not impose a directional bias

The results of this study suggest that tTIS at 5 Hz promotes both potentiation and depression. This effect was consistently observed across a range of Poisson input frequencies and stimulation intensities, as well as under realistic CA3 input conditions. In other stimulation paradigms, such as direct electrical stimulation, low-frequency (1 Hz) stimulation is typically associated with increased depression, whereas stimulation at higher frequencies (>100 Hz) leads to synaptic strengthening (Froc & Racine, 2005). Within this framework, 5 Hz stimulation lies at the LTP/LTD induction boundary, with studies reporting mixed effects (Sammari *et al.*, 2022).

Instead of imposing a directional LTP/LTD bias, 5 Hz tTIS may reinforce ongoing neural patterns. Our data show that most synapses preserved their plasticity direction under low-intensity tTIS relative to the no-stimulation baseline, and their weights remained highly correlated between these conditions. Additional synapses recruited into plasticity by tTIS also exhibited both potentiation and depression, suggesting that tTIS does not uniformly drive synapses towards LTP or LTD and rather facilitates ongoing Hebbian modifications. When applied during the encoding phase, tTIS modulation could strengthen existing activity-dependent representations while preserving their overall structure. This mechanism may contribute to experimentally observed improvements in memory and behavioral performance (Miskey *et al.*, 2025; Ma *et al.*, 2022).

Increases in both potentiated and depressed synapses may contribute to preserving excitation/inhibition (E/I) balance. The balance between neuronal excitation and inhibition maintains neuronal activity within a functional range, preventing both neuronal silencing and excessive excitation associated with pathological states such as epilepsy (Landau *et al.*, 2016). Prior works have suggested that other non-invasive stimulation techniques modulate E/I balance (B. Krause *et al.*, 2013; Estévez-Rodríguez *et al.*, 2025). In our model, tTIS did not alter neuronal firing in response to a test stimulus, suggesting that stimulation did not induce excessive excitation. Under high-frequency synaptic input, we also observed a slight dampening of strongly potentiated synaptic weights, which may indicate that tTIS can modulate synaptic dynamics differently under highly active network states (Miskey *et al.*, 2026). Inhibitory circuitry was not explicitly modeled in this study; therefore, further work is required to fully evaluate the effects of tTIS on E/I balance.

The absence of a directional LTP/LTD bias may also reflect properties of the tTIS wave-

form itself. Although the targeted beat frequency is 5 Hz, the tTIS waveform consists only of high-frequency components, making it difficult to attribute the observed effects purely to the low-frequency modulation. Our model predicts a slight, non-significant decrease in stable synapses under pure carrier stimulation, suggesting that high-frequency carriers may contribute to the observed plasticity effects. Similar concerns regarding carrier influences have been raised in previous studies (Mirzakhali *et al.*, 2020; Esmaeilpour *et al.*, 2021). A more detailed analysis would help disentangle the relative contributions of low-frequency modulation and high-frequency carriers, particularly at the level of individual synapses.

4.3. tTIS efficacy depends on stimulation intensity

Our model predicted that the stimulation intensity required to elicit action potentials in the absence of synaptic input increases with carrier frequency, consistent with prior *in vivo* (Grossman *et al.*, 2017) and computational findings (Missey *et al.*, 2026). Currently, higher carrier frequencies (up to 20 kHz) are being explored for human applications, as they allow for higher stimulation intensities while increasing perception and pain thresholds (Thiele, Tamm, *et al.*, 2024; Y. Wang *et al.*, 2024). However, both our results and prior work suggest that these carrier frequencies would require substantially greater stimulation intensities to achieve comparable modulation. This highlights the importance of validating whether low-intensity tTIS paradigms can induce significant changes in synaptic plasticity.

In our model, significant shifts in synaptic plasticity emerged at approximately 50% of the subthreshold intensity, corresponding to an electric field strength of 74 V/m at the on-target regions. This value is substantially higher than electric field strengths estimated in the human cortex under tTIS, which are typically 0.2–1 V/m when stimulation is applied at 2 mA amplitude at the scalp electrodes (S. Lee *et al.*, 2020; Ness *et al.*, 2025). Notably, similar field strengths are required for modulating hippocampal gamma oscillations (Esmaeilpour *et al.*, 2021), suggesting that relatively high tTIS intensities may be necessary to influence neuronal dynamics. Nonetheless, multiple studies in humans have reported improvements in memory performance under low-intensity tTIS (Violante *et al.*, 2023; Missey *et al.*, 2026). This may reflect the contribution of additional mechanisms, such as network-level dynamics not captured by the single-neuron model (Esmaeilpour *et al.*, 2021; Caldas-Martinez *et al.*, 2024).

