Japanese Journal of Comprehensive Rehabilitation Science (2023)

*Review Article*

# TMS and neocortical neurons: an integrative review on the micro-macro connection in neuroplasticity

**Dongting Tian, MS,[1] Shin-Ichi Izumi, MD, PhD[1,2]**

[1]Department of Physical Medicine and Rehabilitation, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan
[2]Tohoku University Graduate School of Biomedical Engineering, Sendai, Miyagi, Japan

**ABSTRACT**

Tian D, Izumi S. TMS and neocortical neurons: an integrative review on the micro-macro connection in neuroplasticity. Jpn J Compr Rehabil Sci 2023; 14: 1–9.

Neuroplasticity plays a pivotal role in neuroscience and neurorehabilitation as it bridges the organization and reorganization properties of the brain. Among the numerous neuroplastic protocols, transcranial magnetic stimulation (TMS) is a well-established non-invasive protocol to induce plastic changes in the brain. Here, we review the findings of four plasticity-inducing TMS protocols in the human motor cortex with relatively evident mechanisms: conventional repetitive TMS (rTMS), theta-burst stimulation (TBS), quadripulse stimulation (QPS) and paired associative stimulation (PAS). Based on the reviewed evidence and a preliminary TMS neurocytological model proposed in our previous report, we further integrate the neurophysiological evidence and plasticity rules of these protocols to present an updated micro-macro connection model between neocortical neurons and the neurophysiological evidence in TMS. This prototypical model will guide further efforts to understand the neural circuit of the motor cortex, the mechanisms of TMS, and the advance of neuroplasticity technologies and their outcomes.

**Key words:** TMS, cortical neurons, neural plasticity, LTP, LTD

Correspondence: Shin-Ichi Izumi, MD, PhD
Tohoku University Graduate School of Biomedical Engineering, 2−1 Seiryo-cho, Aoba-ku, Sendai, Miyagi 980−8575, Japan.
E-mail: izumis@med.tohoku.ac.jp

Accepted: October 5, 2022.
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.


**doi.org/10.11336/jjcrs.14.1**

## Introduction

Neuroplasticity, generally referred to as the reorganization ability of the brain, is the infrastructure of learning, memory, and functional recovery after central nervous system lesions. In the human motor cortex, neuroplasticity can be induced by non-invasive brain stimulation (NIBS) including transcranial magnetic stimulation (TMS) and transcranial electric stimulation (TES; for a review, see [1]). In our recent review article, we attempted to illustrate the micro-macro connection in the motor cortex by integrating neocortical neurocytological and TMS electrophysiological evidence [2]. However, the outcomes of neuroplasticity studies were not included. As neuroplasticity has vital implications and potential applications in neurorehabilitation and bioengineering, we believe that understanding and establishing a micro-macro model based on the evidence of neuroplasticity is the next step after the preliminary model that we proposed.

By category, plasticity-inducing TMS can be sub-classified into conventional repetitive rTMS (rTMS hereafter), quadripulse stimulation (QPS) and paired associative stimulation (PAS) (Table 1). In the present review, we discuss the "micro-macro connection" of neuroplastic effects and underlying neuronal substrates of these four TMS protocols, as an addition and update to our previous model regarding basic TMS mechanisms [2].

## Corticospinal descending volleys and neocortical neurons

The corticospinal descending volleys are the cortical-originating descending impulses evoked by TMS/TES through the corticospinal tract (CST), consisting of the direct wave (D-wave) and indirect waves (I-waves) recorded from high-cervical epidural electrodes (for a review, see [7]). Both the D-wave and I-waves originate from the neocortex, which has a six-layer laminal structure with a vast number of neurons. In epidural recordings, the initial volley evoked by

**Table 1.** Typical plastic protocols of TMS.
The TMS protocols include conventional rTMS, TBS, QPS and PAS (hereby the peripheral nerve stimulation and TMS protocol, not the corticocortical PAS). Subscript denotes interstimulus interval. Abbreviations: iTBS, intermittent TBS; cTBS, continuous TBS; ITI, inter-train interval; IBI, inter-burst interval; ISI, inter-stimulus interval; MNS, median nerve electrical stimulation at the wrist; M1, primary motor cortex. References: a. [1]; b. [3]; c. [4]; d. [5,6].

| Subcategory | Protocol | Stimulation Pattern | Plastic Effect* |
|---|---|---|---|
| rTMS [a] | High frequency | ≧3 Hz consecutive stimuli + ITI | LTP |
| | Low frequency | ≦1 Hz consecutive stimuli without ITI | LTD |
| TBS [b] | iTBS | 3 pulses at 50 Hz applied at 5 Hz + ITI | LTP |
| | cTBS | 3 pulses at 50 Hz applied at 5 Hz without ITI | LTD |
| QPS [c] | $QPS_{1.5–10}$ | 4 pulses at 1.5–10 ms ISI applied every 5 s | LTP |
| | $QPS_{30–100}$ | 4 pulses at 30–100 ms ISI applied every 5 s | LTD |
| PAS [d] | $PAS_{25}$ | MNS 25 ms prior to M1-TMS | LTP |
| | $PAS_{10}$ | MNS 10 ms prior to M1-TMS | LTD |

*Plasticity effect can be state-dependent (i.e., metaplasticity). If the corticospinal excitability prior to the excitatory protocol is relatively high, the original LTP plastic effect may be reduced or even reversed into LTD and vice versa.

