

Individualized interstimulus interval improves the efficacy and consistency of paired associative stimulation in inducing cortical plasticity

Mattia Campana, Suna D. Günay, Benjamin Pross, Stefan Vestring, Leonhard Schilbach, Matin Mortazavi, Elias Wagner, Wolfgang Strube, Frank Padberg, Alkomiet Hasan

Article - Version of Record

Suggested Citation:

Campana, M., Günay, S. D., Pross, B., Vestring, S., Schilbach, L., Mortazavi, M., Wagner, E., Strube, W., Padberg, F., & Hasan, A. (2026). Individualized interstimulus interval improves the efficacy and consistency of paired associative stimulation in inducing cortical plasticity. *Transcranial Magnetic Stimulation*, 7, Article 100318. <https://doi.org/10.1016/j.transm.2026.100318>

Wissen, wo das Wissen ist.



UNIVERSITÄTS- UND
LANDESBIBLIOTHEK
DÜSSELDORF

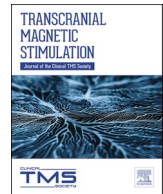
This version is available at:

URN: <https://nbn-resolving.org/urn:nbn:de:hbz:061-20260713-112847-6>

Terms of Use:

This work is licensed under the Creative Commons Attribution 4.0 International License.

For more information see: <https://creativecommons.org/licenses/by/4.0>



Individualized interstimulus interval improves the efficacy and consistency of paired associative stimulation in inducing cortical plasticity

Mattia Campana^{a,b,1,*}, Suna D. Günay^a, Benjamin Pross^c, Stefan Vestring^d, Leonhard Schilbach^{a,b}, Matin Mortazavi^{c,d,e}, Elias Wagner^{c,d,e}, Wolfgang Strube^{c,d,e,f,g}, Frank Padberg^{a,b,c,d,e,f,2}, Alkomiet Hasan^{c,d,e,f,g,2}

^a Department of Psychiatry and Psychotherapy, Medical Faculty, Ludwig Maximilian University, Munich, Germany

^b Department of General Psychiatry 2, LVR-Klinikum Düsseldorf / Kliniken der Heinrich-Heine-Universität, Düsseldorf, Germany

^c Department of Psychiatry, Psychotherapy and Psychosomatics, Medical Faculty, University of Augsburg, Augsburg, Germany

^d Department of Psychiatry and Psychotherapy, Medical Faculty, University of Freiburg, Freiburg, Germany

^e Evidence-based Psychiatry and Psychotherapy, Faculty of Medicine, University of Augsburg, Augsburg, Germany

^f DZPG (German Center for Mental Health), partner site München/Augsburg, LMU Munich, Germany

^g DZPG (German Center for Mental Health), partner site München/Augsburg, University of Augsburg, Germany

ARTICLE INFO

Keywords:

Paired associative stimulation
Cortical plasticity
Individualized neuromodulation
Motor evoked potentials
Transcranial magnetic stimulation
Response variability

ABSTRACT

Purpose: Paired Associative Stimulation (PAS) is a non-invasive neuromodulation tool for inducing changes in motor cortex excitability. However, its applicability is limited by substantial inter-individual variability and inconsistent replicability. We tested whether an individualized PAS protocol (iPAS), in which the interstimulus interval (ISI) is calibrated to each participant's peripheral-cortical conduction time, enhances the efficacy and consistency of cortical plasticity induction compared to the conventional fixed-ISI protocol (PAS25, 25 ms).

Methods: In a randomized, single-blind, within-subject crossover study, 99 healthy participants each completed one PAS25 and one iPAS session in counterbalanced order. Motor evoked potentials (MEP) were recorded at baseline, and at 5-, 10-, and 20-minutes post-stimulation. The primary outcome was MEP amplitude modeled across all timepoints using a linear mixed-effects model with raw MEP amplitudes as the dependent variable.

Results: Both protocols increased MEP amplitudes relative to baseline. The primary analysis revealed a significant Condition $\times$ Timepoint interaction at 20 min ($p = 0.018$). Post hoc contrasts showed that iPAS produced significantly greater MEP amplitudes than PAS25 at 5 min (ratio = 1.15, $p = 0.023$) and 20 min (ratio = 1.23, $p < 0.001$), with a trend at 10 min (ratio = 1.12, $p = 0.058$).

Conclusions: By improving both the magnitude and reliability of motor cortex plasticity induction, and being readily implementable with standard neurophysiological equipment, iPAS may serve as the preferred alternative to conventional PAS, with potential applications in neurology, rehabilitation, and precision psychiatry.

Introduction

Paired associative stimulation (PAS) is a non-invasive brain stimulation technique capable of inducing in vivo, timing-dependent synaptic plasticity in the motor cortex (Carson & Kennedy, 2013; Muller-Dahlhaus et al., 2010; Stefan et al., 2000; Suppa et al., 2017). In classical PAS protocols, repetitive pairings of peripheral nerve stimulation (PNS) rapidly followed by transcranial magnetic stimulation (TMS)

over the contralateral primary motor cortex (M1) result in changes in motor evoked potential (MEP) amplitude (Carson & Kennedy, 2013; Muller-Dahlhaus et al., 2010; Stefan et al., 2000; Suppa et al., 2017). The directionality of these changes is critically dependent on calibrating the interstimulus interval (ISI) between PNS and TMS (Kumpulainen et al., 2012; Weise et al., 2013; Wolters et al., 2005).

The original PAS protocol, which used a 25 ms ISI (from now on referred to as PAS25), has been shown to enhance corticospinal

* Corresponding author at: Department of General Psychiatry 2, LVR-Klinikum Düsseldorf / Kliniken der Heinrich-Heine-Universität, Düsseldorf, Germany.
E-mail address: Mcampana@toh.ca (M. Campana).

¹ Correspondence email reflects current institutional transition; author will be affiliated with the University of Ottawa from May 2026

² These authors contributed equally to this work

excitability in a subset of healthy individuals (Carson & Kennedy, 2013; Stefan et al., 2000). While PAS applications have demonstrated increasing therapeutic potential, particularly in rehabilitation of neurological conditions such as stroke and spinal cord injury (Baroni et al., 2024; Martino Cinnera et al., 2024; Rodionov et al., 2019; Rosso et al., 2022; Shulga et al., 2025; Wahlgren et al., 2024), a major obstacle in clinical adoption remains the considerable inter-individual variability in its effects (Guerra et al., 2020). In line with findings from other neuromodulation modalities (Ziemann & Siebner, 2015), several studies reported a substantial proportion of participants who fail to achieve the expected increase in MEP amplitude following PAS25 (Campana et al., 2019; Hodzic et al., 2025; Lopez-Alonso et al., 2014; Muller-Dahlhaus et al., 2008; Vestring et al., 2024), raising concerns about the robustness and replicability of PAS-induced plasticity.