4.4. Limitations and future directions

The model used in this study captured several key aspects of hippocampal synaptic plasticity, including spike-timing dependence, dendritic voltage dynamics, nonlinear synaptic integration, and interactions between neuronal membrane dynamics and externally applied electric fields. However, several methodological limitations and simplifying assumptions should be considered when interpreting the findings.

The chosen plasticity model used a voltage-based rule rather than an explicit calcium-dependent mechanism; therefore, the model may not have fully captured the nonlinear calcium

dynamics that govern synaptic plasticity and are modulated by subthreshold electric fields. The observed threshold-like emergence of plasticity effects may be influenced by the choice of a fixed dendritic voltage threshold (-20 mV) used to trigger synaptic updates. Additionally, all synapses were assigned identical conductances regardless of their dendritic location. While this simplification reduced the number of free parameters, it neglects location-dependent scaling observed in biological neurons. This may have influenced local depolarization profiles and the likelihood of plasticity induction at individual synapses. Further, the choice of the initial synaptic weight ($w = 0.1$) may have influenced the observed balance between potentiation and depression, as synapses starting near the lower bound have limited capacity for further depression while retaining a broader range for potentiation.

Future work should investigate how these effects emerge at the network level. The use of a single-neuron model limited the ability to capture inhibitory circuitry, oscillatory synchronization, and interactions between multiple neurons that may contribute to tTIS effects in biological systems. Extending the model to hippocampal microcircuits containing both excitatory and inhibitory neurons could help determine how tTIS influences network dynamics, excitation/inhibition balance, and activity-dependent plasticity under more physiologically realistic conditions. Additionally, future studies incorporating explicit calcium-dependent plasticity mechanisms may provide further insight into how weak electric fields interact with intracellular signaling pathways that govern synaptic plasticity. Lastly, the current study was limited to computational modeling and all presented findings remain to be validated in biological systems.

Despite these limitations, our results suggest that tTIS could play a role in shaping synaptic plasticity dynamics in hippocampal neurons. We argue that these interactions arise primarily through modulation of local dendritic mechanisms, leading to a larger number of synapses recruited into plasticity and stronger synaptic weight changes. Our model did not predict a clear potentiating or depressing bias for 5 Hz tTIS, suggesting that stimulation reinforces ongoing synaptic dynamics rather than introducing new patterns. Overall, this study provides a framework for investigating synaptic plasticity mechanisms under low-intensity stimulation and offers a potential mechanistic explanation for the cognitive and long-lasting effects observed following tTIS.

Conclusions

1. A computational model of a CA1 hippocampal pyramidal neuron implementing STDP-based synaptic plasticity and tTIS was developed. The model was tuned to support both low- and high-intensity stimulation regimes.
2. Under artificial Poisson-distributed synaptic inputs, tTIS modulated synaptic plasticity by increasing the number of synapses undergoing synaptic weight changes. These effects emerged alongside elevated dendritic firing rates and were observed without strong increases in somatic spiking under low-intensity stimulation. tTIS enhanced both potentiation and depression and did not introduce a strong directional LTP/LTD bias.
3. The strongest tTIS modulation was observed under low- and intermediate-frequency synaptic inputs. The stimulation effects diminished at higher input frequencies where synaptic input alone was sufficient to drive plasticity.
4. The modulatory effects of tTIS generalized to biologically realistic synaptic input patterns. Under *in vivo* CA3 spike train inputs, tTIS similarly increased the number of synapses undergoing plasticity while preserving the overall balance between potentiation and depression.

Author's personal contribution

The research presented in this Master's thesis was conducted by me under the supervision of Prof. Aušra Saudargienė (Lithuanian University of Health Sciences, Kaunas, Lithuania) and in scientific collaboration with Prof. Michele Migliore (Institute of Biophysics, National Research Council, Palermo, Italy), Dr. Rosanna Migliore (Institute of Biophysics, National Research Council, Palermo, Italy), and Prof. Adam Williamson (St. Anne's University Hospital, Brno, Czech Republic). I performed the literature review, implemented the computational framework used in this thesis, designed the simulation workflow, carried out all simulations and data analyses, interpreted the results, generated the figures, and wrote the thesis manuscript. OpenAI tools were used to assist with grammar refinement and improvement of text flow.

Michele Migliore and Rosanna Migliore provided guidance on computational modeling methodology and model parameter tuning. Adam Williamson contributed to the interpretation of the results and development of the analysis strategy. Scientific supervision and methodological feedback throughout the project were provided by Aušra Saudargienė. The simulations were performed using computational resources provided by the Lithuanian University of Health Sciences.