TES is termed the D-wave, representing the direct excitation of the pyramidal tract neuron (PTN) axons. I-waves are named by their peak latency after the D-wave as I1-wave, I2-wave, I3-wave, etc., which indicates the indirect excitation of the PTN from the input of other pyramidal neurons (PNs) in the cortex. As the descending volleys have long been proven to originate from the neocortex, it is safe to state that neocortical neurons play a vital part in the generation and modulation of the descending volleys [7].

In the present review, we adopt the primary motor cortex (M1) neuron landscape from our previous report [2] and propose the TMS-related neuronal model shown in Figure 1A. Apart from a highly complicated hierarchical taxonomy, cortical neurons can be subcategorized into two populations: the excitatory pyramidal neurons (PNs, including intratelencephalic neuron, IT and pyramidal tract neuron, PTN in Figure 1A) and the inhibitory interneurons (INs) [2]. It has been shown that PNs make synaptic connections with other PNs and INs and form the I-wave pathways. Inhibition impinging on the PNs occurs in three ways, as feedback inhibition (Figure 1A: IT1-BC1-IT1, IT2-BC3-IT2), feedforward inhibition (Figure 1A: IT2-BC4-IT3, DB-ChC2-IT2/3/4) and lateral inhibition (Figure 1A: IT3-MC-IT1/IT2/PTN1/PTN2) [8]. We previously found that paired-pulse short interval intracortical inhibition (SICI) mainly comes from basket cells (BC) and chandelier cells (ChC), while the I-waves are generated in the PN excitatory synaptic chains under the regulation of BC and ChC (Figure 1). However, apart from the synaptic delay between the PNs, the axonal/dendritic conduction time also appears to contribute to the timing of the I-waves [9]. Consequently, we include the axonal/dendritic conduction time in our model of I-wave generation. In patch-clamp neurocytological studies, the action potential-excitatory postsynaptic potential (AP-EPSP) latency (i.e., the latency between presynaptic neuron action potential generation and postsynaptic neuron somatic EPSP rise) can be used as an index of synaptic location at the dendritic tree [9]. As reported by Sjöström et al., the AP-EPSP latency of layer 2/3−layer 5 (L2/3−L5) PN connections was 3.0 ± 0.4 ms [9]. Therefore, the L2/3−L5 AP-EPSP latency of approximately 3 ms cannot catch the initiation of the I1-wave (~1.5 ms). Hence, the majority of L2/3 ITs that target the PTN apical dendrite cannot participate in the I1-wave initiation at the L5 PTN axonal hillock. So, where is the I1-wave generated? Apart from the possibility of action potential backpropagation (BPAP) as proposed by Ugawa et al. [10], another possible origin of the I1-wave may be the fast PTN-PTN synapses at PTN basal dendrites, which can elicit powerful EPSPs near the PTN cell soma and generate the I1-wave in the 1.5 ms time window after the initiation of the D-wave (Figure 1: PTN1-PTN2). Meanwhile, since the L5 PTNs are the pivot of almost all CST output, plasticity in PTN-PTN synapses might be difficult to induce because of a ceiling effect caused by the massive movement-related output in healthy individuals. This hypothesis could explain the small influence of the I1-wave in TMS protocols except continuous theta-burst stimulation (cTBS). Accordingly, in this hypothetical model, we consider that the PTNs generate the I1-wave and the L2/3 ITs generate the late I-waves. Based on this neuron landscape, we explain the neural pathways and plasticity rules of each protocol. However, as the actual mechanisms and neural substrates underlying I-waves are still not understood, further investigations

