

ABSTRACT

Paired associative stimulation (PAS) protocols can be used to induce Hebbian plasticity in the human brain. A modified, cross-modal version, of the PAS (cross-modal PAS, cm-PAS) has been recently developed. The cm-PAS consists in the repetitive pairings of a transcranial magnetic stimulation (TMS) pulse over the primary somatosensory cortex (S1) and a visual stimulus depicting a hand being touched; a 20 ms of inter-stimulus interval (ISI) is required to affect S1 plasticity, in turn modulating tactile acuity and somatosensory evoked potentials. The present study explores the role of **anticipatory simulation** in the cm-PAS efficacy, which could be responsible for a such short ISI. To this aim, we compared the effect of the original, fixed-frequency, cm-PAS to that of a jittered version, in which the time interval between trials was not steady but jittered, hence avoiding the anticipation of the upcoming visual-touch stimulus. Moreover, in the *jittered* PAS, the ISI between the paired stimulations was varied: it could match the early, somatosensory-driven, activation of S1 (20 ms), or the mirror recruitment of S1 by touch observation (150 ms). Results showed that tactile acuity is enhanced by the *fixed-frequency* cm-PAS, with an ISI of 20 ms between paired stimulation (visual-touch stimulus and TMS pulse over S1), and also by the *jittered* cm-PAS but only if the ISI is of 150 ms. These findings **suggest** that the cm-PAS with a jittered frequency, by preventing **an anticipatory** pre-activation of S1, delays the timing of the interaction between the visual-touch stimulus and the cortical pulse. On a broader perspective, our study highlights the **possible** involvement of **sensory anticipation, likely through mirror-like simulation mechanisms**, in tactile mirroring, **as well its influence of the optimal interval between the afferent and the magnetic pulse during PAS protocols.**

KEYWORDS: paired associative stimulation, **sensory anticipation, sensory simulation**, cross-modal plasticity, somatosensory cortex, hebbian learning.

1. INTRODUCTION

Hebbian associative plasticity can be induced non-invasively in the human brain by means of paired associative stimulation (PAS) protocols. These protocols induce forms of long-term potentiation (LTP) and/or long-term depression, accordingly to a Hebbian associative learning rule, through the repeatedly, time-locked, pairings of two stimulations: a peripheral sensory stimulus with a transcranial magnetic stimulation (TMS) pulse applied over the cortical region activated by the same sensory stimulus (Stefan et al., 2000; Suppa et al., 2017; Wolters et al., 2003). In recent years, PAS protocols evolved from those targeting a single brain area (e.g. motor and somatosensory cortex; e.g., Stefan et al., 2000; Wolters et al., 2005) to more complex paradigms targeting cortical networks or by involving different sensory modalities (Arai et al., 2011; Buch et al., 2011; Chiappini et al., 2018; Nord et al., 2019; Santarnecchi et al., 2018; Suppa et al., 2015, 2013).

Recently, our research group developed a new version of the PAS protocol, the cross-modal PAS (cm-PAS) (Zazio et al., 2019), which can modulate somatosensory cortical activity at behavioral and electrophysiological levels likely through the recruitment of a visuo-tactile network with mirror properties (i.e., *tactile mirror system* - Blakemore et al., 2005; Keysers et al., 2010). In particular, in the cm-PAS, we repeatedly coupled two stimuli, a visual stimulus depicting a touch and a TMS pulse over the primary somatosensory cortex (S1). This repetitive, steady, stimulation induces an enhancement of tactile acuity and of somatosensory evoked potentials (the P40 component). Importantly, the cm-PAS is effective only when the inter-stimulus interval (ISI) between the two paired stimulations is of 20 ms (i.e., the visual-touch stimulus precedes the S1-TMS pulse by 20 ms); such time-dependent feature is crucial for the induction of Hebbian learning and plasticity (Caporale and Dan, 2008).