In the search for predictors of response, both biological and technical sources of heterogeneity have been examined. Biological factors include baseline cortical excitability, cortical thickness and connectivity, age, circadian phase, sleep, and alertness/attention; whereas methodological factors include stimulation intensity, number of paired stimuli, and other protocol-specific characteristics (Conde et al., 2012; Corp et al., 2021; Hodzic et al., 2025; Kamke et al., 2012; Kuhn et al., 2016; List et al., 2013; Minkova et al., 2019; Muller-Dahlhaus et al., 2008; Sale et al., 2007; Stefan et al., 2004). However, these potential determinants of response variability were tested mainly in isolation and have not been systematically replicated (Lopez-Alonso et al., 2014; Minkova et al., 2019). Importantly, one plausible and potentially modifiable source of variability is the use of a fixed interstimulus interval, which may fail to account for inter-individual differences in peripheral to cortical conduction timing, possibly contributing directly to variability in PAS efficacy.

To address this limitation, we previously introduced an individualized PAS (iPAS) protocol in an exploratory, proof-of-concept study (Campana et al., 2019), in which the ISI was adjusted for each participant based on individual peripheral-cortical conduction time. In that work, we hypothesized and demonstrated that individualized ISI could lead to a more reliable increase in corticospinal excitability by improving the temporal contiguity of afferent stimuli. Nevertheless, the small sample size ($n = 21$) limited generalizability and the effect did not reach the significance threshold after correcting for multiple comparisons. Small sample sizes are typical in PAS studies (Guerra et al., 2020; Wischnewski & Schutter, 2016), with statistical power and replicability remaining significant concerns in the field. Replication studies are therefore crucial to more reliably assess the robustness and replicability of PAS-induced plasticity (Guerra et al., 2020).

To address these important issues, we sought to replicate and extend our previous findings by directly comparing iPAS with the conventional PAS25 protocol in a highly powered, single blind, within-subject crossover design.

Methods

Study design

After collecting written informed consent, we enrolled psychiatrically healthy adult volunteers between the age of 18 and 65. Exclusion criteria were a current or past psychiatric illness assessed via the M.I.N.I Interview (Sheehan et al., 1998), the presence of TMS contraindications as listed in the work of Rossi et al. (Rossi et al., 2021), current pregnancy as well as the presence of severe neurological and somatic comorbidities assessed by an experienced psychiatrist. Participants were asked not to change their caffeine consumption and their sport habits and to maintain regular sleeping patterns for at least 24 h prior to each session. The local ethics committee of the Medical Faculty of Ludwig-Maximilians University (LMU), Munich, Germany, approved the study protocol in compliance with the Declaration of Helsinki.

After inclusion, each participant underwent a demographic

interview, an assessment of the body-mass-index (BMI), their smoking habits as well as a testing of hand preference using the Edinburgh handedness inventory (Oldfield, 1971).

Following a within-subject design, each participant participated in two experimental sessions, described below, at approximately the same time of the day and separated by at least five days to prevent carry-over effects, with the order of conditions randomized via a computer-generated 1:1 allocation list to ensure counterbalancing. Subjects were blinded to the condition they were receiving. All sessions were conducted by the same experimenter (S.G.).

Stimulation Protocols

Both stimulation protocols involved paired associative stimulation. One protocol, PAS25, employed a fixed ISI of 25 ms, which is the standard and most widely used PAS protocol to date (Stefan et al., 2000). In the iPAS condition, ISI was individually determined as described in our previous publication (Campana et al., 2019). In summary, peripheral-to-cortical conduction time was estimated by subtracting the mean latency of 10 M-waves elicited by ulnar nerve stimulation from the mean latency of 10 TMS-evoked MEPs recorded after stimulation of the cortical representation of the right first dorsal interosseous muscle (FDI) in the left primary motor cortex. The resulting value was then increased by 6 ms to account for the estimated cortico-cortical conduction delay between the primary somatosensory cortex (S1) and M1, in accordance with previous work (Campana et al., 2019; Kennedy & Carson, 2008; Wolters et al., 2003). Both sessions entailed the same number of TMS measurements and were similar in length. Across all participants, stimulation was delivered over the left M1 to evoke responses in the right FDI, irrespective of hand dominance.

Consistent with our past work (Campana et al., 2019), we conducted surface electromyography (EMG) recordings on the right FDI. Raw EMG signals were amplified and bandpass-filtered (2 Hz–3 kHz) using the Digitimer D-360 amplifier setup (Digitimer Ltd, UK). We digitized recordings at 5 kHz using a 1401 data acquisition interface (Cambridge Electronic Design Ltd., Cambridge UK) controlled by Signal Software (Version 5, Cambridge Electronic design, Cambridge UK). MEPs were induced applying TMS to the left primary motor cortex (M1) using a flat figure-of-eight magnetic coil (outer diameter 70 mm) connected to a Magstim Bistim2 stimulator (the Magstim Company Ltd, UK). The coil was positioned above the left M1 and held tangentially to the skull at a 45° angle with the midline achieving a posterior–anterior current flow. Applying suprathreshold stimulation intensities, the motor “hot-spot” of the right FDI was identified and marked on the scalp using a felt tip pen to ensure replicable coil positioning throughout the experiments. We obtained resting motor threshold (RMT) recording the minimum stimulator intensity that resulted in an MEP amplitude of $\geq 50 \mu\text{V}$ in at least five of ten measurements (Rothwell et al., 1999). The stimulation intensity resulting in average peak-to-peak MEP amplitudes of $1.0 \pm 0.3 \text{ mV}$ (SI-1mV) was measured at each session’s baseline and kept unmodified for the duration of the experiment. 30 single pulse MEP measurements with S1 mV intensity were conducted at baseline as well as 5 min, 10 min, and 20 min after PAS.

