Motor-cortex excitability and response variability following paired-associative stimulation: a proof-of-concept study comparing individualized and fixed inter-stimulus intervals

M. Campana[1,2] · I. Papazova[1] · B. Pross[1] · A. Hasan[1] · W. Strube[1]

Abstract
While diverging efficacy and inter-individual response variability have repeatedly been reported for paired-associative stimulation (PAS), approaches to overcome these issues are yet lacking. Hence, the aim of the present study was to determine whether response variability could be reduced through the application of an individualized PAS paradigm. Changes of transcranial magnetic stimulation (TMS) elicited motor-evoked potentials (MEP) following different PAS paradigms were assessed in three experimental conditions. According to a within-subjects design, 21 participants received three consecutive PAS paradigms differing with respect to the applied inter-stimulus intervals (ISI) between peripheral nerve stimulation (PNS) and TMS. Based on foregoing considerations, we compared fixed ISI of 25 ms (PAS 25) and 22 ms (PAS 22) to an individualized PAS paradigm accounting for conduction time differences on the single subject level (iPAS). Overall, we did not observe significantly increased post-stimulation MEP magnitudes in any of the three experimental paradigms. Explorative analyses revealed increased inter-individual response variability in case of PAS 25 and PAS 22 compared to higher rates of expected MEP magnitude increases in case of our iPAS paradigm. The findings of our proof-of-concept study points towards a potential association of decreased inter-individual variability with individually selected ISI that account for differences in conduction time. However, as our findings did not reach the significance threshold, our study highlights the issue of intra-individual variability in PAS paradigms. Further replication studies with larger sample sizes and repetitive designs are needed to confirm our findings.

Keywords Paired-associative stimulation · LTP-like plasticity · Response variability · Individualized non-invasive brain stimulation · Afferent conduction time

Introduction

Paired-associative stimulation (PAS) poses a well-established non-invasive brain stimulation technique, which has been developed to resemble associative plasticity as it employs repetitive near-synchronous pairings of peripheral nerve stimuli (PNS) followed by transcranial magnetic stimulation (TMS) pulses after distinct inter-stimulus intervals (ISI). Dependent on specific ISI timings, PAS has been demonstrated to result in long-lasting LTP- and LTD-like plasticity after-effects within the human motor system, which are considered input-specific dependent (Carson and Kennedy 2013; Muller-Dahlhaus et al. 2008; Stefan et al. 2000) and have been linked to glutamatergic neurotransmission (Stefan et al. 2002). Subsequent to the first report of the PAS technique (Stefan et al. 2000), there have been a wide range of derivative investigations concerning, for example, the ISI that are efficacious (Kumpulainen et al. 2012; Wolters et al. 2005), the motor-cortical representations in which stable after-effects can be elicited (Carson et al. 2013; Stefan et al. 2000; Stinear and Hornby 2005), and variations in the extent to which they can be induced in various clinical populations (Castel-Lacanal et al. 2009; Monte-Silva et al. 2009). As highlighted in a narrative review (Carson and Kennedy 2013), one critical aspect concerning the PAS technique is,

✉ W. Strube
wolfgang.strube@med.uni-muenchen.de

[1] Department of Psychiatry and Psychotherapy, Ludwig-Maximillian's-University of Munich, LMU, Nußbaumstr. 7, 80336 Munchen, Germany

[2] Department of Psychiatry, Catholic University Medical School, Rome, Italy

however, that more recent studies using increased sample sizes of at least 20 subjects were unable to replicate the original findings to the same extent and observed varying numbers of participants displaying the expected after-effects of cortical excitability modulation (Lopez-Alonso et al. 2014; Muller-Dahlhaus et al. 2008). Moreover, the search for reliable outcome predictors has proven difficult. Parameters expressing cortical excitability at rest, the minimum stimulus intensity to elicit a motor-evoked potential of 1 mV, age and time of day have been indicated as outcome predictors in previous studies (Muller-Dahlhaus et al. 2008; Sale et al. 2007). However, those results could not be replicated in a later study investigating a larger sample size (Lopez-Alonso et al. 2014). While numerous candidate mediating factors for the observed inter-individual response variability have been investigated, such as cortical anatomy (Conde et al. 2012), attention (Stefan et al. 2004), or the role of specific genetic polymorphisms (Cheeran et al. 2008), approaches to overcome this issue are yet lacking.