The strict temporal contiguity between the visual-touch stimulus and TMS pulse over S1 required by the cm-PAS is intriguing and thought-provoking: an ISI of 20 ms seems to be a too short time interval to hypothesize a visual recruitment of S1 by touch observation, considering the chronometry of the

visual pathways reaching S1, either via feedforward or feedback connections (Cappe et al., 2009; Driver and Noesselt, 2008). Rather, the visual activation of S1 should occur within a longer time window, especially if mirror networks are involved, as demonstrated by previous electrophysiological and brain stimulation studies (Bolognini et al., 2014; Pihko et al., 2010; Pisoni et al., 2018). Instead, the 20 ms ISI reflects the typical latency of S1 activation by direct somatosensory afference (Allison et al., 1989), which is the optimal timing for the induction of LTP by PAS coupling TMS over S1 with median nerve stimulation (Litvak et al., 2007; Wolters et al., 2005).

A hypothesis put forward (Zazio et al., 2019) was that such short ISI could reflect an anticipatory mirror-touch effect. In this perspective, the cm-PAS could act by generating a reafference ‘tactile’ prediction signal from the observed touch, anticipating the time-course of putative neural interaction in S1 between the visual-touch stimulus (i.e., the observed touch) and the TMS-induced somatosensory activation. Indeed, due to the repetitiveness of the cm-PAS, that requires a regular frequency of 0.1 Hz between a couple of paired stimuli and the following ones for 25 minutes, participants, after a few trials, may implicitly start to anticipate the visual-touch stimulus before its actual occurrence. The result would be an early interaction between the expected touch and the cortical somatosensory TMS pulse, which would explain the effectiveness of the 20 ms ISI. During the cm-PAS, learned contingencies between cm-PAS events (the visual-touch stimulus and the S1 activation by the TMS pulse) could **give rise to an anticipatory simulation of the incoming touch. Thanks to the anticipation of the touch stimulus, after a few trials, tactile mirroring in S1 may occur earlier, hence before actual touch observation, consequently anticipating at 20 ms the interaction between the S1 activation induced by touch observation and that induced by the TMS pulse.** It is worth to mention that this **anticipatory simulation, which can be seen as a form of sensory prediction**, can be learned rapidly, even over the course of an experiment and it may affect earliest stages of sensory cortical processing (Den Ouden et al., 2009; Kok et al., 2012).

In order to verify the role of sensory predictions in the cm-PAS, in the present study we have manipulated participants’ **predictions** about the upcoming visual-touch stimulus: we hypothesized

that, if **simulation mechanisms** are involved in the cm-PAS efficacy, a cm-PAS where the frequency between trials (paired stimuli) is not fixed should avoid an anticipatory, expectation-based, activation of S1 by the visual-touch stimulus. If this is the case, a longer ISI would become effective for the induction of Hebbian plasticity in S1, namely matching more closely the mirror recruitment of S1 by touch observation (150 ms) (Bolognini et al., 2014; Pisoni et al., 2018). Conversely, if the effects of the cm-PAS are not mediated by sensory **anticipation**, even an unpredictable cm-PAS would be effective with the ISI of 20 ms, in the same way of the *fixed-frequency* cm-PAS.