We applied PNS through a bipolar electrode (cathode proximal) to the ulnar nerve of the right hand using a CE-certified DS7A peripheral nerve stimulator (Digitimer Ltd, UK). Square wave pulses were applied for 1 ms duration and stimulation intensity were set at 300% of the individual perceptual threshold (Carson & Kennedy, 2013). To retain a consistent level of attention to improve the stability and the extent of PAS after-effects (Stefan et al., 2004), participants were instructed to stay alert, watch their right hand, silently count the number of TMS stimuli and to report this cumulative number to the examiner upon completion of PAS stimulation. While the true number of stimuli delivered was always 180, participants were falsely informed that the cumulative number was different for each of the two experimental conditions. No positive or negative feedback was supplied when the

cumulative number was reported, which was used as an approximate measure for sustained levels of attention (Campana et al., 2019; Stefan et al., 2000, 2004). To reduce anticipation of the stimulus pairs and its potential influence on MEP responses, both PAS paradigms used a 10% jitter between each stimulus pair.

Statistical analyses

Statistical analyses were conducted in R (version 4.4.3). Prior to hypothesis testing, all data were screened for missing values. MEP measurements were visually inspected prior to averaging. Trials were excluded when pre-stimulus EMG activity indicative of muscular contraction was identifiable in the epoch preceding the TMS pulse. No trials were removed on the basis of MEP amplitude cut-offs. Participants were excluded from analysis if they completed only one experimental condition. All tests were two-tailed, and statistical significance was set at $p < 0.05$.

Descriptive statistics were calculated for demographic, physiological, and stimulation-related variables. Normality was assessed using the Shapiro–Wilk test. Differences in baseline characteristics between stimulation conditions and between responder groups were evaluated using independent samples *t*-tests or Wilcoxon rank-sum tests for continuous variables, and Chi-square or Fisher's exact tests for categorical variables, as appropriate.

MEPs served as the primary outcome measure of cortical excitability. For each time point, MEP amplitudes were averaged across 30 pulses. The primary linear mixed-effects model (LMM) was fitted using the `lmer()` function from the `lme4` package in R, with repeated MEP amplitudes across Baseline, 5 min, 10 min, and 20 min as the dependent variable. LMM was chosen for its flexibility in handling the covariance structure among MEP repeated measures across time points without the restrictive sphericity assumption of a classical ANOVA. Moreover, LMM allows the explicit modeling of inter-individual variability in the treatment response through the inclusion of random slopes. A natural log transformation ($\ln(\text{MEP})$) was applied to the dependent variable to better meet model assumptions of normality and homoscedasticity of residuals. Fixed effects included Condition (PAS25 vs. iPAS), Timepoint (Baseline, 5 min, 10 min, 20 min), and their interaction. Covariates were included to account for between-subject differences in overall MEP responsiveness and included age, sex, BMI, smoking status and RMT. Subject-level variability was modeled with random intercepts and random slopes for Condition to account for the repeated-measures crossover design. Model estimation was performed via restricted maximum likelihood (REML). Model assumptions were assessed by visual inspection of residuals, Q–Q plots, and residual-vs-fitted scatterplots (Supplemental Figure S1). A sensitivity analysis using the same model structure on untransformed MEP amplitudes was additionally performed and is reported in the Supplemental Material.

Post hoc pairwise comparisons were conducted on estimated marginal means (EMMs) using the `emmeans` package. EMMs were computed on the log scale and back-transformed to the original scale, yielding geometric mean MEPs and contrast ratios. Pairwise comparisons were corrected using Tukey's method. Within-condition contrasts assessed temporal changes relative to baseline, whereas between-condition contrasts compared PAS25 and iPAS at each time point. Baseline-referenced ratios were not used as the dependent variable in the primary LMM, but only for post hoc interpretation and descriptive/exploratory analyses.

As an exploratory analysis, we examined whether the absolute deviation of the individualized ISI from the conventional 25-ms interval predicted the hypothesized relative benefit of iPAS over PAS25. To this end, we fitted a linear regression model with the between-condition difference in 20-min post-stimulation MEP amplitude (iPAS minus PAS25) as the dependent variable and the absolute deviation of the individualized ISI from 25 ms as the predictor.

Responder analysis was performed as a secondary exploratory analysis using the grand-average approach. Participants were

categorized as responders if their average post-stimulation MEPs amplitude (mean of all post-intervention time points) was $\geq 120\%$ that of baseline in accordance to our previous study (Campana et al., 2019). Responder proportions were compared between PAS25 and iPAS conditions using Chi-square tests. Exploratory comparisons of demographic and stimulation parameters between responders and non-responders were performed using appropriate parametric or non-parametric methods. To examine potential predictors of PAS response using baseline-referenced grand-average as a continuous outcome, we additionally fitted exploratory linear models separately for PAS25 and iPAS, with age, sex, BMI, RMT, and smoking status as predictors. All exploratory analyses are reported in the Supplemental Material.

A priori power analysis was conducted using G*Power (version 3.1.9.7) to determine the minimum required sample size for detecting an interaction effect in a 2×4 (condition $\times$ time) repeated-measures ANOVA with within-between factors. The analysis was based on an alpha level of 0.05, a desired statistical power of 0.90, a small-to-moderate effect size ($f = 0.15$) and a correlation among repeated measures of 0.5. The analysis indicated that a minimum of 82 participants would be required to detect a statistically significant interaction effect under these assumptions. Estimating a dropout rate of 15% we aimed at recruiting over 97 participants. A LMM was ultimately chosen over an ANOVA for its statistical advantages. The targeted sample size provides comparable statistical power for either model, as this study features a balanced design with no missing data.

Results

Participant Characteristics and Baseline Comparisons

A total of $n = 102$ participants were enrolled in the study. One participant withdrew after experiencing nausea during the baseline measurements of the first session. Another participant was excluded due to the inability to evoke MEPs at baseline. Finally, one participant withdrew after completing the first session due to personal reasons, adverse events and tolerability issues were denied. Thus, a total of $n = 99$ participants (71 female; mean age = 27.23 ± 7.33 years) completed both stimulation sessions and were included in the analysis. The majority were right-handed ($n = 91$) and non-smokers ($n = 68$). Mean individualized ISI for the iPAS condition was 24.60 ± 1.71 ms (range: 19.30–28.80 ms). For a visualization of the ISI distribution for the iPAS condition please refer to Figure S3.

Neurophysiological and stimulation parameters at baseline did not differ significantly between PAS25 and iPAS conditions. No group differences were observed for RMT, SI-1mV, baseline MEP amplitude, PAS intensity or for number of paired stimuli counted by the subject (all

Table 1

Comparisons of baseline excitability and PAS parameters, presented separately for both PAS25 and iPAS conditions. Values reported as mean $\pm$ standard deviation.