During the preparation of this thesis, I completed a two-month research visit at the Institute of Biophysics, National Research Council in Palermo, Italy, and a one-week research visit at the Institute for Systems Neurosciences, Aix-Marseille University in Marseille, France. I also participated in and presented parts of this work at the following international conferences:

1. Kerševičiūtė I., Migliore M., Migliore R., Saudargienė A., Williamson A. *Subthreshold extracellular electric fields alter how neurons respond to naturally occurring synaptic inputs in temporal interference stimulation*. CNS 2025, Florence, Italy, July 5–9, 2025 (poster presentation).
2. Kerševičiūtė I., Migliore M., Migliore R., Saudargienė A., Williamson A. *Temporal interference stimulation alters long-term hippocampal synaptic plasticity*. Bernstein Conference 2025, Frankfurt, Germany, September 29–October 2, 2025 (poster presentation).

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VILNIAUS UNIVERSITETAS
GYVYBĖS MOKSLŲ CENTRAS

Ieva Kerševičiūtė

Magistro baigiamasis darbas

**Laikine interferencija paremtos stimuliacijos poveikis ilgalaikiam
sinaptiniam plastiškumui CA1 hipokampo neuronuose**

SANTRAUKA

Transkranijinė laikine interferencija paremta stimuliacija (angl. *transcranial temporal interference stimulation*, tTIS)—tai naujas neinvazinis smegenų stimuliacijos metodas, paremtas aukšto dažnio elektrinių laukų laukų interferencija ir taip leidžiantis pasiekti giliausias smegenų struktūras. Hipokampo tTIS stimuliacija pagerina atmintį ir turi išliekantį ilgalaikį poveikį, todėl spėjama, kad ši stimuliacija gali paveikti ilgalaikio sinapsinio plastiškumo procesus. Vis dėlto tikslus šių sąveikų pobūdis yra mažai ištirtas, taigi šiame darbe siekiama nustatyti, ar mažo ir didelio intensyvumo 5 Hz tTIS turi įtaką ilgalaikio sinapsinio plastiškumo procesams hipokampe. Tyrimas buvo atliktas naudojant kompiuterinį biofiziškai realistišką CA1 hipokampo piramidinio neurono modelį su skirtingais Šaferio kolateralių nervinių impulsų tipais: įvairaus dažnio dirbtinėmis sekomis, sugeneruotomis pagal Puasono skirstinį, arba biologiškai realistiškomis *in vivo* įrašytomis CA3 neuronų veikimo potencialų sekomis. Modeliavimas parodė, kad tTIS reikšmingai padidino plastiškumą patiriančių sinapsių skaičių net esant mažo intensyvumo stimuliacijai, kuri lėmė aukštesnį dendritinių, bet ne somatinių, veikimo potencialų dažnį. Stimuliacijos metu sinapsės patyrė didesnius sinapsinių svorių pokyčius, tačiau stimuliacija išlaikė bendrą svorių dinamiką ir nesukėlė stipraus potencijuojančio ar slopinančio poveikio. Stimuliacija turėjo didžiausią poveikį, kai sinapsės gavo žemo ir vidutinio dažnio nervinius impulsus. Tai rodo, kad tTIS stimuliacija gali sustiprinti vykstančius sinapsinius procesus ir paskatinti plastiškumą esant silpnam sinapsiniam aktyvumui. Panašūs rezultatai buvo gauti ir naudojant biologiškai realistiškus *in vivo* sinapsinius nervinius impulsus. Šie rezultatai rodo, kad tTIS gali paveikti hipokampo sinapsinį plastiškumą ir suteikia įžvalgų apie galimus mechanizmus, lemiančius mažo intensyvumo tTIS kognityvinius efektus.

VILNIUS UNIVERSITY
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Ieva Kerševičiūtė

Master's thesis

The Effects of Temporal Interference Stimulation on Long-Term Synaptic Plasticity in CA1 Hippocampal Neurons