To prevent the anticipation of the upcoming visual-touch stimulus we have developed a sort of jittered version of the cm-PAS (*jittered* cm-PAS) by varying the inter-trials frequency, thus avoiding that participants could make predictions about the time-course of the next trial (hence, about the arrival of the paired stimulations). Participants underwent the original version of the cm-PAS (Zazio et al., 2019), with a fixed frequency of 0.1 Hz (i.e., paired stimuli occur regularly every 10 s), and an ISI of 20 ms between the visual-touch and the TMS pulse; they also underwent, in different sessions, two versions of the *jittered* cm-PAS, both featured by the paired stimuli occurring at varying intervals (5, 10 or 15 s), but differing with respect to the ISI between the onset of the visual-touch stimulus and the TMS pulse over S1: in one version, as in the *fixed-frequency* cm-PAS, the ISI was of 20 ms (*jittered* cm-PAS_{20ms}), in the other version, the ISI was of 150 ms (*jittered* cm-PAS_{150ms}), hence matching the mirror activation of S1 by the sight of tactile stimuli **as documented by a TMS study assessing the chronometry of S1 activation by touch observation (Bolognini et al., 2014)**. **Noteworthy, from a Hebbian learning account, associative plasticity should be induced only when contingency between the activation of two neurons, at a microscopic level, or cortical areas/circuits, at a macroscopic level, is achieved (Song et al., 2000). Thus, the ISI of 150 ms was selected because, from the available literature, it seems to be the more appropriate interval to obtain the required temporal contingency between the TMS pulse and the visual-touch in S1.**

2. MATERIALS and METHODS

We report how we determined our sample size, all data exclusions, all inclusion/exclusion criteria, whether inclusion/exclusion criteria were established prior to data analysis, all manipulations, and all measures in the study. Database and the tasks/stimuli used in the present study are publicly archived at: <https://osf.io/5vtkz/>

2.1. Participants

Eighteen healthy volunteers took part in the study (5 males, mean age \pm standard deviation - SD: 25.4 \pm 2.9 years). Two participants dropped out during the experimental sessions, leaving the final analyzed sample to 16 subjects. **This sample size is in line with our previous study which assessed the effectiveness of the cm-PAS protocol (Zazio et al., 2019).** Before taking part in the study all participants gave their written informed consent and underwent a screening questionnaire for eligibility in a TMS study according to the international standard criteria (Rossi et al., 2009). The study was carried out according to the ethical standards of the Declaration of Helsinki and was approved by the local Ethics Committee of Milano-Bicocca University.

2.2. cm-PAS protocols

To investigate the contribution of predictive coding mechanisms during the cm-PAS, we employed both our original version of the cm-PAS protocol (*fixed-frequency* cm-PAS; Zazio et al., 2019) and a modified version in which the time interval between paired stimulations was not fixed (*jittered* cm-PAS), but it could vary in a random fashion among three different values being of 5, 10 and 15 s. In both protocols, every trial began with a fixation frame showing a left-hand palm in an egocentric perspective (15 X 24° of visual angle); participants were asked to look at a red asterisk in the center of the fixation frame. As soon as the fixation frame ended the visual-touch stimulus appeared. It consisted of a frame showing, from an allocentric perspective, the index finger of a right hand touching the palm of the left hand (i.e., visual-touch frame, 15 X 25° of visual angle). The two frames appeared consequently, giving an impression of apparent motion. The TMS pulse over right S1 was

timed with the onset of the second frame, namely with the contact between the index finger and the palm, and it was delivered at 150% of the resting motor threshold (rMT – see Par. 2.4), according to previous PAS experiments (Wolters et al., 2005; Zazio et al., 2019).

In the *fixed-frequency* cm-PAS, the fixation frame lasted for 9.7 s and the visual-touch one lasted 300 ms, thus the frequency of the stimulation was fixed at 0.1 Hz (i.e., 1 trial – visual stimulus and TMS pulse - every 10 s). After 20 ms from the onset of the visual-touch frame, the TMS pulse was delivered (Zazio et al., 2019) (**Figure 1**).

In the *jittered* cm-PAS, the fixation frame could last 4.7, 9.7 or 14.7 s (50 trials for each duration, given in random order) while the visual-touch frame still lasted 300 ms. Therefore, during this protocol, PAS frequency varied among 0.2 Hz (i.e., 1 trial every 5 s), 0.1 Hz (i.e., 1 trial every 10 s) and 0.067 Hz (i.e., 1 trial every 15 s). To assess the timing-dependency, two different versions of the *jittered* cm-PAS were tested: in one session, the TMS pulse over right S1 was delivered after 20 ms (*jittered* cm-PAS_{20ms}), as in the *fixed-frequency* cm-PAS, while in another session it was delivered after 150 ms (*jittered* cm-PAS_{150ms}) (**Figure 1**). Both the *fixed-frequency* cm-PAS and the *jittered* cm-PAS consisted of 150 trials (i.e., 150 paired stimulations) and lasted 25 minutes.