In this context, a characteristic feature of the PAS stimulation technique poses its attributed spike-time dependency on two associative stimuli whose cortical integration is supposed to induce synaptic plasticity according to Hebbian principles (Cooke and Bliss 2006; Stefan et al. 2000; Wolters et al. 2005). Given this property, it has been hypothesized that individualized ISI could potentially result in more stable and less variable after-effects compared to fixed ISI (Carson and Kennedy 2013). This hypothesis is based on the proposed neuronal mechanisms underlying PAS, which are viewed to depend on optimal spike-timing to allow synchronous (or near-synchronous) pairings of the afferent and cortical stimuli (Bliss and Collingridge 1993; Cooke and Bliss 2006; Muller et al. 2007; Stefan et al. 2000; Wolters et al. 2003, 2005). Against this background, the aim of the present study was to investigate the efficacy of three excitability enhancing PAS protocols and to determine whether the inherent inter-individual response variability would be reduced through the application of an individualized ISI PAS paradigm and comparing its efficacy to induce MEP magnitude changes to PAS conditions employing fixed ISI. For this purpose, we used previously established variants of the PAS protocol (PAS 25 and PAS 22), which conceptually differed with respect to the ISI applied between PNS and TMS pulses (Fixed ISI of 25 ms and 22 ms) and compared their efficacy and response variability to a PAS paradigm employing individual ISI based ISI on individual measurements of conduction time delays (iPAS). In case of the PAS 25 paradigm, the applied ISI were fixed at 25 ms based on foregoing seminal publications that had demonstrated distinct facilitatory after-effects (Stefan et al. 2002, 2000; Wolters et al. 2003). In case of the second condition (PAS 22), we used a fixed ISI of 22 ms, which served as an approximate paradigm of foregoing individualized PAS applications that aimed to account for the N20-latency of somatosensory-evoked cortical potentials and the transmission delay to the motor cortex by adding 2 ms (N20 + 2) (Cash et al. 2017; Heidegger et al. 2010; Ilic et al. 2011; Korchounov and Ziemann 2011; Muller-Dahlhaus et al. 2008; Voytovych et al. 2012). These two fixed ISI PAS conditions were then compared to an individualized iPAS paradigm. Regarding the experimental design of this paradigm, we followed previously published work (Kennedy and Carson 2008) regarding the individual determination of conduction time delays between the peripheral nerve stimulation and cortical TMS pulses (see "Methods" section). By comparing LTP-like plasticity changes between PAS paradigms using fixed inter-stimulus intervals (ISI) and a PAS instantiation that based ISI on individual conduction time delays, the aim of the present study was to determine whether response variability could be reduced through the application of an individualized PAS paradigm.

Methods

Participants and study design

In total, 21 participants were included in this study after giving written informed consent. Subjects were aged between 18 and 48 years (mean: 28.8 ± 6.1), $n = 14$ were female (66.7%), $n = 6$ were not-right handed (28.6%), and body height ranged from 158 to 187 cm (mean: 171.5 ± 7.4 cm). Further, all subjects were non-smokers and medication free. The sample size was based both on a respective power analysis using G*Power (Faul et al. 2007) specifying an expected effect size of $f = 0.3$, a power of $\beta = 0.8$, a correlation among repeated measures of 0.5, and an alpha error probability of $\alpha = 0.05$ for the main 6×3 repeated measures ANOVA model ('time course × condition' RM-ANOVA) detailed in the "Statistics" section, which resulted in a required sample size of $n = 18$ participants for an between-within-group interaction. Second, as most of foregoing PAS studies had tested at least 20 participants [see (Carson and Kennedy 2013) for review] we decided to further increase the sample size to 21 to allow for comparability of our findings with foregoing PAS experiments. The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Ludwig-Maximilian's-University of Munich (LMU). Upon inclusion, all participants underwent a standardized biographic interview, assessments of body height and weight, testing of hand preference by the Edinburgh inventory (Oldfield 1971), and a comprehensive interview to rule out exclusion criteria of neurological or psychiatric illness. Further, participants with contraindication to TMS (Rossi et al. 2011) or peripheral nerve stimulation were also excluded. According to a within-subject design, the study was comprised of three different testing

sessions for each participant, where TMS assessments and different PAS stimulation paradigms, as further described below, were administered in a subject-blind randomized manner based on a predefined computer-generated randomization list. Each session was at least 5 days apart from the previous one and at approximately the same time of the day. All experiments were conducted by the same experimenter (M.C.).

TMS recordings

Subjects were examined while sitting in half-reclined position with their arms suspended passively by armrests. Surface electromyography (EMG) recordings were conducted by applying electrodes on the right first dorsal interosseous muscle (FDI). Raw EMG signals were amplified and band-pass-filtered (2 Hz–3 kHz) using the Digitimer D-360 amplifier setup (Digitimer Ltd, UK). Recordings were digitized 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). Motor-evoked potentials (MEP) 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 Bistim^2 stimulator (the Magstim Company Ltd, UK). Throughout every experiment, the coil was positioned above M1 and held tangentially to the skull, with the longer axis forming a 45° angle with the midline achieving a posterior–anterior current flow. Applying supra-threshold stimulation intensities, the optimal stimulation site eliciting stable motor-evoked potentials (MEP) in the right FDI was identified and marked on the scalp using a felt tip pen to ensure replicable coil positioning throughout the experiments. Resting motor threshold (RMT) was defined as the minimum stimulator intensity that resulted in an MEP amplitude of ≥ 50 µV 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 ± 0.3 mV (S1 mV) was measured at each session's baseline and kept unmodified for the duration of the experiment. To monitor induced after-effects, single pulse MEP measurements employing the S1 mV stimulation intensity were conducted at baseline at the time points 0 min, 5 min, 10 min, 20 min and 30 min after PAS (30 stimuli at each time point).