ABSTRACT

Transcranial temporal interference stimulation (tTIS) is a novel non-invasive brain stimulation technique that employs multiple high-frequency interfering electric fields to enable stimulation at depth. Multiple studies applying tTIS to the human hippocampus have reported improvements in memory and persistent aftereffects, suggesting that tTIS may interact with long-term synaptic plasticity processes. However, the exact nature of these interactions remains unknown. Here, we applied computational modeling to investigate whether low- and high-intensity 5 Hz tTIS could modulate long-term synaptic plasticity in the hippocampus. We used a biophysical model of a CA1 hippocampal pyramidal neuron receiving Schaffer collateral synaptic input, driven either by Poisson spike trains at multiple frequencies or by biologically realistic *in vivo* CA3 firing patterns. Our model showed a significant increase in the number of synapses undergoing plasticity under low-intensity tTIS that increased dendritic but not somatic spiking. On average, stimulation led to larger changes in synaptic weights while preserving overall synaptic weight dynamics and without introducing a clear potentiating or depressing bias. These effects were most pronounced under low and intermediate synaptic input frequencies, suggesting that tTIS could enhance ongoing synaptic dynamics and promote plasticity under weak synaptic input conditions. Similar effects were observed under biologically realistic *in vivo* synaptic input. Together, these findings suggest that tTIS may influence hippocampal synaptic plasticity and provide additional insight into the potential mechanisms underlying the cognitive effects of low-intensity tTIS.

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Appendices

Appendix A. Computational tools used in this thesis

Python v3.11.6

NEURON v8.2.2 (Carnevale & Hines, 2006)

Python packages:

NumPy v1.26.4 (Harris *et al.*, 2020)

Pandas v2.1.4 (pandas development team, 2023)

Matplotlib v3.8.1 (Hunter, 2007)

SciPy v1.11.4 (Virtanen *et al.*, 2020)

Seaborn v0.13.2 (Waskom, 2021)

scikit-learn v1.6.1 (Pedregosa *et al.*, 2011)

statsmodels v0.14.4 (Seabold & Perktold, 2010)

Jupyter v7.5.5 (Thomas *et al.*, 2016)

Appendix B. Subthreshold amplitudes

Table B1. Stimulation intensities for fractions of subthreshold amplitude for 5 Hz tTIS with a 1000 Hz carrier.

Subthreshold amplitude fraction, %	Intensity, V/m
10	14.9
20	29.8
30	44.6
40	59.5
50	74.4
60	89.3
70	104.2
80	119.0
90	133.9
100	148.8

Table B2. Subthreshold stimulation amplitudes for parallel electric field orientation.

Beat	Carrier 1000 Hz	Carrier 2000 Hz	Carrier 5000 Hz	Carrier 9000 Hz
0.0	512.5	794.4	1566.5	2339.4
0.5	149.9	233.2	459.2	687.9
1.0	149.9	231.9	457.8	685.7
2.0	149.2	231.2	455.8	682.4
3.0	148.8	230.8	454.6	680.4
4.0	148.8	230.8	454.6	679.8
5.0	148.8	231.2	454.6	680.4
6.0	149.2	231.9	455.8	681.2
7.0	149.9	231.9	457.4	683.1
8.0	149.9	233.2	458.5	685.7
9.0	150.6	233.9	460.4	689.0
10.0	151.1	235.1	463.3	692.1
15.0	155.0	241.7	476.1	711.7
20.0	158.9	247.6	487.8	731.9
25.0	162.3	253.4	499.5	750.4
30.0	165.5	258.5	510.5	767.8
35.0	168.3	263.2	520.3	783.6
40.0	170.7	267.1	528.8	796.4
45.0	172.6	270.3	533.4	804.2
50.0	173.3	271.7	535.9	808.1
60.0	175.3	274.2	539.8	814.5
70.0	176.5	275.8	543.2	819.1
80.0	177.2	277.5	545.7	822.4
90.0	178.5	278.8	548.3	825.7
100.0	179.2	280.8	550.3	828.2
110.0	180.4	281.6	552.2	830.4
120.0	181.2	282.7	554.2	832.8
130.0	182.0	283.9	555.4	834.4
140.0	183.1	284.7	557.0	836.1

Table B3. Subthreshold stimulation amplitudes for perpendicular electric field orientation.

Beat	Carrier 1000 Hz	Carrier 2000 Hz	Carrier 5000 Hz	Carrier 9000 Hz
0	877.3	1359.7	2669.4	3999.5
0.5	255.4	396.7	778.8	1174.6
1	255.4	396.0	776.9	1171.4
2	254.3	394.0	774.2	1166.7
3	253.4	394.0	772.9	1164.4
4	253.4	394.0	772.2	1163.6
5	254.3	394.0	772.9	1164.8
6	254.6	395.3	774.9	1166.7
7	255.4	396.0	776.9	1170.3
8	256.2	397.9	778.8	1173.9
9	257.3	398.8	782.0	1178.1
10	258.2	400.8	785.5	1183.7
15	263.9	409.7	803.5	1211.7
20	269.0	419.4	821.0	1239.7
25	274.9	427.2	837.4	1265.1
30	278.8	435.1	851.1	1287.5
35	283.3	442.1	864.0	1307.4
40	286.6	448.7	875.2	1324.6
45	289.8	452.6	882.9	1335.4
50	291.4	454.6	886.2	1340.6
60	293.7	458.5	892.1	1348.4
70	295.7	461.7	896.0	1354.2
80	297.6	464.4	899.9	1359.7
90	299.6	466.3	903.8	1364.0
100	301.0	468.8	907.0	1368.7
110	302.2	470.9	909.7	1371.8
120	304.2	472.8	912.5	1375.4
130	305.4	474.6	914.8	1378.4
140	306.2	476.1	917.5	1381.6