In both protocols, the exact timing of visual-touch stimuli with respect to the TMS pulses was ensured using a photodiode.

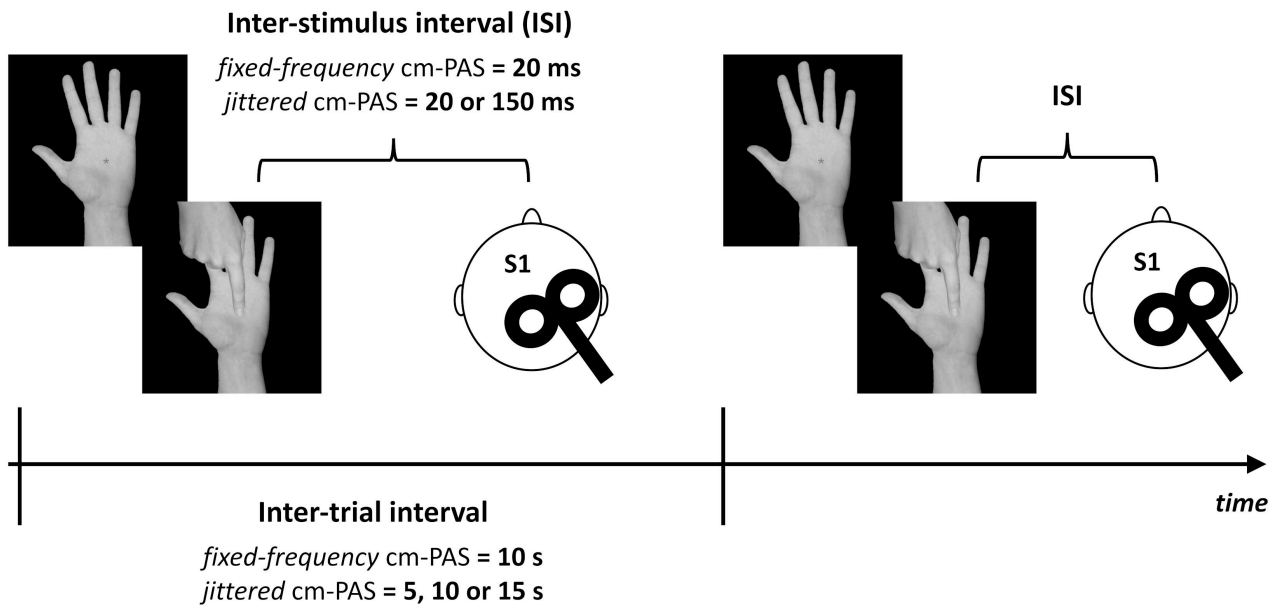


Figure 1. cm-PAS protocols. In the *fixed-frequency* cm-PAS, a visual stimulus depicting a hand being touched is paired with a TMS pulse over right S1. The visual stimulus is made of two frames: a fixation frame lasting for 9700 ms and a visual-touch frame lasting for 300 ms. The TMS pulse is triggered at the visual-touch frame onset, with an ISI of 20 ms. Couples of paired stimuli occur regularly every 10 s. The *jittered* cm-PAS entails the same above-mentioned paired stimuli, but the paired stimuli occur at varying intervals (5, 10 or 15 s). The ISI between the TMS pulse and the visual-touch frame could be 20 or 150 ms.