PAS stimulation paradigms

All experimental conditions consisted of 180 pairs of single electric stimuli to the ulnar nerve at the level of the wrist followed by TMS pulses at specific inter-stimulus intervals (ISI). In case of the PAS 25 paradigm, ISI were fixed at 25 ms based on respective publications (Stefan et al. 2000, 2002; Wolters et al. 2003). In case of the second condition (PAS 22), we used a fixed ISI of 22 ms. As only two of these foregoing studies had reported data on the obtained N20 latencies (Cash et al. 2017; Ilic et al. 2011), ranging from 18.7 ms to 21.0 ms (reported mean 19.85 ms, $n = 14$ participants) and from 19.0 ms to 19.8 ms (reported mean 19.45 ms, $n = 14$ participants), respectively, we based our fixed ISI of 22 ms on adding 2 ms to the rounded mean value of observed N20 latencies (pooled average 19.7 ms in $n = 28$ reported subjects) to generate an approximation of the N20 + 2 protocol. For the iPAS paradigm, we recorded ten supra-threshold test stimuli of peripheral nerve stimulation that were able to elicit stable motor responses (*M* waves) of ≥ 100 µV magnitudes in the right FDI. By subtracting the time-interval between the peripheral electrical stimulus and these elicited motor responses (Measure II: time PNS to *M* wave) from the time-interval between the TMS pulse applied to the left motor-cortex and the consecutively recorded MEP (Measure I: time TMS pulse to MEP) we obtained a direct and millisecond accurate measure of nerval conduction time for the distance from the peripheral stimulation site to the motor cortex [Δ conduction time = (Measure I − Measure II), see Table 1]. We then added a 6 ms estimate to account for the conduction delay between the somatosensory and the primary motor cortex. As detailed in Table 1, the resulting stimulation latencies [Δ conduction time + 6 ms = (Measure I − Measure II) + 6 ms, see Table 1, column 4] ranged from 22.20 to 28.28 ms (mean: 24.14 ± 1.78 ms). The applied 6 ms estimate was based on a supporting mathematical model (Wolters et al. 2003) which described an ISI dependence of PAS after-effects in the somatosensory cortex (S1) in relationship to measured effects over the hand area of the primary motor cortex (M1). Based on their model, the authors proposed that activation of cortico–cortical connections from the somatosensory cortex onto the primary motor cortex was mediated by a conduction time latency ranging between 6 and 7 ms. While this mathematical approach only allows indirect characterization of the ipsilateral latencies governing S1–M1 communication, it was previously suggested (Kennedy and Carson 2008) that adding 6 ms to individually obtained conduction times—as obtained by the above described method [Δ conduction time = (Measure I − Measure II)]—would account for cortico–cortical latencies between S1 and M1.

To maintain blinding, subjects could not see the examiner analyzing the individual data and were not informed of the differences between the three protocols. Additionally, individual ISI for iPAS stimulation were always obtained in the first experimental session, yet the different paradigms were investigated in randomized order using a list generated by A.H. In all three PAS conditions, peripheral nerve stimuli were applied at the level of the wrist through a bipolar electrode (cathode proximal) to the ulnar nerve using a CE-certified DS7A peripheral nerve stimulator (Digitimer Ltd,

Table 1 Individual conduction times: the second column displays the time-interval between the TMS pulse applied to the left motor-cortex and the consecutively recorded MEP (Measure I, abbreviated M I)

Subject no.	Time TMS pulse to MEP (Measure I)	Time PNS to *M* wave (Measure II)	ISI (M I − M II) + 6 ms
1	24.28 (1.64)	2.00 (0.33)	28.28
2	19.84 (0.47)	3.52 (0.56)	22.32
3	21.20 (1.52)	3.90 (0.34)	23.30
4	20.16 (0.99)	3.78 (0.29)	22.38
5	21.10 (1.32)	4.12 (0.45)	22.98
6	20.62 (0.94)	2.72 (0.53)	23.90
7	20.48 (1.67)	3.64 (0.13)	22.84
8	25.78 (0.93)	4.84 (0.58)	26.94
9	20.14 (1.25)	2.84 (0.48)	23.30
10	23.72 (0.37)	2.68 (0.19)	27.04
11	19.32 (1.16)	3.12 (0.17)	22.20
12	21.04 (0.76)	3.72 (0.73)	23.32
13	23.62 (0.47)	5.38 (0.50)	24.24
14	23.28 (0.62)	3.28 (0.23)	26.00
15	20.98 (1.79)	4.02 (0.66)	22.96
16	22.04 (1.04)	3.90 (0.34)	24.14
17	24.28 (1.15)	7.18 (0.43)	23.10
18	24.64 (0.66)	3.80 (0.28)	26.84
19	23.44 (0.73)	5.18 (0.24)	24.26
20	21.16 (1.15)	4.24 (0.20)	22.92
21	21.50 (1.62)	3.76 (0.47)	23.74
Mean	22.03 (1.06)	3.89 (0.39)	24.14 (1.78)