	Conditions		Statistics	
	PAS25	iPAS	Wilcoxon rank-sum test	p-value
Resting motor threshold (%)	34.99 $\pm$ 7.97	35.31 $\pm$ 7.60	W = 5069.5	0.676
SI-1mV (%)	43.42 $\pm$ 9.39	44.06 $\pm$ 9.27	W = 5118.0	0.590
Baseline MEP amplitude (mV)	1.03 $\pm$ 0.17	1.05 $\pm$ 0.18	W = 5411.5	0.205
PAS intensity (mA)	8.75 $\pm$ 3.86	9.41 $\pm$ 3.84	W = 5583.5	0.089
Stimulus count	177.73 $\pm$ 11.62	177.77 $\pm$ 12.82	W = 5099.5	0.612

$p \geq 0.089$). For further results, please refer to [Table 1](#).

Effects of PAS25 and iPAS on Corticospinal Excitability

Results from LMM analysis showed no main effect of Condition ($p = 0.661$), with none of the covariates reaching statistical significance (all $p \geq 0.121$). Fixed-effect estimates indicated that MEP amplitudes were significantly higher at 10 min and 20 min relative to Baseline (both $p < 0.0001$), but not at 5 min ($p = 0.662$). In addition, the model showed a significant Condition $\times$ Timepoint interaction at 20 min ($p = 0.018$), indicating differential temporal profiles of excitability modulation between PAS25 and iPAS. A sensitivity analysis using the same model structure on untransformed MEP amplitudes yielded a comparable overall pattern of results. Detailed model outputs are provided in the [Supplementary Material](#).

Post hoc EMM pairwise comparisons, back-transformed from the log scale, showed that in the PAS25 condition, geometric mean MEPs were significantly increased relative to Baseline at 10 min (ratio = 1.232, $p < 0.001$) and 20 min (ratio = 1.339, $p < 0.0001$), but not at 5 min (ratio = 0.977, $p = 0.662$). Similarly, in the iPAS condition, geometric mean MEPs were significantly increased relative to Baseline at 10 min (ratio = 1.346, $p < 0.0001$) and 20 min (ratio = 1.600, $p < 0.0001$), but not at 5 min (ratio = 1.093, $p = 0.094$).

Between-condition contrasts showed that MEP amplitudes were significantly greater following iPAS than PAS25 at 5 min (ratio = 1.15, $p = 0.023$) and 20 min (ratio = 1.23, $p < 0.001$). A statistical trend was observed at 10 min (ratio = 1.12, $p = 0.058$), while no difference was found at baseline (ratio = 1.03, $p = 0.661$). For a graphical representation, please see [Fig. 1](#). For detailed EMMs outputs please refer to [Table S1](#).

In the exploratory linear regression, the absolute deviation of the individualized ISI for the iPAS condition from 25 ms was not significantly associated with the between-condition difference in 20-min MEP

amplitude (iPAS minus PAS25) ($\beta = -0.091$, $SE = 0.089$, $p = 0.307$, $R^2 = 0.011$).

In a secondary exploratory analysis, iPAS yielded a significantly higher responder rate than PAS25 (65.7% vs. 50.5%, $p = 0.044$). The distribution of percentage MEP change relative to baseline following iPAS, shown in [Fig. 2](#), further revealed an increased proportion of responders and an amplified response magnitude in those who did respond, as the density curve is right-shifted and seems to be indicative of a heterogeneous distribution pattern. More detailed exploratory analyses of responder-related demographic and stimulation parameters are reported in the [Supplementary Material](#).

Discussion

This study provides robust evidence that an individualized PAS protocol, in which ISIs are tailored to individual conduction latencies, induces greater increases in corticospinal excitability and a reduced variability of response than the conventional PAS25 protocol. Conceptually, this finding suggests that one important source of variability in PAS is the fixed interstimulus interval. By accounting for inter-individual differences in afferent conduction timing, iPAS likely reduces temporal mismatch between peripheral input and cortical stimulation, thereby increasing the probability of inducing facilitatory plasticity or enhancing the magnitude of the effect thereof. Importantly, we replicated our previous findings in a larger, independent sample. To our knowledge, this study employs the largest sample size to date in PAS research, thus lending substantial evidence for the replicability and efficacy of the iPAS protocol in inducing cortical plasticity in M1. Given the considerable inter-individual variability historically reported with PAS ([Lopez-Alonso et al., 2014](#); [Muller-Dahlhaus et al., 2008](#)), the improved response rate and efficacy observed with iPAS mark a methodological advancement in the field of in vivo neuromodulation. An improved reliability may enhance the interpretability of future