During the entire protocol, participants were told to keep their left hand in the same posture and position as the one shown in the visual frame. Moreover, in order to be sure that participants were paying attention to the stimuli, a critical condition for the success of a PAS protocol (Stefan et al., 2004; Zazio et al., 2019), they were engaged in a task to catch rare visual events (i.e., trials depicting a double touch). These rare trials consisted of a visual-touch frame presented twice, sequentially: a first visual-touch frame presented for 180 ms, followed by a second visual-touch frame lasting 300 ms (time interval between the two visual-touch frames = 180 ms); in this condition, the TMS pulse was applied on the second visual-touch frame. In both versions of the cm-PAS, catch trials were 15

of 150. Performance was not analyzed. During the protocol, participants heard white noise in a pair of headphones to mask the sound made by the TMS.

Trial randomization and timing of the TMS pulses were controlled using the software E-prime (2.0, Psychology Software Tool, Inc.)

2.3. 2-Point Discrimination Task (2-PDT)

As in our previous study, the efficacy of the cm-PAS was assessed at a behavioral level, by assessing changes of tactile acuity with a 2-point discrimination task (2-PDT; Case et al., 2017, 2016; Zazio et al., 2019). Participants were blindfolded and rested comfortably in an armchair while an experimenter touched them with one or two tips of an aesthesiometer (North Coast Medical, Morgan Hill, USA). The experimenter was trained and instructed to touch always the same **site** – previously marked with a soft tip - on the participant's left-hand palm and to apply always the same pressure. The location of the tactile stimulation corresponded to the same **site** showed in the visual-touch frame of the cm-PAS (**namely the center of the left-hand palm**). The task consisted in reporting verbally if one or two tips were felt, and the responses were recorded by a second experimenter on a PC. **The second experimenter also certified that the task was administered properly.** The distances between the plastic tips ranged from 3 to 15 mm **in steps of 1 mm**, so the 2-PDT trials were arranged in blocks of 10 trials, half with one tip and half with two tips. The order of one and two tips stimulations was randomized within blocks. Blocks were administered following a descendent order, starting from the longer distances (i.e., from 15 mm to 3 mm). Each block was repeated 3 times per distance (30 trials for each distance) with a brief one-minute pause between each repetition. The total number of trials was 390. The task lasted approximately 15 minutes.

2.4. TMS

TMS was delivered using a figure-of-eight 70 mm coil connected to a Magstim Rapid 2 biphasic stimulator (Magstim Company, Whitland, UK). Resting motor threshold (rMT) of each participant

was assessed at the beginning of every session. rMT was defined as the minimum TMS intensity that elicited a visually detectable muscle twitch in the left thumb in 5 out of 10 TMS pulses (Bolognini et al., 2013; Rossi et al., 2009). The mean rMT was 52.2 ± 4.7 % (mean \pm SD) of the maximal output stimulator (no difference between sessions $p = 0.395$).

After the assessment of the individual rMT, the coil was moved 2 cm posterior to the motor hotspot, thus targeting S1. We followed this procedure to locate S1 accordingly with previous PAS protocols targeting S1 and for consistency with the methodology of the cm-PAS used in previous studies (Gorgoni et al., 2015; Litvak et al., 2007; Wolters et al., 2005; Zazio et al., 2019). As a control measure of correct coil positioning, after S1 was located, a few pulses at rMT intensity were delivered to be sure that no muscle twitches were elicited in the contralateral hand.

TMS positioning was assisted with the SofTaxic 2.0 neuronavigation system (E.M.S., Bologna, Italy, www.softaxic.com).

2.5. Experimental procedure

The experiment comprised three sessions that differed only for the cm-PAS protocol used: *fixed-frequency* cm-PAS; *jittered* cm-PAS_{20ms} and *jittered* cm-PAS_{150ms}. The order of the three sessions was counterbalanced among participants. The experimental procedure was the same in every session: each session started with the administration of the 2-PDT and the assessment of the individual rMT. Then the cm-PAS was administered and immediately after the stimulation, a second assessment of participants' tactile acuity was performed. On average, a session lasted 1 hour and 30 minutes. The three sessions were held at the same moment of the day (in the morning or in the afternoon) and at least 72 hours passed between them, thus to prevent an overlapping of stimulation effects (Sale et al., 2007).