The third column reports the time-interval between the peripheral electrical stimulus and the elicited motor responses (Measure II, abbreviated M II). Both measures, M I and M II, for each participant are presented as means (± standard deviation) of ten recordings (see "Methods" section). The fourth column presents the individual inter-stimulus intervals (ISI) used for each subject in case of the iPAS condition, i.e.[mean Measure I − mean Measure II] + the described 6 ms estimate for cortico–cortical connections from premotor cortical areas onto the primary motor cortex (see "Methods" section). Data describing conduction times are presented in milliseconds (± standard deviations in brackets)

UK). Square wave pulses were applied for 1 ms duration and stimulation intensity was set at 300% of the individual perceptual threshold as specified in most previous PAS experiments (Carson and Kennedy 2013). All TMS stimuli were applied to the identified optimal coil placement site to elicit stable MEP measurements. Further, as retaining a consistent level of attention and its focus have been demonstrated to impact the stability and the extent of PAS after-effects (Stefan et al. 2004), participants were instructed 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 three experimental conditions and that it could be either odd or even. 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 (Stefan et al. 2002, 2004). To control for fatigue due to monotonous stimulation, all PAS paradigms used a 10% jitter between each stimulus pair.

Statistics

For statistical analyses, we used IBM SPSS 25 and set the level of significance at $\alpha = 0.05$. Descriptive statistical analyses were conducted on sociodemographic characteristics. As the assumption of normal data distribution was met for our main outcome variables (MEP measures at baseline and at all time points following stimulation; Kolmogorov–Smirnov tests: all $p \geq 0.073$), repeated measures analyses of variance (RM-ANOVAs) with the within-subjects factor 'condition' were employed to compare baseline differences between the three experimental sessions concerning TMS (RMT, S1 mV, MEP) and PAS stimulation parameters (peripheral nerve stimulation intensity, PAS stimulus count). To test the time course of MEP amplitude, changes over time across experimental conditions, a RM-ANOVA (3 × 6) with the main factor 'time course' (baseline, 0 min, 5 min, 10 min, 20 min, 30 min) and 'condition' (PAS25, PAS22, and iPAS) was performed. Since there was no 'time course × condition' interaction, we averaged all time points following the respective stimulation conditions to give a mean post-PAS excitability measure for all consecutive statistical analyses. Following this approach, an RM-ANOVA with the factor 'time' (baseline, average MEP change post-stimulation) and 'condition' (PAS25, PAS22, and iPAS) was computed. Sphericity was tested using the Mauchly's test and, if necessary (Mauchly's test < 0.05), Greenhouse–Geisser correction was applied. Data in tables are presented as mean values ± standard deviation and in all figures, error bars refer to the standard error.

Results

Descriptive statistics and baseline excitability parameters

RM-ANOVAs obtained no significant main effects on 'condition' comparing baseline excitability parameters (RMT, S1 mV, MEP) and PAS parameters across the three experimental sessions (see Table 2). As outlined by foregoing publications the overall correct stimulus count was used as an approximate for sufficient levels of attention in all three experimental conditions (Rajji et al. 2013; Stefan et al. 2004)

Table 2 Within-subject comparisons of baseline excitability and PAS parameters across the three stimulation conditions

	Conditions			Statistics	
	PAS 25	PAS 22	iPAS	*F*	*p* values
RMT (%)	36.0 (11.8)	36.0 (11.8)	36.2 (12.6)	$F_{(2,40)} = 0.067$	0.935
S1 mV (%)	40.3 (8.0)	39.6 (8.9)	41.0 (9.5)	$F_{(2,40)} = 2.339$	0.109
MEP (mV)	1.04 (0.09)	1.05 (0.12)	1.01 (0.15)	$F_{(1.3,26.8)} = 0.497$	0.540
PAS intensity (mA)	5.7 (1.8)	6.0 (1.9)	6.1 (1.7)	$F_{(2,40)} = 0.244$	0.785
Stimulus count	176.9 (5.5)	180.5 (13.0)	177.7 (6.8)	$F_{(1.3,26.1)} = 1.014$	0.345

Data presented as mean (±standard deviation in brackets)

RMT and S1MV stimulation intensities to elicit resting motor-threshold and 1 mV sized MEPs (given as percentage stimulator output), *MEP* motor-evoked potentials (presented in millivolt), *PAS* paired-associative stimulation, *PASintensity mA* peripheral nerve stimulation intensity during PAS given in milliampere

and did also not differ between the three experimental sessions ($F_{(1.3,26.1)} = 1.014$, $p = 0.345$).