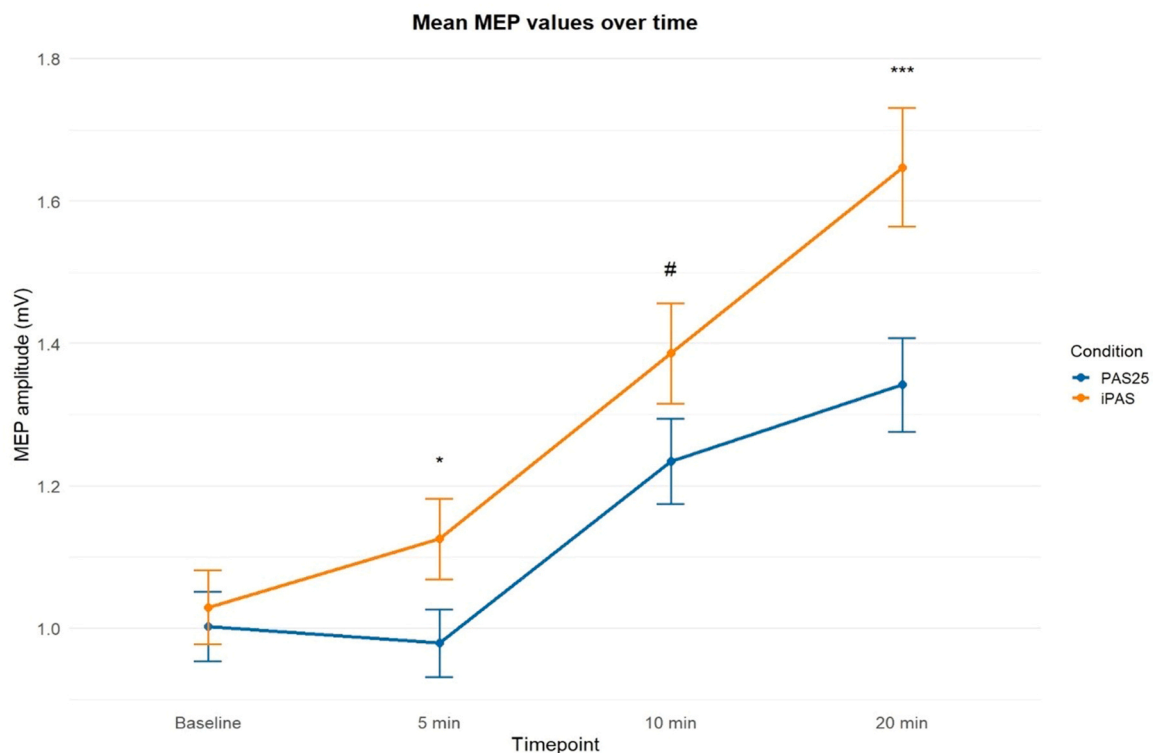


Fig. 1. Time course of motor evoked potential (MEP) amplitudes following PAS25 and iPAS stimulation. Back-transformed estimated marginal means (geometric mean MEP amplitudes $\pm$ SEM) from the primary linear mixed-effects model are shown at Baseline and 5, 10, and 20 min after stimulation for PAS25 (blue) and iPAS (orange). Asterisks indicate significant between-condition post hoc contrasts at the corresponding time points (* $p < 0.05$, *** $p < 0.001$); the hash symbol (#) indicates a statistical trend ($p = 0.058$).

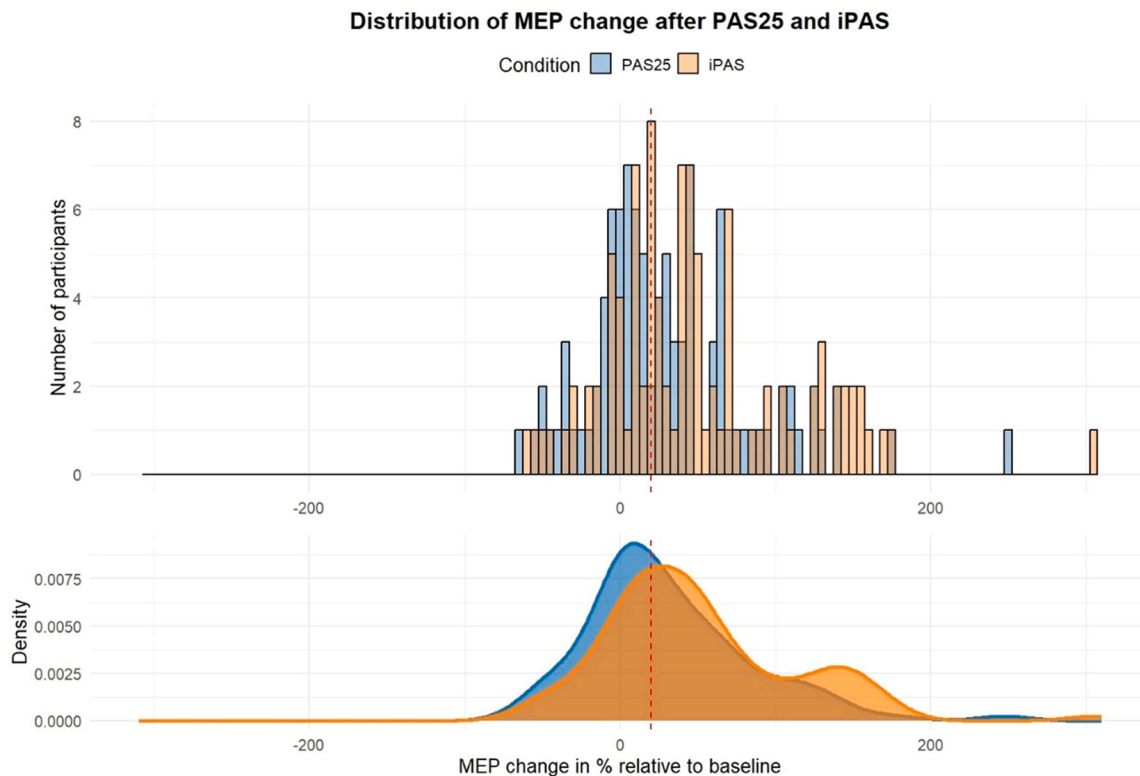


Fig. 2. Exploratory distribution of MEP percentage change after PAS25 and iPAS. Histograms (top panel) and density curves (bottom panel) show the distribution of percentage change in MEP amplitude ($(\text{Post} - \text{Pre}) / \text{Pre} \times 100$) relative to baseline for PAS25 (blue) and iPAS (orange). The dashed vertical line at 20% marks the responder threshold.

mechanistic or interventional studies by reducing variability and limiting the proportion of participants who fail to show any facilitatory response.

These results provide compelling evidence that individualized temporal parameters can optimize motor cortex plasticity induction in healthy subjects. The greater efficacy of iPAS supports the spike-timing-dependent plasticity (STDP) framework, in which associative plasticity is dependent on the synchronicity between presynaptic and postsynaptic activation (Andrade-Talavera et al., 2023; Dan & Poo, 2004; Suppa et al., 2017; Wolters et al., 2005). Interestingly, the absolute deviation of the individualized ISI from 25 ms did not predict the relative benefit of iPAS over PAS25, suggesting that the superiority of iPAS is unlikely to reflect a graded, dose-dependent relationship between the magnitude of ISI correction and plasticity gain. A possible interpretation is that PAS efficacy follows a threshold-like relationship with temporal precision, whereby individualization of the ISI, irrespective of its exact deviation from the conventional interval, increases the probability of achieving sufficient temporal contiguity between afferent input and cortical stimulation to engage STDP-like mechanisms. Accordingly, ISI individualization should be understood as a probabilistic improvement in conditions favorable for plasticity induction, rather than a precise correction of a measurable deficit. However, our findings are exploratory and should be interpreted cautiously (for graphical representation please see figure S2-S3).

In exploratory analyses using continuous and dichotomous PAS outcome measures, we did not identify robust associations between response magnitude and age, sex, BMI, smoking status, or RMT in either condition. This null finding adds to a growing body of literature indicating that response variability in PAS is unlikely to be explained by static demographic or physiological traits alone (Minkova et al., 2019). Instead, it may reflect dynamic neural states or momentary fluctuations in cortical excitability and network synchrony, which are not addressed in a classic open-loop stimulation (Karabanov et al., 2016; Ziemann &

Siebner, 2015).