2.6. Statistical analysis

As in previous experiments (Zazio et al., 2019), the behavioral effects on the 2-PDT performance were assessed according to signal-detection theory, calculating a perceptual sensitivity index (d') (Green and Swets, 1966). A sensory threshold estimation was calculated using a logistic function fitting on perceptual sensitivity (R, version 3.3.1 – R Core Team, 2019). Sensory threshold was set as the distance in mm at which performance was 50%. To evaluate the performance at the 2-PDT we considered also the response criterion (c) regardless of the distance in mm, thus to investigate possible subject-related response bias, and the global performance, calculated as the mean perceptual sensitivity. Before running the analyses, normality of data was confirmed by the Shapiro-Wilk test. Three separate repeated-measures analysis of variance (3X2 rmANOVA) were conducted on sensory threshold, response criterion and global performance with Session (*fixed-frequency* cm-PAS, *jittered* cm-PAS_{20ms}, *jittered* cm-PAS_{150ms}) and Time (pre cm-PAS, post cm-PAS) as within-subjects factors. The sphericity requirements were assessed by using Mauchly's test. As effect size index, partial eta-squared (ηp^2) values are reported. To further explore ANOVAs results, post hoc analysis with Tuckey correction for multiple comparisons were used. Whenever data were not normally distributed, nonparametric tests were used (i.e. Friedman's ANOVA). Statistical significance was set at $p < 0.05$ and, for each variable, mean \pm standard error is reported. Statistical analysis was performed using Statistica software (version 10, StatSoft).

3. RESULTS

The rmANOVA on the sensory threshold showed a significant main effect of Time ($F_{1,15} = 14.63$, $p = 0.002$, $\eta p^2 = 0.49$). Moreover, we found a significant Session by Time interaction ($F_{2,30} = 7.07$, $p = 0.003$, $\eta p^2 = 0.32$). Post-hoc analyses highlighted a decrease in the sensory threshold after the *fixed* cm-PAS (Pre *fixed-frequency* cm-PAS = 9.59 ± 1.14 mm, Post *fixed-frequency* cm-PAS = 8.33 ± 1.40 mm, $t = 4.13$, $p = 0.002$). Interestingly, a change in the same direction was found after the *jittered*

cm-PAS_{150ms} (Pre *jittered* cm-PAS_{150ms} = 9.55 ± 1.36 mm, Post *jittered* cm-PAS_{150ms} = 8.48 ± 1.12 mm, $t = 3.52$, $p = 0.012$). Post-hoc also revealed a difference in the sensory threshold after the *fixed-frequency* cm-PAS and the threshold after the *jcm-PAS*_{20ms} (Post *fixed-frequency* cm-PAS = 8.33 ± 1.40 mm vs. Post *jittered* cm-PAS_{20ms} = 9.50 ± 1.46 mm, $t = -3.40$, $p = 0.005$) and between the *jittered* cm-PAS_{20ms} and the *jittered* cm-PAS_{150ms} (Post *jittered* cm-PAS_{150ms} = 8.48 ± 1.12 mm vs. Post *jittered* cm-PAS_{20ms} = 9.50 ± 1.46 mm, $t = 2.98$, $p = 0.017$). **The sensory threshold after the *fixed-frequency* cm-PAS (8.33 ± 1.40 mm) was lower as compared to that after the *jittered* cm-PAS₂₀ (9.50 ± 1.46 mm, $t = -3.40$, $p = 0.005$).** Noteworthy, no difference in sensory threshold were observed before each stimulation session ($p > 0.05$). No other significant effects were found (Session: $F_{2,30} = 1.63$, $p = 0.213$, $\eta^2 = 0.09$) (**Figure 2**).