MEP changes over time

The RM-ANOVA with the within-subjects factor 'time course' and the main factor 'condition' revealed no significant main effects on 'time course' ($F_{(3.0,60.0)} = 2.05$, $p = 0.117$) or 'condition' ($F_{(2,40)} = 1.12$, $p = 0.335$) and no 'time course × condition' interaction ($F_{(10,200)} = 1.01$, $p = 0.441$). Since we did not observe a significant interaction, we further explored the mean after-effects following our different PAS paradigms by averaging MEP amplitudes across all time points following PAS stimulation, thereby giving a mean post-PAS excitability measure for all consecutive statistical analyses. The subsequent RM-ANOVA with main factors 'condition' (PAS 25, PAS 22, and iPAS) and 'time' (baseline, average MEP change post-stimulation) revealed no effect on 'condition' ($F_{(2,40)} = 0.81$, $p = 0.454$) and no interaction of both factors ($F_{(2,40)} = 1.59$, $p = 0.217$), but a significant main effect on 'time' ($F_{(1,20)} = 4.44$, $p = 0.048$).

As our PAS 22 approach did only approximate established PAS techniques (N20 + 2) we then conducted a confirmatory analysis and excluded the acquired PAS 22 data from subsequent analyses. The repeated RM-ANOVA with main factors 'condition' (PAS 25 and iPAS) and 'time' (baseline, average MEP change post-stimulation) again obtained a significant main effect on 'time' ($F_{(1,20)} = 4.94$, $p = 0.038$), but no significant effect 'condition' ($F_{(1,20)} = 0.37$, $p = 0.551$) or interaction ($F_{(1,20)} = 0.14$, $p = 0.710$).

Subsequent explorative paired-samples *t* tests showed that the observed effects on 'time'—both in the overall analysis and in the confirmatory analysis—were driven by significantly increased average post-stimulation MEP magnitudes in case of the iPAS condition ($t_{(20)} = -2.28$, $p = 0.034$), while the same analysis obtained only trend level or no significant differences in case of the PAS 25 ($t_{(20)} = -1.77$, $p = 0.092$) and the PAS 22 ($t_{(20)} = -0.50$, $p = 0.621$) paradigms, respectively. Correction for multiple comparisons using the Sidak method resulted in trend-level differences for the iPAS paradigm ($p = 0.098$) and obtained no significant differences both for PAS 25 and PAS 22 (all $p > 0.203$) (see Fig. 1).

Contributing factors to PAS after-effects

To better understand potential contributors to the divergent after-effects following applied PAS paradigms, we further conducted a set of explorative analyses. For this purpose, we identified participants displaying expected MEP increases following PAS using the grand average approach (MEP increase > 120% relative to baseline defines response), which revealed 57.1% expected responders ($n = 12$) in case of the iPAS condition, while lower frequencies were observed for the PAS 25 (38.1%, $n = 8$) and the PAS 22 paradigm (28.6%, $n = 6$). The distribution of responders/non-responders did not differ across groups ($Chi^2_{(2)} = 3.667$, $p = 0.160$). Next, we compared potential measures driving this effect between expected responders and their respective peers (showing not-expected MEP courses following stimulation) using independent samples *t* test. This explorative approach, using independent samples *t* tests and Chi^2 tests where appropriate, revealed no significant differences for candidate contributors, such as demographic variables (age, gender, weight, height, handedness), baseline excitability measures (RMT, S1 mV, Baseline MEP), or PAS parameters (peripheral nerve stimulation intensities, stimulus count, individual inter-stimulus intervals) in case of all three experimental conditions (PAS 25: all $p > 0.182$; PAS 22: all $p > 0.065$; iPAS: all $p > 0.061$). Further, although our experimental conditions differed regarding the aspect of fixed versus individualized ISI, we explored intra-individual variability across the three experimental paradigms. Again using the grand average approach (MEP increase > 120% relative to baseline defines response) we observed only two participants (9.5%) displaying expected MEP magnitude increase following all three paradigms and four participants (19.0%) showing no MEP magnitude changes above the defined threshold