Appendix C. Synaptic weight distribution under 5 Hz Poisson synaptic input

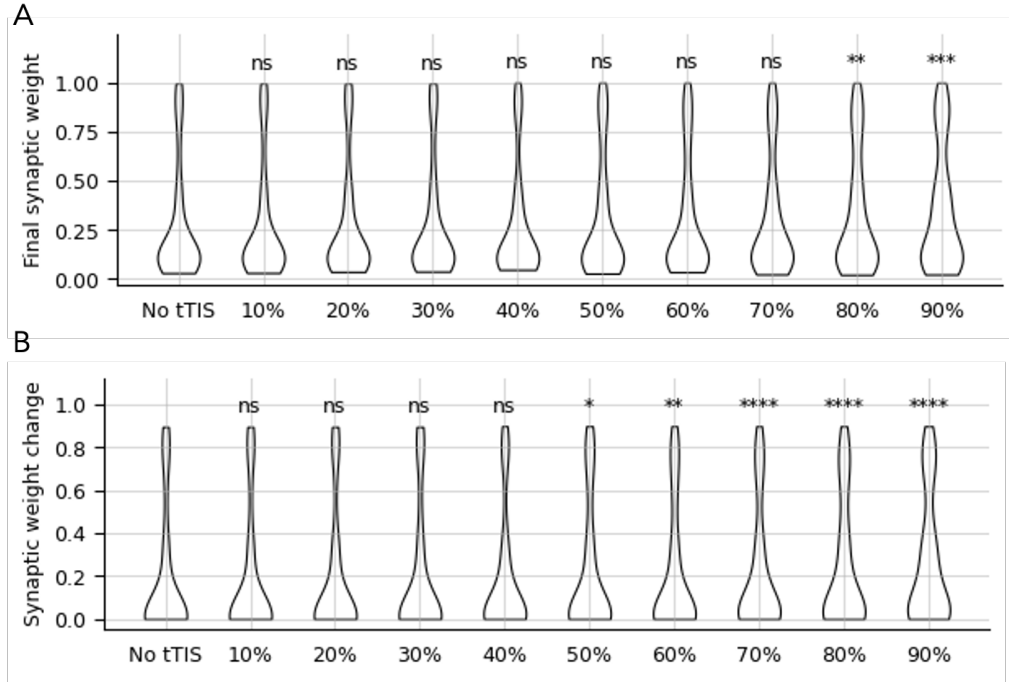


Figure C1. Stimulation intensities below 50% of the subthreshold stimulation amplitude produced non-significant changes in synaptic weight distributions. (A) Distribution of final synaptic weights at the end of the stimulation period. (B) Distribution of absolute synaptic weight changes ($|w_{\text{final}} - w_{\text{init}}|$). Kolmogorov-Smirnov (K-S) test; all significance markers denote comparisons to the No tTIS condition (*not significant*, *ns*: $p \geq 0.05$; *: $p < 0.05$; **: $p < 10^{-2}$; ***: $p < 10^{-3}$; ****: $p < 10^{-4}$).

Appendix D. Influence of pure carrier stimulation under 5 Hz Poisson synaptic input