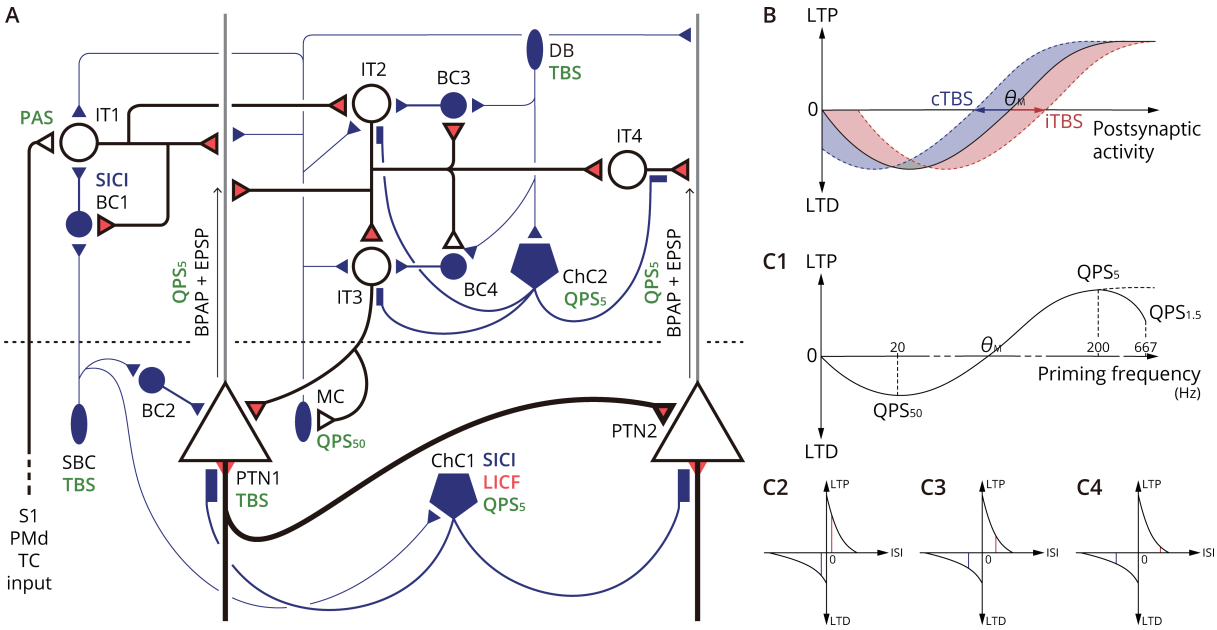


**Figure 1.** Schematic landscape of TMS-related neocortical neuron landscape and plasticity rules.
**(A)** Schematic neuron landscape in the M1. Horizontal dotted line indicates the boundary of neocortical supragranular layers (L1−L3, above) and infragranular layers (L5−L6, below). Neurons are labeled and numbered next to the cell soma. Interneurons and related synapses are colored in dark blue while black open shapes denote pyramidal neurons and related synapses. BCs target the perisomatic dendrites while ChCs target mainly the axonal initial segment (AIS). Gray vertical lines indicate the apical dendrites of the PTNs, solid lines denote axons at different conduction speed (line thickness). Red-colored synapses denote the excitatory synapses generating I-waves (SICF) in single-pulse TMS. Red triangles below the PTNs denote the axon hillock generating the D-wave. The neuron substrates for paired-pulse SICI (blue text), LICF (red text) are labeled next to the No.1 neuron of each type. The selective targets of TMS plasticity are denoted next to the neuron. **(B)** BCM rule in cTBS and iTBS. As the stimulation dose increases, the threshold for LTP-LTD shift ($\theta_M$) slides to the right/left as the general excitability of the cortex increases/decreases in iTBS (red)/cTBS (blue), eventually causing plasticity reversal in a homeostatic manner. **(C1−C4)** Integration of BCM and STDP rules in QPS. At shorter ISIs, the degree of LTP (length of the red line) outweighs that of LTD (length of the blue line), causing net potentiation (C2). At longer ISIs, LTD gradually outweighs LTP due to the slower decay, and turns potentiation into depression (C4). At the time point where the degree of LTP equals that of LTD, the effect is completely counterbalanced to zero (C1: $\theta_M$, C3). For detailed interpretation and related references in this figure, the reader is referred to the main text. Abbreviations: BPAP, backpropagating action potential; EPSP, excitatory postsynaptic potential; S1, somatosensory cortex; PMd, dorsolateral premotor cortex; TC, thalamocortical; IT, intratelencephalic neuron; BC, basket cell; MC, Martinotti cell; SBC, small basket cell; PTN, pyramidal tract neuron; ChC, chandelier cell; DB, double-bouquet cell.

are needed on the empirical specification of the I-wave related neural substrates.

## Conventional repetitive TMS (rTMS)

### 1. Neurophysiological evidence

It is widely accepted that high-frequency (≥ 3-Hz) rTMS potentiates cortical excitability through long-term potentiation (LTP) and low-frequency (≤ 1-Hz) rTMS depresses the excitability via long-term depression (LTD) [1]. The frequency, delivery period and aftereffects of rTMS correspond with the evidence of experimental LTP/LTD generation in animal studies [11]. However, although rTMS has been subcategorized by stimulation frequency [1], the explicit frequency-dependent effects inside high- and low-frequency rTMS remain obscure. Moreover, modulation of paired-pulse cortical inhibition and facilitation circuits after rTMS is also highly inconsistent (for a review, see [12]). In light of the rTMS effect on the TMS-evoked cortical descending volleys, Di Lazzaro et al. reported that 5-Hz rTMS facilitated the "proximal D-wave" (i.e., the D-wave evoked by non-focal or biphasic TMS pulse, which has a slightly longer latency than the monophasic-TMS-evoked D-wave; this D-wave is considered to originate from the axonal location that is more proximal to the PTN cell soma) and late I-waves while 1-Hz rTMS inhibited the late I-waves [13]. This evidence suggested that rTMS may induce neuroplasticity in the neural population responsible for generating the late I-waves.

Another factor affecting the rTMS-induced plasticity

is the inter-train interval (ITI). ITI in high-frequency rTMS is necessary as it may be related to neuron response failure proposed in a cellular study [14]. Neuron response failure is the phenomenon whereby a neuron cannot maintain synchronic firing in response to suprathreshold stimuli when the stimulation rate exceeds a certain frequency, unless a long interval is intersected [14]. Consistent with the neurocytologic evidence, Rothkegel et al. reported that MEP decreased significantly after consecutive 5-Hz rTMS, compared to the MEP increase after 5-Hz rTMS with 60-s ITI [15]. Another study using 10-Hz rTMS with different stimulation durations yielded similar results. While the 10-Hz rTMS with 55-s ITI induced LTP aftereffects, the effects turned into LTD if the ITI was 5 s [16]. This reverse from LTP to LTD was also observed in TBS [3]. These lines of evidence indicate the influence of ITI in rTMS, which is also evident in TBS. This suggests that an ITI that is significantly longer than the membrane time constant of the neurons may be necessary for rTMS and TBS to induce LTP.

**2. Neuroplasticity rule**

A few insights can be drawn from the frequency-dependent LTP/LTD rule. We noted that the highest frequency of safe rTMS in human studies (≦ 20 Hz [1]) is much lower than the frequencies adopted in laboratory LTP induction (up to 400 Hz [17]). As the membrane time constant of neocortical PNs is shorter than 50 ms (the NeuroElectro Project, https://neuroelectro.org/), we can attribute the rTMS-induced LTP to the repetitive firing by single-pulse TMS, with an abundant interval left for the stimulated neurons to repolarize and return to their rest potentials between stimuli.