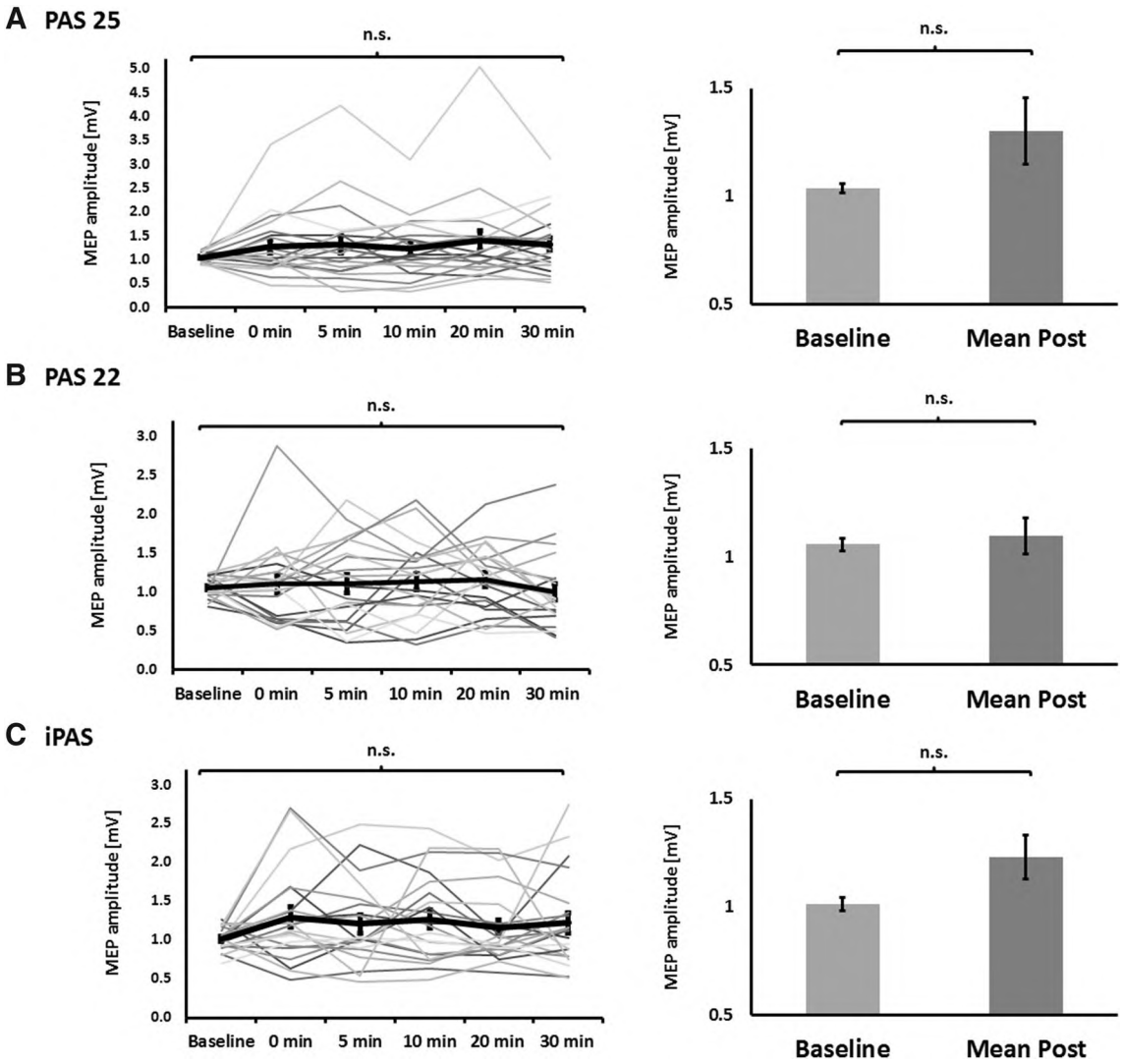

Fig. 1 Presentation of MEP values at baseline, across time points and mean post-MEP values following PAS 25 (**a**), PAS 22 (**b**) and iPAS (**c**). Mean MEP were increased compared to baseline following iPAS stimulation ($p = 0.034$), but was rendered not significant after correction for multiple comparisons ($p = 0.098$) (n.s. indicates not-significant results). Thick black lines indicate respective group mean values. MEP values represent raw values and are displayed scaled in millivolt (mV); error bars represent standard errors of the mean

(> 120% relative to baseline MEP) in any of the applied PAS conditions. However, when we again excluded the acquired PAS 22 data from this analysis (as our PAS 22 approach did only approximate the established PAS N20 + 2 paradigm) we observed MEP increase > 120% relative to baseline in five participants (23.8%) and no expected MEP increase to both PAS paradigms in six participants (28.6%).

Discussion

To our knowledge, the present study is the first to compare LTP-like plasticity changes between PAS paradigms using fixed inter-stimulus intervals (ISI) and a PAS instantiation that based ISI on individual conduction time delays. As a main finding, we observed only an effect of 'time' ($F_{(1,20)} = 4.44$, $p = 0.048$), but no effects of 'condition' and no 'time course × condition' interaction (all $p \geq 0.217$). These results suggest overall MEP magnitude increases across all three experimental paradigms; however, no dependency of PAS efficacy on the application of individualized ISI compared to fixed ISI. While exploratory analyses revealed MEP increase for the average of MEP changes within the post-stimulation observational period in case of the individualized PAS paradigm (iPAS), this result was not significant following correction for multiple comparisons. In case of the two other PAS conditions (PAS 25 and PAS 22), no significant MEP magnitude increases were

observed averaging all time points following stimulation compared to baseline. Our findings thereby coincide with several more recent studies that did not observe significant MEP increases on the group level following the same (in the case of PAS 25) or other facilitatory PAS protocols, which used N20 + 2 ms ISI (Lopez-Alonso et al. 2014; Muller-Dahlhaus et al. 2008). At the same time, however, our observations contrast to earlier studies, which had either observed stable facilitatory after-effects on the group level following PAS 25 and PAS N20 + 2, respectively (Fratello et al. 2006; Heidegger et al. 2010; Ilic et al. 2011; Sale et al. 2007) or did not find significant MEP increases in the case of PAS paradigms employing ISI that were also based on individual latencies between the peripheral nerve stimulation and TMS (Kennedy and Carson 2008). Against this background our findings thereby contribute to the growing number of studies reporting divergent efficacy following different PAS paradigms (Carson and Kennedy 2013).