Regarding the response criterion, we performed a Friedman ANOVA because data in one condition (Post *fixed-frequency* cm-PAS) were not normally distributed. No significant effects were found ($\chi^2_5 = 10.9$, $p = 0.053$).

Results on global performance showed a significant main effect of Time ($F_{1,15} = 13.55$, $p = 0.002$, $\eta^2 = 0.48$) and of the interaction Session by Time ($F_{2,30} = 7.11$, $p = 0.003$, $\eta^2 = 0.32$). Post-hoc showed an improvement in participants' global performance after the *fixed-frequency* cm-PAS (Pre *fixed-frequency* cm-PAS: $d' = 1.68 \pm 0.28$; Post *fixed-frequency* cm-PAS: $d' = 2.00 \pm 0.32$; $t = -4.41$, $p = 0.001$). A significant improvement was found also after the *jittered* cm-PAS_{150ms} (*jittered* cm-PAS_{150ms}: $d' = 1.68 \pm 0.36$; Post *jittered* cm-PAS_{150ms}: $d' = 1.89 \pm 0.34$; $t = -2.98$, $p = 0.045$). No difference was found after the *jittered* cm-PAS_{20ms} (Pre *jittered* cm-PAS_{20ms}: $d' = 1.74 \pm 0.35$; Post *jittered* cm-PAS_{20ms}: $d' = 1.69 \pm 0.38$; $t = 0.59$, $p = 0.99$). No other significant effects were found (Session: $F_{2,30} = 1.40$, $p = 0.262$, $\eta^2 = 0.09$).

Taken together, our results show an enhancement of tactile acuity selectively after the *fixed-frequency* cm-PAS with an ISI of 20 ms, and after the *jittered* cm-PAS with an ISI of 150 ms.

Finally, possible effect of the sessions' order was ruled out by a further 3X2 rm-ANOVA, including the session Order as a covariate which did not show any effect of this factor (Global performance: $F_{2,13} = 0.33, p = 0.73, \eta p^2 = 0.05$; sensory threshold: $F_{2,13} = 0.15, p = 0.863, \eta p^2 = 0.02$; response criterion: $F_{2,13} = 1.23, p = 0.323, \eta p^2 = 0.16$).