**3. Neurocytological model**

Based on the plasticity rule model, the stimulation pattern indicates that rTMS appears to induce plasticity in the general neural circuit involved in single-pulse TMS, in a frequency-dependent manner. Specifically, in Figure 1A, we propose that high/low-frequency rTMS induces LTP/LTD in all typical I-wave-related IT-PTN and BC/ChC-IT synaptic connections. While LTP in the IT-IT synapses might be counteracted by feedback inhibition (IT-BC-IT), the enhancement of IT-PTN synapses can result in late I-wave facilitation, thus accounting for the neurophysiological evidence.

## Theta-burst stimulation (TBS)

**1. Neurophysiological evidence**

The difference in protocol between TBS and rTMS lies in the integration of θ rhythm (4−12 Hz) oscillation with high-frequency LTP-inducing bursts up to 50 Hz, which enables TBS to induce plastic effects with different properties compared to rTMS. Technically, θ-frequency-matched stimulation should entrain and augment the θ band activity and increase the firing synchronicity of these two neural populations. Consistent with this inference, studies investigating neural oscillation changes by TBS revealed that cTBS (corresponding to the θ frequency) significantly increased θ band power in awake electroencephalography (EEG) [18], while the entrainment of intermittent theta-burst stimulation (iTBS) was not significant [19]. For the frequency-dependent plasticity induction, even though the dose-dependent effects of TBS resembled those of rTMS at lower pulse numbers [20], doubling the stimulation dose of both cTBS and iTBS resulted in plasticity reversal, showing MEP suppression after iTBS and facilitation after cTBS [21,22]. In addition, although TBS combines the two LTP induction processes, cTBS was found to be suppressive [3]. This disparity may also come from the necessity of an ITI to prevent neuron response failure, as the ITI duration of iTBS (8−10 s [21]) largely overlaps with that of rTMS. For corticospinal descending volleys, while iTBS facilitated the late I-waves as did high-frequency rTMS, cTBS suppressed the I1-wave instead of the late I-waves [23,24]. Given that the late I-waves, instead of the I1-wave, are selectively suppressed by paired-pulse intracortical inhibition (ICI) [25], the authors suggested that cTBS induced LTD from pathways other than the late-I-wave targeting ICI circuits, such as in the excitatory PN chains [24]. Interestingly, evidence also suggested that SICI decreased after cTBS (i.e., disinhibition of SICI) and increased after iTBS (i.e., more inhibition of SICI) [3], which showed a simultaneous increase in both SICI and the late I-waves in iTBS.

**2. Neuroplasticity rule**

Apart from the involvement of frequency-dependent LTP induced by the 50-Hz bursts, the plasticity reversal upon double-dose TBS [21,22] conforms to another rule named the BCM theory [26] (Figure 1B). Specifically, the induction of LTP or LTD largely depends on the postsynaptic neuron excitability, showing as a gradual alteration from suppression (LTD) to potentiation (LTP) as the postsynaptic neuron excitability increases. In TBS, as the stimulation dose increases, the threshold for LTP-LTD shift ($\theta_M$) slides to the right/left as the general excitability of the cortex increases/decreases in iTBS/cTBS, eventually causing plasticity reversal in a homeostatic manner.

**3. Neurocytological model**

Viewed microscopically, cortical θ rhythm stems from two populations: L5 PN (PTN in the M1) and regular-spiking interneuron (RS-IN, e.g., small basket cells and double-bouquet cells) [27,28] (Figure 1A). The aforementioned EEG evidence [18,19] indicates that cTBS may produce greater entrainment to the θ rhythm-related PTN and RS-IN than iTBS. Nevertheless, insufficient ITI of cTBS may also induce PTN response failure, turn the PTN-related LTP at IT-PTN and even

PTN-PTN synapses into LTD [14], and thus inhibit the I1-wave. However, the LTD at the IT-PTN synapses (responsible for late I-waves) could be lifted to some extent, as RS-INs that mainly inhibit inhibitory BCs can in turn disinhibit the ITs [29] (i.e., cause the decrease of SICI), eventually resulting in little change in the late I-waves. For iTBS, we infer from the weaker modulation of θ rhythm [19] that fewer response failures occur in PTNs, along with the RS-INs being less synchronized. The I1-wave can be kept stable due to the PTN ceiling effect, while the late I-wave-related synapses are strengthened by high-frequency LTP, including the synapses involved in feedback inhibition (IT-BC-IT synapses). The simultaneous enhancement of I-wave-related excitatory and inhibitory circuits therefore accounts for the late I-wave facilitation along with SICI increase in iTBS.