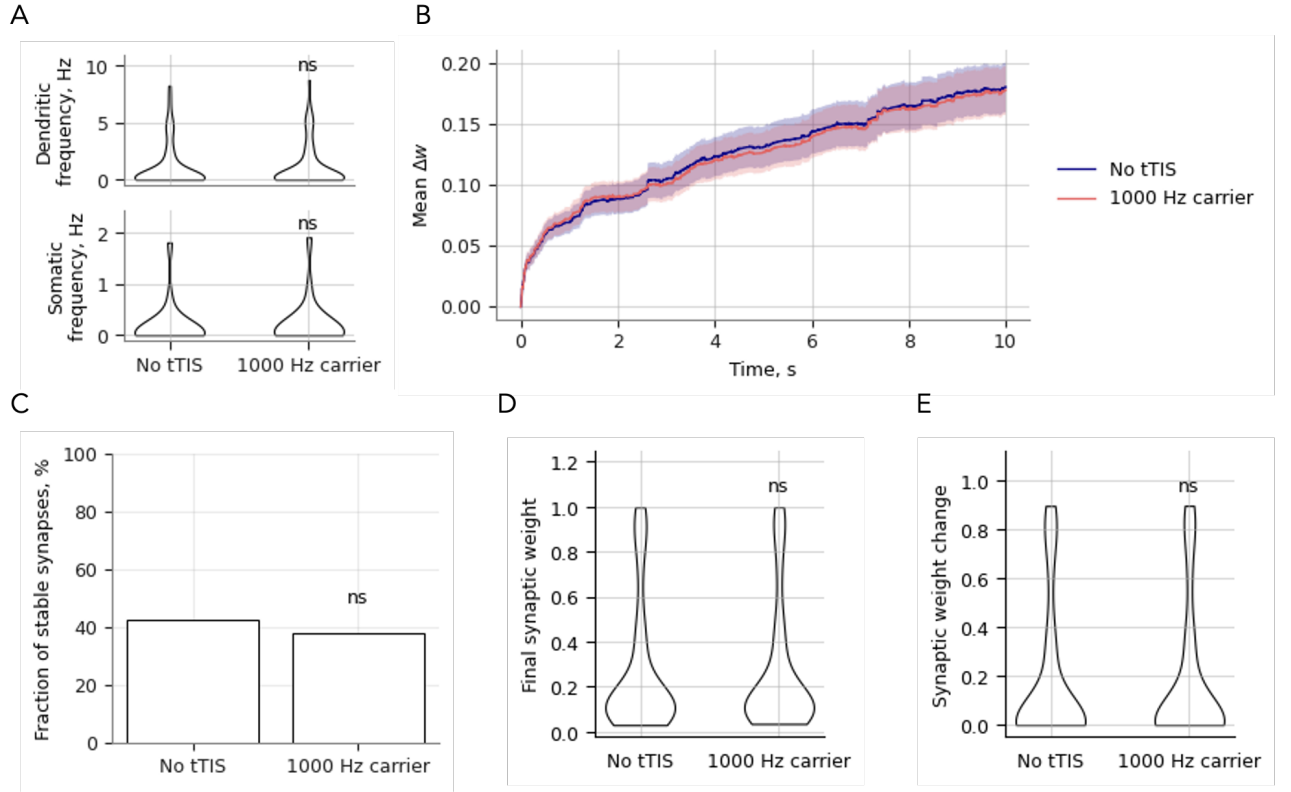


Figure D1. Pure carrier stimulation (1000 Hz) at field strengths comparable to the 90% tTIS condition produces minimal effects under 5 Hz Poisson synaptic input. (A) Dendritic and somatic firing frequency (Mann-Whitney U test). (B) The dynamics of the average change in weight ($\Delta w_t = |w_t - w_0|$ for each time point t , $w_0 = 0.1$) for all synapses throughout the simulation period (10 s). (C) Fraction of stable synapses (Chi-square test). (D) Distribution of final synaptic weights at the end of the stimulation period (Kolmogorov-Smirnov (K-S) test). (E) Distribution of absolute synaptic weight changes ($|w_{\text{final}} - w_{\text{init}}|$) (Kolmogorov-Smirnov (K-S) test). All significance markers denote comparisons to the No tTIS condition (*not significant*, *ns*: $p \geq 0.05$).

Appendix E. Synaptic weight dynamics during 5 Hz Poisson synaptic input

The double exponential model used to fit the average synaptic weight dynamics:

$$\Delta w(t) = A_1 (1 - e^{-t/\tau_1}) + A_2 (1 - e^{-t/\tau_2})$$

Table E1. Double exponential fit parameters describing the average absolute synaptic weight change over time.

Stimulation	A_1	τ_1	A_2	τ_2
No tTIS	0.05	0.18	0.20	9.83
50% tTIS	0.06	0.18	0.24	10.00
90% tTIS	0.09	0.55	0.28	9.31

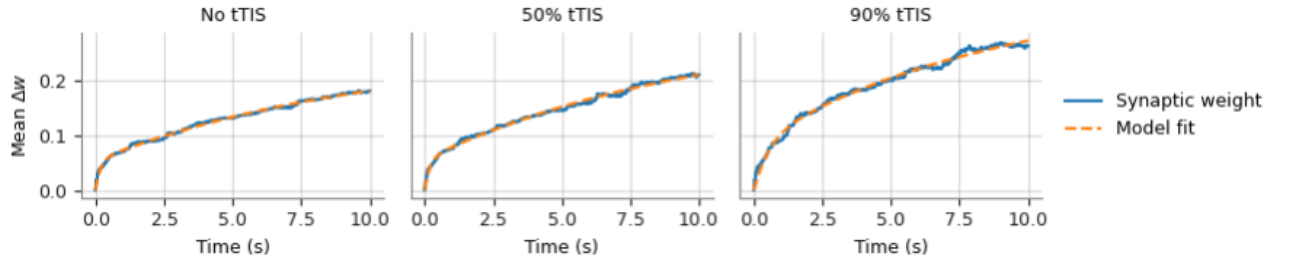


Figure E1. Average synaptic weight change over time and the corresponding double exponential fit.