Further, explorative analyses of response rates using the grand average approach (MEP increase > 120% relative to baseline defines response) revealed lower rates for expected MEP magnitude increases in the case of the PAS 25 (38.1% responder) and PAS 22 (28.6% responder) conditions, whereas numerically higher rates were observed for the iPAS paradigm (57.1% responder). By comparison, applying individualized ISI (in the case of the iPAS paradigm) that accounted for differences in conduction time on the single subject level thereby appeared to have resulted in decreased inter-individual response variability defined as increased response rates. Our findings thereby substantiate circumstantial evidence for a potential association of decreased inter-individual variability with individually selected ISI. A hypothetical explanation for this finding might be constituted by the proposed neuronal mechanisms underlying PAS, which are viewed to depend on optimal spike-timing to allow synchronous (or near-synchronous) pairings of the afferent and cortical stimuli (Bliss and Collingridge 1993; Cooke and Bliss 2006; Muller et al. 2007; Stefan et al. 2000; Wolters et al. 2003, 2005). Thus, one of the reasons why we observed a diminished inter-individual variability following the application of an individualized ISI PAS protocol could reside in the fact that such a paradigm might more accurately mimic a synchronous integration of two stimuli than standardized ISI paradigms. Such an assumption would gain support from foregoing considerations by groups aiming to match the applied ISI more closely to physiological parameters such as the N20 latency (Cash et al. 2017; Heidegger et al. 2010; Ilic et al. 2011; Korchounov and Ziemann 2011; Muller-Dahlhaus et al. 2008; Voytovych et al. 2012) or individual conduction times (Kennedy and Carson 2008). While it would be intriguing to follow this line of reasoning, we are aware that other PAS parameter configurations aside of different ISI could have contributed to the observed differences in efficacy and variability, such as the peripheral target nerve (Carson and Kennedy 2013).

Our study has several limitations. First, despite having a sample size within the range of other studies in the field, our main analysis did not show significant MEP magnitude increases following iPAS as well as following both fixed ISI PAS paradigms. However, the frequency of responders and the significant main effect on time in the second RM-ANOVA could to some limited extend support a careful discussion of a possible superiority of iPAS with respect to its capability to reduce response variability on the group level compared to the other employed conditions. Second, our PAS 22 paradigm was only derivative of the established PAS N20 + 2 technique and thus represents a paradigm that has not previously been used in the literature. However, as detailed above, we used this pragmatic approach as second control condition in addition to the established PAS 25 paradigm. Third, despite being in the range of the field, a sample size of 21 subjects could still be too small to account for the established variability of various PAS approaches. Either studies with larger sample sizes or enriched samples (e.g. a group of subjects with established response to PAS 25) are needed to reconfirm our findings.

In summary, the findings of our proof-of-concept study might point toward a potential association of decreased inter-individual variability with individually selected ISI. Given the limitations of our proof-of-concept study, further replication studies addressing these issues with larger sample sizes and a repetitive design are needed to confirm the relationship between individualizing ISIs and efficacy of PAS.

Acknowledgements We would like to thank all participants for so generously giving their time. This work was supported by a research grant of the Münchner Medizinische Wochenschrift (MMW, Grant number 5/14-WS). The funder had no role in the design and conduct of the study, the collection and management of the data as well as their respective analysis and the preparation or approval of the manuscript.

Author contributions Conceptualization: MC, WS, and AH. Formal analysis: MC, WS, and AH. Funding acquisition: MC and WS. Investigation: MC. Methodology: WS, TB, BP, IP, and AH. Project administration: WS, BP, IP, and AH. Software: WS and BP. Supervision: WS, IP, and AH. Writing—original draft: MC, WS, and AH. Writing—review and editing: all the authors.

Compliance with ethical standards

Conflict of interest WS has received speaker's honorarium from Mag & More GmbH. AH has received a paid speakership from Janssen-Cliag, Otsuka and Lundbeck. He was member of an advisory board of Roche, Janssen-Cliag, Otsuka and Lunbeck. All the other authors declare no conflicts of interests.

References

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39. https://doi.org/10.1038/361031a0

Carson RG, Kennedy NC (2013) Modulation of human corticospinal excitability by paired associative stimulation. Front Hum Neurosci 7:823. https://doi.org/10.3389/fnhum.2013.00823

Carson RG, Nelson BD, Buick AR, Carroll TJ, Kennedy NC, Cann RM (2013) Characterizing changes in the excitability of corticospinal projections to proximal muscles of the upper limb. Brain Stimul 6:760–768. https://doi.org/10.1016/j.brs.2013.01.016

Cash RFH, Jegatheeswaran G, Ni Z, Chen R (2017) Modulation of the direction and magnitude of hebbian plasticity in human motor cortex by stimulus intensity and concurrent inhibition. Brain Stimul 10:83–90. https://doi.org/10.1016/j.brs.2016.08.007