## Quadripulse stimulation (QPS)

### 1. Neurophysiological evidence

QPS is a novel stimulation protocol inducing a stronger and more distinct plasticity effect with less inter-individual variability compared to rTMS and TBS [4]. Although the paradigm of facilitatory 1.5-ms QPS ($QPS_{1.5}$) resembled another protocol termed I-wave periodicity rTMS (iTMS, applying 1.5-ms inter-stimulus interval (ISI) suprathreshold paired-pulses every 5 s repetitively) [30], the plastic effects of $QPS_{1.5}$ significantly outweighed those of same-intensity iTMS at either matched pulse or train number [31]. This evidence suggested that whereas excitatory QPS used the same ISI as iTMS, QPS is not a simple intensification of iTMS but is able to recruit certain neural populations other than iTMS to induce such strong and long-lasting plasticity. At ISIs ranging from 1.5 to 1250 ms, the most prominent LTP and LTD induction occurred at 5 ms ($QPS_5$) and 50 ms ($QPS_{50}$) [32]. Excitatory $QPS_5$ increased and inhibitory $QPS_{50}$ decreased single-pulse MEP amplitude as well as paired-pulse short interval intracortical facilitation (SICF) and long interval intracortical facilitation (LICF) significantly without affecting SICI [32]. In terms of the interhemispheric effects, in 2013, Tsutsumi et al. [33] firstly investigated the transcallosal effect of QPS. The study revealed that applying excitatory $QPS_5$ to the left motor cortex resulted in an MEP increase in both hemispheres (i.e., induced left-to-right interhemispheric facilitation (IHF)) whereas inhibitory $QPS_{50}$ did not affect MEP in the non-stimulated hemisphere [33]. Regarding the IHF effect, it is possible that the LTP plasticity induced by QPS might be strong enough to overcome the regulation of IHI-related inhibitory INs and result in IHF. This speculation is based on the observation of a paradoxical IHF effect in the paired-pulse IHI paradigm, when the conditioning stimulus was large enough [34]. However, a crucial issue regarding QPS mechanisms has not been addressed: evidence regarding the change of corticospinal descending volleys in QPS remains absent. As the descending volleys can provide more direct information about the change in the neocortex than the MEPs, empirical evidence is needed in order to examine the underlying mechanisms.

### 2. Neuroplasticity rule

QPS also involves two plasticity rules at the same time: the ISI-dependent BCM-like nonlinear plasticity and spike-timing dependent plasticity (STDP, time window ± 100 ms) [4,26,32]. According to Hamada et al. [32], the effect spectrum of QPS fitted well with the BCM curve (Figure 1C1) [4]. The possibility of combining BCM with STDP comes from the quadripulse protocol, which allows the pulse sequence to be a variable range depending on the burst ISI. According to experimental data [35], the STDP curve is not symmetric. Instead, it shows a different decay pattern of LTP/LTD as the ISI lengthens, where LTP decreases more sharply than LTD (Figure 1C2–4). We regard the quadripulse to distribute equally both pre- and post-neuron firing of each PN in the I-wave pathways. At shorter ISIs (regardless of firing sequence), the LTP effect outweighs LTD and results in net potentiation; at longer ISIs the LTP decays more than LTD, resulting in net inhibition. Accordingly, the nature of combining frequency-dependent LTP/LTD, BCM and STDP rules at the same time enables QPS to further promote neuroplasticity than other protocols.

### 3. Neurocytological model

Microscopic perspectives on neuronal selectivity can be made separately from $QPS_5$ and $QPS_{50}$ with the most prominent effects (Figure 2A). In $QPS_5$, the ISI (5 ms) allows the latter pulse to arrive at the IT-PTN synapse when the IT-PTN apical dendrite BPAP induced by the former pulse returns to the IT-PTN synapse (a round-trip propagation in the apical dendrite between the IT1/2-PTN synapse and the PTN cell soma) [36]. This repeated coincidence of presynaptic depolarization and BPAP induces strong LTP at distal synapses along the apical dendrite where most of the L2/3 IT neurons form synapses with the PTN [9], showing as the increase of single-pulse MEP (late I-waves by inference, evidence needed) and SICF. In inhibitory QPS, the effect of BPAP would be less where IT-PTN synapses at the distal dendrites tend to undergo LTD at longer ISIs [9]. Regarding the LICF increase without affecting SICI, we suggest the possibility of low-threshold ChC-selective axonal potentiation. That is, when the PN membrane potential is depolarized but does not reach the firing threshold, the subthreshold conditioning of 5-ms ISI enables ChCs to elicit depolarizing GABAergic postsynaptic potentials (GPSPs) and increase the PN spike probability [37], which was proposed in the previous model as a neural substrate of paired-pulse LICF [2].

This selective potentiation of ChC by 5-ms bursts should account for the LICF increase and also partly for the L2/3 IT potentiation in $QPS_5$, whereas BCs (related to SICI) are less sensitive to this ISI [38]. Simultaneously, the high-frequency action potential firing of the L2/3 IT axons would also affect the transcallosal projection targeting the unstimulated hemisphere, overwhelm the regulation of IN inhibition (IHI) and result in IHF, if the excitation becomes strong enough. On the other hand, $QPS_{50}$ decreased MEP the most with less IHI modulation [4]. As already proven the callosal fiber is excitatory, consisting of axons from IT neurons in all cortical layers [2]. The modulation absence of IHI in $QPS_{50}$ therefore suggests little increase in excitability in the IT neurons in the stimulated hemisphere. Since $QPS_{50}$ also has little effect on SICI [32] and the ISI of 50 ms is consistent with the firing frequency of neocortical Martinotti cells (MC, mediating lateral inhibition) [39], the strong inhibition might come from the synchronization of MC spiking and selective potentiation of MC-IT/PTN synapses, along with the LTD in IT-PTN synapses.