We acknowledge several limitations in the interpretability of our results. First, our individualized ISI estimates were based on well-established electrophysiological principles; however, they remain indirect proxies for the timing of monosynaptic afferent arrival at cortex and do not account for other sources of individual variability in synaptic integration or network dynamics, such as polysynaptic pathways (Davis et al., 2022; Umeda et al., 2019). As we did not employ more advanced applications such as concurrent EEG to directly assess impulse propagation, mechanistic insight into the precise underpinnings of the observed effects is limited. At the same time, this pragmatic approach was chosen to maximize feasibility and clinical applicability, although future studies should directly compare it with somatosensory evoked potentials recordings that may provide a more precise estimate of afferent cortical arrival timing. Second, because the present study was conducted exclusively in healthy participants and assessed only short-term physiological effects, future work will be required to determine whether the relative advantage of iPAS generalizes to patient populations and translates into more durable or clinically meaningful outcomes. We also did not measure the impact of variables such as menstrual cycle (Campana et al., 2024; Hattemer et al., 2007) or neuroimaging markers (Minkova et al., 2019; Silbert et al., 2006). Third, although iPAS yielded a higher responder rate than PAS25, this finding should be interpreted cautiously because the $\geq 120\%$ threshold represents a pragmatic but inherently arbitrary dichotomization of a continuous response distribution. Finally, although the correct coil position was consistently visually inspected, we did not employ neuronavigation and thus, we are not able to report on coil error data, as some variability could have been due to shifts of the coil from the M1 “hotspot”.

Nevertheless, by replicating our previous findings in a large, well powered sample, we provide strong evidence for the role of ISI individualization in increasing plasticity of the motor cortex and reducing response variability. These results support the implementation of iPAS

as a methodologically improved alternative to the conventional PAS25. This may be particularly relevant in clinical settings, where reducing non-response and improving reliability are critical for trial design and treatment optimization. By being readily implementable with standard neurophysiological equipment, iPAS offers a more robust experimental framework for probing and improving corticospinal excitability and may prove useful in future stroke or spinal cord injury rehabilitation trials (Baroni et al., 2024; Martino Cinnera et al., 2024; Rosso et al., 2022; Shulga et al., 2025; Wahlgren et al., 2024). More broadly, the ability to non-invasively and more reliably assess STDP-like plasticity may also have important implications for psychiatry. Alterations in plasticity-related processes have been implicated in neurodevelopmental, neurodegenerative, and psychiatric disorders (Brzosko et al., 2019). A more reliable neurophysiological assay of such processes could contribute to improved patient characterization and, in the future, support response prediction in neurostimulation or pharmacological trials (Silverstein et al., 2019), thereby informing neurophysiology-based stratification and precision medicine approaches in psychiatry.

CRedit authorship contribution statement

Alkomiet Hasan: Writing – review & editing, Supervision, Methodology, Conceptualization. **Frank Padberg:** Writing – review & editing, Supervision, Resources, Project administration. **Wolfgang Strube:** Writing – review & editing, Methodology, Conceptualization. **Elias Wagner:** Writing – review & editing, Supervision. **Matin Mortazavi:** Writing – review & editing, Formal analysis. **Leonhard Schilbach:** Writing – review & editing. **Stefan Vestring:** Writing – review & editing. **Benjamin Pross:** Writing – review & editing, Methodology. **Suna D. Günay:** Writing – review & editing, Project administration, Investigation, Data curation. **Mattia Campana:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT v5.1. to assist with language editing to improve readability as well as for code refinement for data visualization. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The data presented in this manuscript are part of the doctoral thesis of S.G.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.transm.2026.100318](https://doi.org/10.1016/j.transm.2026.100318).

References

Andrade-Talavera, Y., Fisahn, A., & Rodriguez-Moreno, A. (2023). Timing to be precise? An overview of spike timing-dependent plasticity, brain rhythmicity, and glial cells interplay within neuronal circuits. *Mol Psychiatry*, 28(6), 2177–2188. <https://doi.org/10.1038/s41380-023-02027-w>