Appendix F. Computing synaptic weight differences between No tTIS and tTIS conditions across different input frequencies

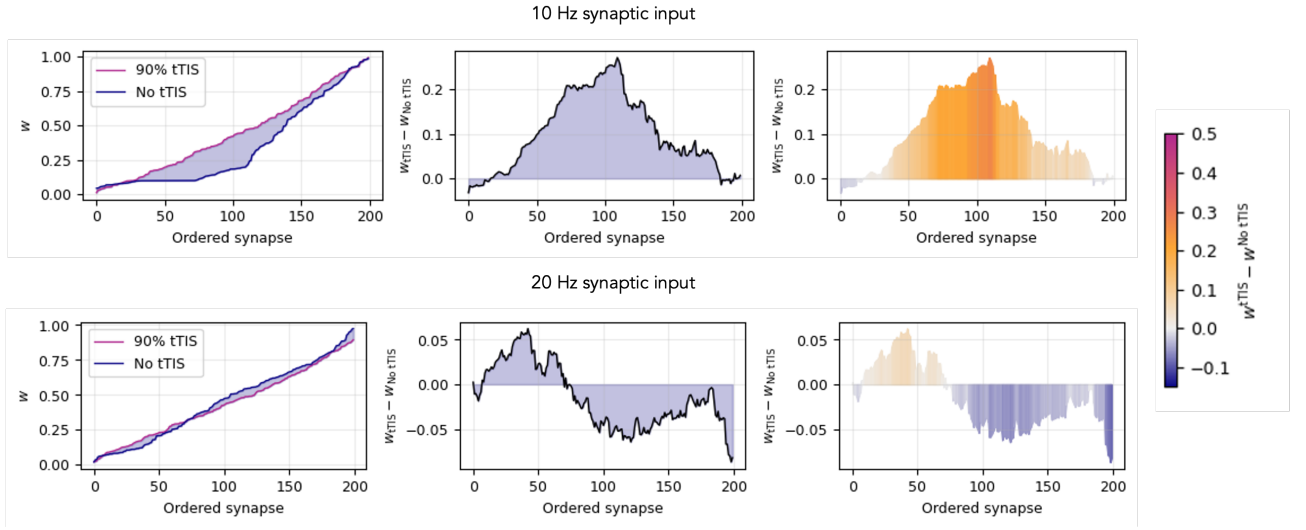


Figure F1. Example synaptic weight distribution differences for high-intensity (90%) tTIS under 10 Hz and 20 Hz Poisson-distributed synaptic inputs, illustrating the computation of the heatmap in Figure 13.