## Paired Associative Stimulation (PAS)

### 1. Neurophysiological evidence

Compared to the frequency-dependent LTP/LTD-mediated rTMS/TBS/QPS, the highly timing-dependent PAS is likely to induce neuroplasticity via STDP [40,41], together with the feature of TMS-induced current selectivity. In light of the I-waves, research by Di Lazzaro et al. showed that $PAS_{10}$/$PAS_{25}$ significantly inhibited/enhanced the late I-waves without significant change of the I1-wave [42,43]. Although the modulation of the late I-waves by PAS resembles rTMS and iTBS, empirical evidence also points to the selectivity of TMS-induced current direction. Changing the TMS current direction alters the cortical descending volleys as well as the MEP latency (for a review, see [44]). Specifically, the D-wave is preferentially evoked by TMS-induced current parallel to the precentral gyrus in a lateromedial direction, while the I-waves are prominently evoked by posterior-to-anterior (PA-) and anterior-to-posterior (AP-) TMS, in which the induced current flows perpendicular to the precentral gyrus. Although evidence has indicated that PA-TMS preferentially recruits both the I1-wave and the late I-waves whereas AP-TMS recruits late I-waves only, the late I-waves generated by PA- and AP-TMS are thought to come from different neural populations [44]. In PAS, it has been reported that $PAS_{21.5}$ potentiated MEP either with PA or AP TMS-induced current [45]. However, when the TMS pulse was conditioned by another subthreshold pulse in a SICI manner, only the MEP increase induced by AP-$PAS_{21.5}$ was cancelled. The facilitation of PA-$PAS_{21.5}$ was not affected by PA-SICI conditioning [45]. However, the cancellation of PA-PAS aftereffects by PA-SICI was observed elsewhere, as the effects of PA-$PAS_{25}$ were cancelled by PA-$SICI_{2ms}$ [46] and PA-$PAS_{10}$ by PA-$SICI_{3ms}$ [47]. Conversely, PA-$SICI_{3ms}$ also decreased after PA-$PAS_{25}$ [48], suggesting the involvement of the current-specific SICI circuits in both PA- and AP-PAS. Furthermore, in a study by Hamada et al. [49], the authors proposed the insight that $PAS_{21.5}$ and $PAS_{25}$ targeted the PA- and AP-sensitive population respectively, as the MEP facilitation of $PAS_{25}$ was cancelled by cerebellar tDCS (which is thought to target the AP-sensitive circuits) while the potentiation of $PAS_{21.5}$ was not affected by the DC stimulation.

### 2. Neuroplasticity rule

STDP is the most related and evident plasticity rule in PAS. The polarity of STDP plasticity by PAS relies highly on the timing of the afferent input to the primary somatosensory cortex (S1), the S1-M1 relay, and the TMS pulse timing [40]. If the TMS pulse is delivered before the afferent input arrives at S1 (i.e., the N20 timing), PAS induces LTD in M1 and suppresses MEP. Conversely, if the timing of the TMS pulse lags the arrival by 1.5−4.8 ms (allowing the signal to be relayed to M1), PAS induces LTP and potentiates MEP [1].

### 3. Neurocytological model

The neurocytological model of PAS is mainly based on TMS-induced current selectivity. Although consensus on the neuronal basis of TMS current selectivity has not been reached, it has been convincingly proposed that the main locations affected by motor cortex TMS are the dorsal premotor cortex (PMd) and M1 in the precentral gyrus. Specifically, the M1 hand area is usually located in the posterior bank of the precentral gyrus, while the anterior bank is mostly PMd with strong connectivity with the posterior M1 and can be selectively excited by AP-TMS. Conversely, the M1 hand area located in the posterior bank can be excited by PA-TMS at lower intensity than AP-TMS, as the induced current flow is parallel to the corticospinal axon descending direction [2,50]. Accordingly, in PAS we consider that the peripheral afferent input firstly passes the thalamus, then is relayed to M1 directly or relayed to S1, and finally arrives at L2/3 in M1 indirectly, with or without passing PMd. Therefore, the thalamus-M1, S1-M1 relay and S1-PMd-M1 relay account for the timing and induced current selectivity of $PAS_{21.5}$ and $PAS_{25}$. The STDP-related LTP/LTD would first occur at L2/3 IT synapses (in Figure 1A, the incoming synapse targeting IT1) as cortical inputs are mainly processed in L2/3 [51]. The SICI priming pre-activates the fast-spiking BCs and ChCs, and therefore cancels the effect of STDP of both PA- and AP-PAS.

## Implications and future directions

In neuroscience, the aim is to "decipher the brain" while in neurorehabilitation the aim is to "reorganize the brain." In these processes, neuroplasticity is the overlap of these two disciplines. As reviewed in the present paper, many neuromodulation protocols have been devised, yielding prominent results both in research and clinical practice. As the techniques in neurocytology and NIBS are advancing rapidly, it will become necessary to combine cellular and neurophysiological evidence in the future. However, although we reviewed the M1-TMS plastic protocols with relatively clear mechanical evidence, during the literature search and model construction we felt there was a severe lack of evidence to establish a precise causal link between evidence from the micro and macro scale. Therefore, many explanations of the present model come from analogy and need further investigation from both neurocytological and neurophysiological perspectives. Although it is probably too early to propose the hypothetical micro-macro connection model, we believe that the "ice-breaking" and preliminary infrastructure presented in this paper may serve as a prototype for explorations to decipher the brain and neuromodulation mechanisms, and lead to technical advances in neuroscience and neurorehabilitation.

## Funding

This work was supported by JST SPRING, Grant Number JPMJSP2114.

## Acknowledgments

The authors would like to acknowledge Mr. Fuping Han for technical assistance on the literature collection and visualization.