- Baroni, A., Antonioni, A., Fregna, G., Lamberti, N., Manfredini, F., Koch, G., D'Ausilio, A., & Straudi, S. (2024). The Effectiveness of Paired Associative Stimulation on Motor Recovery after Stroke: A Scoping Review. *Neurol Int*, 16(3), 567–589. <https://doi.org/10.3390/neurolint16030043>
- Brzosko, Z., Mierau, S. B., & Paulsen, O. (2019). Neuromodulation of Spike-Timing-Dependent Plasticity: Past, Present, and Future. *Neuron*, 103(4), 563–581. <https://doi.org/10.1016/j.neuron.2019.05.041>
- Campana, M., Papazova, I., Pross, B., Hasan, A., & Strube, W. (2019). Motor-cortex excitability and response variability following paired-associative stimulation: a proof-of-concept study comparing individualized and fixed inter-stimulus intervals. *Exp Brain Res*, 237(7), 1727–1734. <https://doi.org/10.1007/s00221-019-05542-x>
- Campana, M., Schneider-Axmann, T., Wobrock, T., Malchow, B., Langguth, B., Landgrebe, M., Eichhammer, P., Frank, E., Cordes, J., Wolwer, W., Gaebel, W., Winterer, G., Hajak, G., Ohmann, C., Verde, P. E., Rietschel, M., Ahmed, R., Mortazavi, M., Strube, W., ... Wagner, E. (2024). Assessing the impact of sex on high-frequency repetitive transcranial magnetic stimulation's clinical response in schizophrenia - results from a secondary analysis. *World J Biol Psychiatry*, 25(4), 233–241. <https://doi.org/10.1080/15622975.2024.2327028>
- Carson, R. G., & Kennedy, N. C. (2013). Modulation of human corticospinal excitability by paired associative stimulation. *Front Hum Neurosci*, 7, 823. <https://doi.org/10.3389/fnhum.2013.00823>
- Conde, V., Vollmann, H., Sehm, B., Taubert, M., Villringer, A., & Ragert, P. (2012). Cortical thickness in primary sensorimotor cortex influences the effectiveness of paired associative stimulation. *Neuroimage*, 60(2), 864–870. <https://doi.org/10.1016/j.neuroimage.2012.01.052>
- Corp, D. T., Bereznicki, H. G. K., Clark, G. M., Youssef, G. J., Fried, P. J., Jannati, A., Davies, C. B., Gomes-Osman, J., Kirkovski, M., Albein-Urios, N., Fitzgerald, P. B., Koch, G., Di Lazzaro, V., Pascual-Leone, A., Enticott, P. G., & Big, T. M. S. D. C. (2021). Large-scale analysis of interindividual variability in single and paired-pulse TMS data. *Clin Neurophysiol*, 132(10), 2639–2653. <https://doi.org/10.1016/j.clinph.2021.06.014>
- Dan, Y., & Poo, M. M. (2004). Spike timing-dependent plasticity of neural circuits. *Neuron*, 44(1), 23–30. <https://doi.org/10.1016/j.neuron.2004.09.007>
- Davis, M., Wang, Y., Bao, S., Buchanan, J. J., Wright, D. L., & Lei, Y. (2022). The Interactions Between Primary Somatosensory and Motor Cortex during Human Grasping Behaviors. *Neuroscience*, 485, 1–11. <https://doi.org/10.1016/j.neuroscience.2021.11.039>
- Guerra, A., Lopez-Alonso, V., Cheeran, B., & Suppa, A. (2020). Variability in non-invasive brain stimulation studies: Reasons and results. *Neurosci Lett*, 719, Article 133330. <https://doi.org/10.1016/j.neulet.2017.12.058>
- Hattemer, K., Knake, S., Reis, J., Rochon, J., Oertel, W. H., Rosenow, F., & Hamer, H. M. (2007). Excitability of the motor cortex during ovulatory and anovulatory cycles: a transcranial magnetic stimulation study. *Clin Endocrinol (Oxford)*, 66(3), 387–393. <https://doi.org/10.1111/j.1365-2265.2007.02744.x>
- Hodzik, K., Thordstein, M., Strandberg, J., Jerlhag, E., & Wass, C. E. (2025). A Methodological Evaluation of Four Different Paired Associative Stimulation Paradigms in Healthy Controls. *Brain Sci*, 15(5). <https://doi.org/10.3390/brainsci15050461>
- Kamke, M. R., Hall, M. G., Lye, H. F., Sale, M. V., Fenlon, L. R., Carroll, T. J., Riek, S., & Mattingley, J. B. (2012). Visual attentional load influences plasticity in the human motor cortex. *J Neurosci*, 32(20), 7001–7008. <https://doi.org/10.1523/JNEUROSCI.1028-12.2012>
- Karabanov, A., Thielscher, A., & Siebner, H. R. (2016). Transcranial brain stimulation: closing the loop between brain and stimulation. *Curr Opin Neurol*, 29(4), 397–404. <https://doi.org/10.1097/WCO.0000000000000342>
- Kennedy, N. C., & Carson, R. G. (2008). The effect of simultaneous contractions of ipsilateral muscles on changes in corticospinal excitability induced by paired associative stimulation (PAS). *Neurosci Lett*, 445(1), 7–11. <https://doi.org/10.1016/j.neulet.2008.08.064>
- Kuhn, M., Wolf, E., Maier, J. G., Mainberger, F., Feige, B., Schmid, H., Burklin, J., Maywald, S., Mall, V., Jung, N. H., Reis, J., Spiegelhalder, K., Kloppel, S., Sterr, A., Eckert, A., Riemann, D., Normann, C., & Nissen, C. (2016). Sleep recalibrates homeostatic and associative synaptic plasticity in the human cortex. *Nat Commun*, 7, Article 12455. <https://doi.org/10.1038/ncomms12455>
- Kumpulainen, S., Mrachacz-Kersting, N., Peltonen, J., Voigt, M., & Avela, J. (2012). The optimal interstimulus interval and repeatability of paired associative stimulation when the soleus muscle is targeted. *Exp Brain Res*, 221(3), 241–249. <https://doi.org/10.1007/s00221-012-3165-x>
- List, J., Kubke, J. C., Lindenberg, R., Kulzow, N., Kerti, L., Witte, V., & Floel, A. (2013). Relationship between excitability, plasticity and thickness of the motor cortex in older adults. *Neuroimage*, 83, 809–816. <https://doi.org/10.1016/j.neuroimage.2013.07.033>
- Lopez-Alonso, V., Cheeran, B., Rio-Rodriguez, D., & Fernandez-Del-Olmo, M. (2014). Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain Stimul*, 7(3), 372–380. <https://doi.org/10.1016/j.brs.2014.02.004>
- Martino Cinnera, A., Bonanno, M., Calabro, R. S., Bisirri, A., D'Arienzo, M., D'Acunto, A., Ciancarelli, I., Morone, G., & Koch, G. (2024). Paired associative stimulation to enhance motor outcome in spinal cord injury: a systematic review of first evidence. *Expert Rev Med Devices*, 1–12. <https://doi.org/10.1080/17434440.2024.2358048>
- Minkova, L., Peter, J., Abdulkadir, A., Schumacher, L. V., Kaller, C. P., Nissen, C., Kloppel, S., & Lahr, J. (2019). Determinants of Inter-Individual Variability in Corticomotor Excitability Induced by Paired Associative Stimulation. *Front Neurosci*, 13, 841. <https://doi.org/10.3389/fnins.2019.00841>
- Muller-Dahlhaus, F., Ziemann, U., & Classen, J. (2010). Plasticity resembling spike-timing dependent synaptic plasticity: the evidence in human cortex. *Front Synaptic Neurosci*, 2, 34. <https://doi.org/10.3389/fnsyn.2010.00034>