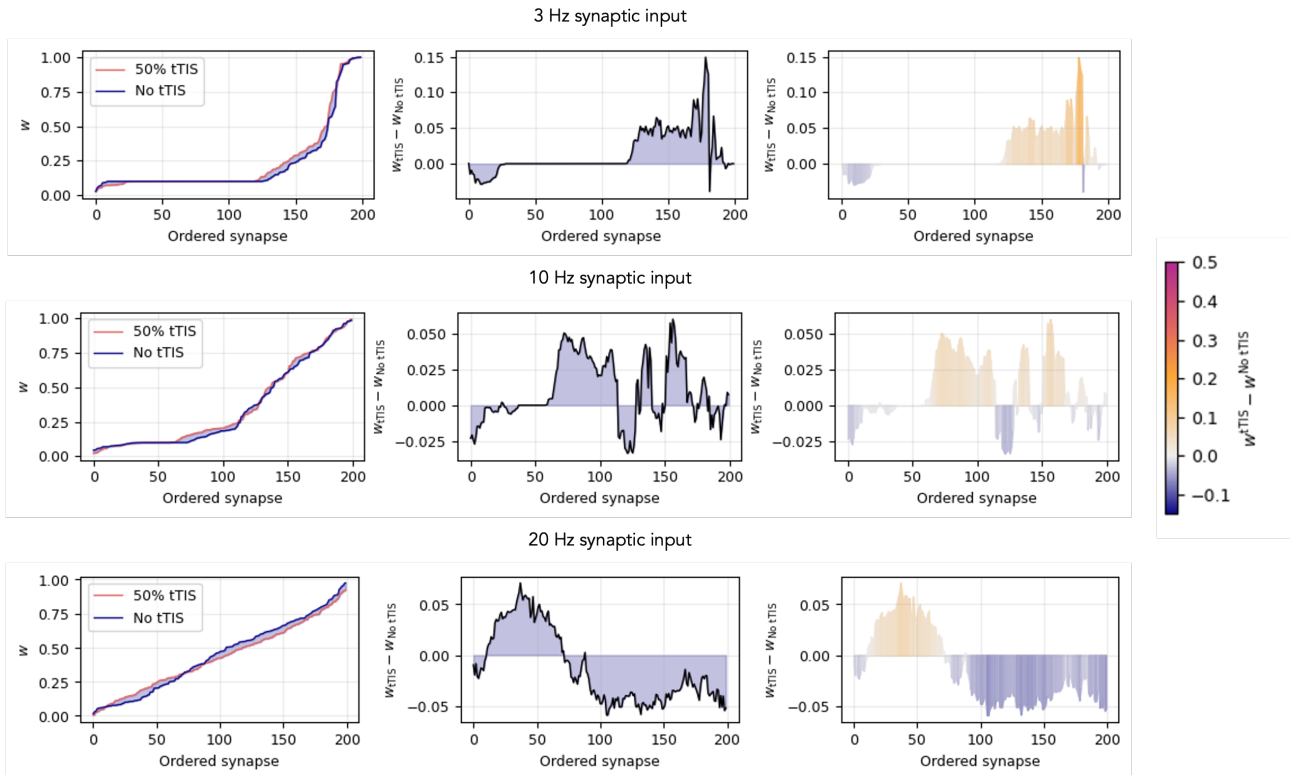


Figure F2. Example synaptic weight distribution differences for low-intensity (50%) tTIS under 3 Hz, 10 Hz and 20 Hz Poisson-distributed synaptic inputs, illustrating the computation of the heatmap in Figure 13.

Appendix G. Synaptic weight dynamics during *in vivo* synaptic input

The single exponential model used to fit the average synaptic weight dynamics:

$$\Delta w(t) = A \left(1 - e^{-t/\tau}\right)$$

Table G1. Single exponential fit parameters describing the average absolute synaptic weight change over time.

Stimulation	A	τ
No tTIS	0.14	105.63
50% tTIS	0.16	102.59
90% tTIS	0.20	98.27

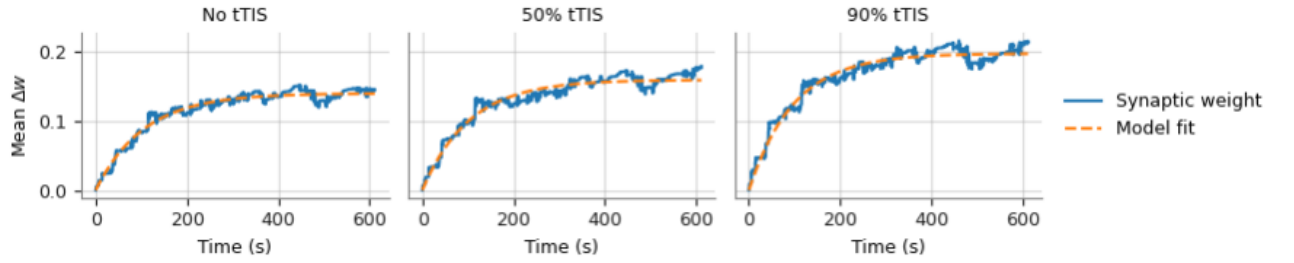


Figure G1. Average synaptic weight change over time and the corresponding single exponential fit.

Appendix H. Post-stimulation neuronal excitability under *in vivo* synaptic inputs

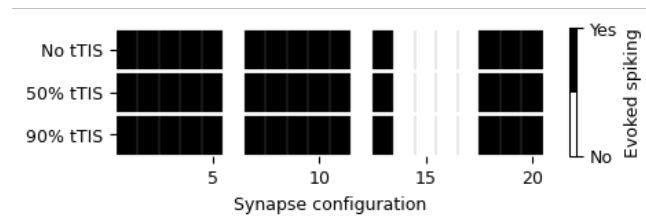


Figure H1. Neuronal excitability was tested separately for each synaptic configuration by delivering a single test stimulus simultaneously to all synapses, using the final synaptic weights obtained under different stimulation conditions. Stimulation was not applied during the test. The neuronal response pattern remained identical across all tested conditions.