Castel-Lacanal E et al (2009) Induction of cortical plastic changes in wrist muscles by paired associative stimulation in the recovery phase of stroke patients. Neurorehabil Neural Repair 23:366–372. https://doi.org/10.1177/1545968308322841

Cheeran B et al (2008) A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. J Physiol 586:5717–5725. https://doi.org/10.1113/jphysiol.2008.159905

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 60:864–870. https://doi.org/10.1016/j.neuroimage.2012.01.052

Cooke SF, Bliss TV (2006) Plasticity in the human central nervous system. Brain 129:1659–1673. https://doi.org/10.1093/brain/awl082

Faul F, Erdfelder E, Lang A-G, Buchner A (2007) G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39:175–191

Fratello F et al (2006) Modulation of corticospinal excitability by paired associative stimulation: reproducibility of effects and intraindividual reliability. Clin Neurophysiol 117:2667–2674. https://doi.org/10.1016/j.clinph.2006.07.315

Heidegger T, Krakow K, Ziemann U (2010) Effects of antiepileptic drugs on associative LTP-like plasticity in human motor cortex. Eur J Neurosci 32:1215–1222. https://doi.org/10.1111/j.1460-9568.2010.07375.x

Ilic NV, Milanovic S, Krstic J, Bajec DD, Grajic M, Ilic TV (2011) Homeostatic modulation of stimulation-dependent plasticity in human motor cortex. Physiol Res 60(Suppl 1):S107–S112

Kennedy NC, Carson RG (2008) The effect of simultaneous contractions of ipsilateral muscles on changes in corticospinal excitability induced by paired associative stimulation (PAS). Neurosci Lett 445:7–11. https://doi.org/10.1016/j.neulet.2008.08.064

Korchounov A, Ziemann U (2011) Neuromodulatory neurotransmitters influence LTP-like plasticity in human cortex: a pharmaco-TMS study. Neuropsychopharmacology 36:1894–1902. https://doi.org/10.1038/npp.2011.75

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 221:241–249. https://doi.org/10.1007/s00221-012-3165-x

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 7:372–380. https://doi.org/10.1016/j.brs.2014.02.004

Monte-Silva K, Kuo MF, Thirugnanasambandam N, Liebetanz D, Paulus W, Nitsche MA (2009) Dose-dependent inverted *U*-shaped effect of dopamine (D2-like) receptor activation on focal and non-focal plasticity in humans. J Neurosci 29:6124–6131. https://doi.org/10.1523/JNEUROSCI.0728-09.2009

Muller JF, Orekhov Y, Liu Y, Ziemann U (2007) Homeostatic plasticity in human motor cortex demonstrated by two consecutive sessions of paired associative stimulation. Eur J Neurosci 25:3461–3468. https://doi.org/10.1111/j.1460-9568.2007.05603.x

Muller-Dahlhaus JF, Orekhov Y, Liu Y, Ziemann U (2008) Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Exp Brain Res 187:467–475. https://doi.org/10.1007/s00221-008-1319-7

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97–113

Rajji TK, Sun Y, Zomorrodi-Moghaddam R, Farzan F, Blumberger DM, Mulsant BH, Fitzgerald PB, Daskalakis ZJ (2013) PAS-induced potentiation of cortical-evoked activity in the dorsolateral prefrontal cortex. Neuropsychopharmacology 38(12):2545

Rossi S, Hallett M, Rossini PM, Pascual-Leone A (2011) Screening questionnaire before TMS: an update. Clin Neurophysiol 122:1686. https://doi.org/10.1016/j.clinph.2010.12.037

Rothwell J, Hallett M, Berardelli A, Eisen A, Rossini P, Paulus W (1999) Magnetic stimulation: motor evoked potentials. Electroencephalogr Clin Neurophysiol Suppl 52:97–103

Sale MV, Ridding MC, Nordstrom MA (2007) Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp Brain Res 181:615–626

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J (2000) Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123(Pt 3):572–584

Stefan K, Kunesch E, Benecke R, Cohen LG, Classen J (2002) Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J Physiol 543:699–708

Stefan K, Wycislo M, Classen J (2004) Modulation of associative human motor cortical plasticity by attention. J Neurophysiol 92:66–72. https://doi.org/10.1152/jn.00383.2003

Stinear JW, Hornby TG (2005) Stimulation-induced changes in lower limb corticomotor excitability during treadmill walking in humans. J Physiol 567:701–711. https://doi.org/10.1113/jphysiol.2005.090654

Voytovych H, Krivanekova L, Ziemann U (2012) Lithium: a switch from LTD- to LTP-like plasticity in human cortex. Neuropharmacology 63:274–279. https://doi.org/10.1016/j.neuropharm.2012.03.023

Wolters A et al (2003) A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. J Neurophysiol 89:2339–2345. https://doi.org/10.1152/jn.00900.2002

Wolters A et al (2005) Timing-dependent plasticity in human primary somatosensory cortex. J Physiol 565:1039–1052. https://doi.org/10.1113/jphysiol.2005.084954