- Muller-Dahlhaus, J. F., Orekhov, Y., Liu, Y., & Ziemann, U. (2008). Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. *Exp Brain Res*, 187(3), 467–475. <https://doi.org/10.1007/s00221-008-1319-7>
- Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, 9(1), 97–113. [https://doi.org/10.1016/0028-3932\(71\)90067-4](https://doi.org/10.1016/0028-3932(71)90067-4)
- Rodionov, A., Savolainen, S., Kirveskari, E., Makela, J. P., & Shulga, A. (2019). Restoration of hand function with long-term paired associative stimulation after chronic incomplete tetraplegia: a case study. *Spinal Cord Ser Cases*, 5, 81. <https://doi.org/10.1038/s41394-019-0225-5>
- Rossi, S., Antal, A., Bestmann, S., Bikson, M., Brewer, C., Brockmoller, J., Carpenter, L. L., Cincotta, M., Chen, R., Daskalakis, J. D., Di Lazzaro, V., Fox, M. D., George, M. S., Gilbert, D., Kimiskidis, V. K., Koch, G., Ilmoniemi, R. J., Lefaucheur, J. P., Leocani, L., & basis of this article began with a Consensus Statement from the Ifcn Workshop on "Present, F. o. T. M. S. S. E. G. S. O. u. t. A. (2021). Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. *Clin Neurophysiol*, 132(1), 269–306. <https://doi.org/10.1016/j.clinph.2020.10.003>
- Rosso, C., Moulton, E. J., Kemlin, C., Leder, S., Corvol, J. C., Mehdi, S., Obadia, M. A., Obadia, M., Yger, M., Meseguer, E., Perlberg, V., Valabregue, R., Magno, S., Lindberg, P., Meunier, S., & Lamy, J. C. (2022). Cerebello-Motor Paired Associative Stimulation and Motor Recovery in Stroke: a Randomized, Sham-Controlled, Double-Blind Pilot Trial. *Neurotherapeutics*, 19(2), 491–500. <https://doi.org/10.1007/s13311-022-01205-y>
- Rothwell, J. C., Hallett, M., Berardelli, A., Eisen, A., Rossini, P., & Paulus, W. (1999). Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl*, 52, 97–103. (<https://www.ncbi.nlm.nih.gov/pubmed/10590980>).
- Sale, M. V., Ridding, M. C., & Nordstrom, M. A. (2007). Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. *Exp Brain Res*, 181(4), 615–626. <https://doi.org/10.1007/s00221-007-0960-x>
- Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., & Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*, 59 20, 22–33. (<https://www.ncbi.nlm.nih.gov/pubmed/9881538>). ;quiz 34-57.
- Shulga, A., Natkynmaki, A., Pelkonen, A. L., Pohjonen, M., Savolainen, S., Kirveskari, E., Brandstack, N., Makela, J. P., & Arokoski, J. (2025). Paired associative stimulation improves outcomes when applied at the subacute stage after incomplete cervical spinal cord injury. *Neurotherapeutics*, Article e00778. <https://doi.org/10.1016/j.neurot.2025.e00778>
- Silbert, L. C., Nelson, C., Holman, S., Eaton, R., Oken, B. S., Lou, J. S., & Kaye, J. A. (2006). Cortical excitability and age-related volumetric MRI changes. *Clin Neurophysiol*, 117(5), 1029–1036. <https://doi.org/10.1016/j.clinph.2006.02.003>
- Silverstein, J., Cortes, M., Tsagaris, K. Z., Climent, A., Gerber, L. M., Oromendia, C., Fonzei, P., Ratan, R. R., Kitago, T., Iacoboni, M., Wu, A., Dobkin, B., & Edwards, D. J. (2019). Paired Associative Stimulation as a Tool to Assess Plasticity Enhancers in Chronic Stroke. *Front Neurosci*, 13, 792. <https://doi.org/10.3389/fnins.2019.00792>
- Stefan, K., Kunesch, E., Cohen, L. G., Benecke, R., & Classen, J. (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain*, 123(Pt 3), 572–584. <https://doi.org/10.1093/brain/123.3.572>
- Stefan, K., Wycislo, M., & Classen, J. (2004). Modulation of associative human motor cortical plasticity by attention. *J Neurophysiol*, 92(1), 66–72. <https://doi.org/10.1152/jn.00383.2003>
- Suppa, A., Quartarone, A., Siebner, H., Chen, R., Di Lazzaro, V., Del Giudice, P., Paulus, W., Rothwell, J. C., Ziemann, U., & Classen, J. (2017). The associative brain at work: Evidence from paired associative stimulation studies in humans. *Clin Neurophysiol*, 128(11), 2140–2164. <https://doi.org/10.1016/j.clinph.2017.08.003>
- Umeda, T., Isa, T., & Nishimura, Y. (2019). The somatosensory cortex receives information about motor output. *Sci Adv*, 5(7), Article eaaw5388. <https://doi.org/10.1126/sciadv.aaw5388>
- Vestring, S., Wolf, E., Dinkelacker, J., Frase, S., Hessling-Zeinen, C., Insan, S., Kümlehn, M. M., Feige, B., Domschke, K., Normann, C., & Frase, L. (2024). Lasting effects of transcranial direct current stimulation on the inducibility of synaptic plasticity by paired-associative stimulation in humans. *J Neuroengineering Rehabil*, 21 (1), 162. <https://doi.org/10.1186/s12984-024-01459-x>
- Wahlgren, C., Levi, R., & Thordstein, M. (2024). Paired associative stimulation improves motor function in the upper extremity in chronic incomplete spinal cord injury: a corroborative study. *J Rehabil Med*, 56, jrm41021. <https://doi.org/10.2340/jrm.v56.41021>
- Weise, D., Mann, J., Ridding, M., Eskandar, K., Huss, M., Rumpf, J. J., Di Lazzaro, V., Mazzone, P., Ranieri, F., & Classen, J. (2013). Microcircuit mechanisms involved in paired associative stimulation-induced depression of corticospinal excitability. *J Physiol*, 591(19), 4903–4920. <https://doi.org/10.1113/jphysiol.2013.253989>
- Wischnewski, M., & Schutter, D. (2016). Efficacy and time course of paired associative stimulation in cortical plasticity: Implications for neuropsychiatry. *Clin Neurophysiol*, 127(1), 732–739. <https://doi.org/10.1016/j.clinph.2015.04.072>
- Wolters, A., Sandbrink, F., Schlottmann, A., Kunesch, E., Stefan, K., Cohen, L. G., Benecke, R., & Classen, J. (2003). A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. *J Neurophysiol*, 89(5), 2339–2345. <https://doi.org/10.1152/jn.00900.2002>
- Wolters, A., Schmidt, A., Schramm, A., Zeller, D., Naumann, M., Kunesch, E., Benecke, R., Reiners, K., & Classen, J. (2005). Timing-dependent plasticity in human primary somatosensory cortex. *J Physiol*, 565(Pt 3), 1039–1052. <https://doi.org/10.1113/jphysiol.2005.084954>
- Ziemann, U., & Siebner, H. R. (2015). Inter-subject and Inter-session Variability of Plasticity Induction by Non-invasive Brain Stimulation: Boon or Bane? *Brain Stimul*, 8 (3), 662–663. <https://doi.org/10.1016/j.brs.2015.01.409>