## References

1. Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an IFCN Committee. Clin Neurophysiol 2015; 126: 1071–107.
2. Tian D, Izumi SI. Transcranial Magnetic Stimulation and Neocortical Neurons: The Micro-Macro Connection. Review. Front Neurosci 2022; 16: 866245.
3. Huang Y-Z, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta Burst Stimulation of the Human Motor Cortex. Neuron 2005; 45: 201–6.
4. Matsumoto H, Ugawa Y. Quadripulse stimulation (QPS). Exp Brain Res 2020; 238: 1619–25.
5. Wolters A, Schmidt A, Schramm A, Zeller D, Naumann M, Kunesch E, et al. Timing-dependent plasticity in human primary somatosensory cortex. J Physiol 2005; 565: 1039–52.
6. Guidali G, Roncoroni C, Bolognini N. Paired associative stimulations: Novel tools for interacting with sensory and motor cortical plasticity. Behav Brain Res 2021; 414: 113484.
7. Ziemann U. I-waves in motor cortex revisited. Exp Brain Res 2020; 238: 1601.
8. Hu H, Gan J, Jonas P. Fast-spiking, parvalbumin+ GABAergic interneurons: From cellular design to microcircuit function. Science 2014; 345(6196): 1255263.
9. Sjöström PJ, Häusser M. A Cooperative Switch Determines the Sign of Synaptic Plasticity in Distal Dendrites of Neocortical Pyramidal Neurons. Neuron 2006; 51: 227–38.
10. Ugawa Y, Rothwell JC, Paulus W. Possible role of backpropagating action potentials in corticospinal neurons in I-wave periodicity following a TMS pulse. Neurosci Res 2020; 156: 234–6.
11. Levy WB, Steward O. Temporal contiguity requirements for long-term associative potentiation/depression in the hippocampus. Neuroscience 1983; 8: 791–7.
12. Fitzgerald PB, Fountain S, Daskalakis ZJ. A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clin Neurophysiol 2006; 117: 2584–96.
13. Di Lazzaro V, Profice P, Pilato F, Dileone M, Oliviero A, Ziemann U. The effects of motor cortex rTMS on corticospinal descending activity. Clin Neurophysiol 2010; 121: 464–73.
14. Halawa I, Goldental A, Shirota Y, Kanter I, Paulus W. Less Might Be More: Conduction Failure as a Factor Possibly Limiting the Efficacy of Higher Frequencies in rTMS Protocols. Front Neurosci 2018; 12: 358.
15. Rothkegel H, Sommer M, Paulus W. Breaks during 5Hz rTMS are essential for facilitatory after effects. Clin Neurophysiol 2010; 121: 426–30.
16. Jung SH, Shin JE, Jeong Y-S, Shin H-I. Changes in motor cortical excitability induced by high-frequency repetitive transcranial magnetic stimulation of different stimulation durations. Clin Neurophysiol 2008; 119: 71–9.
17. Okubo-Suzuki R, Saitoh Y, Shehata M, Zhao Q, Enomoto H, Inokuchi K. Frequency-specific stimulations induce reconsolidation of long-term potentiation in freely moving rats. Mol Brain 2016; 9: 36.
18. Noh NA, Fuggetta G, Manganotti P, Fiaschi A. Long Lasting Modulation of Cortical Oscillations after Continuous Theta Burst Transcranial Magnetic Stimulation. PLOS ONE 2012; 7: e35080.
19. Bai Z, Zhang J, Fong KNK. Intermittent Theta Burst Stimulation to the Primary Motor Cortex Reduces Cortical Inhibition: A TMS-EEG Study. Brain Sci 2021; 11: 1114.
20. Nettekoven C, Volz LJ, Kutscha M, Pool E-M, Rehme AK, Eickhoff SB, et al. Dose-dependent effects of theta burst rTMS on cortical excitability and resting-state connectivity of the human motor system. J Neurosci 2014; 34: 6849–59.
21. Gamboa OL, Antal A, Moliadze V, Paulus W. Simply longer is not better: reversal of theta burst after-effect with prolonged stimulation. Exp Brain Res 2010; 204: 181–7.

22. Gentner R, Wankerl K, Reinsberger C, Zeller D, Classen J. Depression of Human Corticospinal Excitability Induced by Magnetic Theta-burst Stimulation: Evidence of Rapid Polarity-Reversing Metaplasticity. Cerebral Cortex 2008; 18: 2046−53.
23. Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, et al. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol 2008; 586: 3871−9.
24. Di Lazzaro V, Pilato F, Saturno E, Oliviero A, Dileone M, Mazzone P, et al. Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. J Physiol 2005; 565(Pt 3): 945−50.
25. Ni Z, Gunraj C, Wagle-Shukla A, Udupa K, Mazzella F, Lozano AM, et al. Direct demonstration of inhibitory interactions between long interval intracortical inhibition and short interval intracortical inhibition. J Physiol 2011; 589(Pt12): 2955−62.
26. Bienenstock EL, Cooper LN, Munro PW. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci 1982; 2: 32.
27. Silva Laurie R, Amitai Y, Connors Barry W. Intrinsic Oscillations of Neocortex Generated by Layer 5 Pyramidal Neurons. Science 1991; 251(4992): 432−5.
28. Callaway EM, Dong H-W, Ecker JR, Hawrylycz MJ, Huang ZJ, Lein ES, et al. A multimodal cell census and atlas of the mammalian primary motor cortex. Nature 2021; 598(7879): 86−102.
29. Hioki H, Sohn J, Nakamura H, Okamoto S, Hwang J, Ishida Y, et al. Preferential inputs from cholecystokinin-positive neurons to the somatic compartment of parvalbumin-expressing neurons in the mouse primary somatosensory cortex. Brain Res 2018; 1695: 18−30.
30. Thickbroom GW, Byrnes ML, Edwards DJ, Mastaglia FL. Repetitive paired-pulse TMS at I-wave periodicity markedly increases corticospinal excitability: a new technique for modulating synaptic plasticity. Clin Neurophysiol 2006; 117: 61−66.
31. Hamada M, Hanajima R, Terao Y, Arai N, Furubayashi T, Inomata-Terada S, et al. Quadro-pulse stimulation is more effective than paired-pulse stimulation for plasticity induction of the human motor cortex. Clin Neurophysiol 2007; 118: 2672−82.
32. Hamada M, Terao Y, Hanajima R, Shirota Y, Nakatani-Enomoto S, Furubayashi T, et al. Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. J Physiol 2008; 586: 3927−47.
33. Tsutsumi R, Hanajima R, Terao Y, Shirota Y, Ohminami S, Shimizu T, et al. Effects of the motor cortical quadripulse transcranial magnetic stimulation (QPS) on the contralateral motor cortex and interhemispheric interactions. J Neurophysiol 2013; 111: 26−35.
34. Belyk M, Banks R, Tendera A, Chen R, Beal DS. Paradoxical facilitation alongside interhemispheric inhibition. Exp Brain Res 2021; 239: 3303−13.
35. Bi G-q, Poo M-m. Synaptic Modification by Correlated Activity: Hebb's Postulate Revisited. Annu Rev Neurosci 2001; 24: 139−66.
36. Larkum ME, Zhu JJ, Sakmann B. A new cellular mechanism for coupling inputs arriving at different cortical layers. Nature 1999; 398 (6725): 338−41.
37. Woodruff AR, McGarry LM, Vogels TP, Inan M, Anderson SA, Yuste R. State-Dependent Function of Neocortical Chandelier Cells. J Neurosci 2011; 31(49): 17872.
38. Fong P-Y, Spampinato D, Rocchi L, Hannah R, Teng Y, Di Santo A, et al. Two forms of short-interval intracortical inhibition in human motor cortex. Brain Stimulation 2021; 14: 1340−52.
39. Obermayer J, Heistek TS, Kerkhofs A, Goriounova NA, Kroon T, Baayen JC, et al. Lateral inhibition by Martinotti interneurons is facilitated by cholinergic inputs in human and mouse neocortex. Nature Commun 2018; 9: 4101.
40. Suppa A, Quartarone A, Siebner H, Chen R, Di Lazzaro V, Del Giudice P, et al. The associative brain at work: Evidence from paired associative stimulation studies in humans. Clin Neurophysiol 2017; 128: 2140−64.
41. Rizzo V, Bove M, Naro A, Tacchino A, Mastroeni C, Avanzino L, et al. Associative cortico-cortical plasticity may affect ipsilateral finger opposition movements. Behav Brain Res 2011; 216: 433−9.
42. Di Lazzaro V, Dileone M, Pilato F, Profice P, Oliviero A, Mazzone P, et al. Associative motor cortex plasticity: direct evidence in humans. Cereb Cortex 2009; 19: 2326−30.
43. Di Lazzaro V, Dileone M, Profice P, Pilato F, Oliviero A, Mazzone P, et al. LTD-like plasticity induced by paired associative stimulation: direct evidence in humans. Exp Brain Res 2009; 194: 661.
44. Opie GM, Semmler JG. Preferential Activation of Unique Motor Cortical Networks With Transcranial Magnetic Stimulation: A Review of the Physiological, Functional, and Clinical Evidence. Neuromodulation: Technology at the Neural Interface 2021; 24: 813−28.
45. Ni Z, Cash RFH, Gunraj C, Bercovici E, Hallett M, Chen R. Involvement of different neuronal components in the induction of cortical plasticity with associative stimulation. Brain Stimul 2019; 12: 84−6.
46. Elahi B, Gunraj C, Chen R. Short-interval intracortical inhibition blocks long-term potentiation induced by paired associative stimulation. J Neurophysiol 2012; 107: 1935−41.
47. Weise D, Mann J, Ridding M, Eskandar K, Huss M, Rumpf J-J, et al. Microcircuit mechanisms involved in paired associative stimulation-induced depression of corticospinal excitability. J Physiol 2013; 591: 4903−20.
48. Stefan K, Kunesch E, Benecke R, Cohen LG, Classen J. Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J Physiol 2002; 543: 699−708.
49. Hamada M, Galea JM, Di Lazzaro V, Mazzone P, Ziemann U, Rothwell JC. Two distinct interneuron circuits in human motor cortex are linked to different subsets of

physiological and behavioral plasticity. J Neurosci 2014; 34: 12837–49.
50. Siebner HR, Funke K, Aberra AS, Antal A, Bestmann S, Chen R, et al. Transcranial magnetic stimulation of the brain: What is stimulated? – a consensus and critical position paper. Clin Neurophysiol 2022; doi:https://doi.org/10.1016/j.clinph.2022.04.022
51. Mao T, Kusefoglu D, Hooks Bryan M, Huber D, Petreanu L, Svoboda K. Long-Range Neuronal Circuits Underlying the Interaction between Sensory and Motor Cortex. Neuron 2011; 72: 111–23.