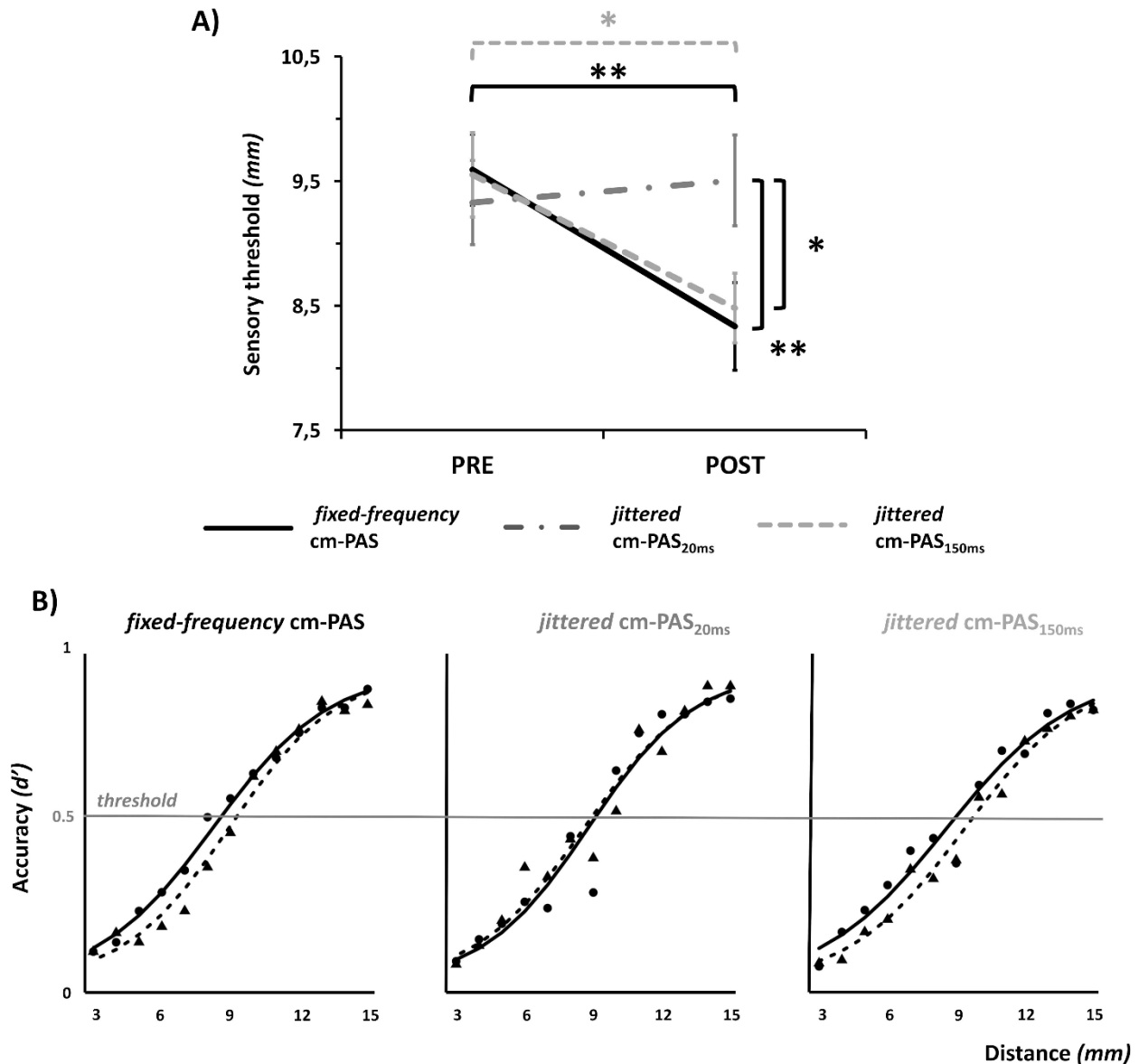


Figure 2. Results. [A] Effects of the different tested versions of the cm-PAS on tactile acuity at the 2-PDT: significantly lower threshold after the *fixed-frequency* cm-PAS (black straight line) and the *jittered* cm-PAS_{150ms} (light grey dotted line) compared to *jittered* cm-PAS_{20ms} (dark grey straight-dot

line). Asterisks indicate statistical significance in post-hoc tests corrected for multiple comparisons (* $p < 0.05$, ** $p < 0.01$).

[B] Psychometric functions obtained from logistic fitting to raw data, before (dotted line, triangles) and after (black line, dots) *fixed-frequency* cm-PAS, *jittered* cm-PAS_{20ms} and *jittered* cm-PAS_{150ms}.

4. DISCUSSION

In our previous work, we developed a visuo-tactile PAS protocol (cm-PAS) modulating S1 functioning through the visual modality and the putative recruitment of a mirror tactile system (Zazio et al., 2019). In the present study, we further explored whether **anticipatory** mechanisms may be responsible for the short, effective, cm-PAS interval between the two paired stimulations (i.e., ISI of 20 ms between the visual-touch stimulus and the S1-TMS pulse) successfully modulating tactile processing.

The present results confirm the enhancement of tactile acuity after administration of the *fixed-frequency* cm-PAS with an ISI of 20 ms (Zazio et al., 2019), but they also show that the same enhancement effect is induced by a cm-PAS with a jittered frequency between the delivery of paired stimulations. However, under this condition, where the appearance of the visual-touch stimulus becomes almost unpredictable, the cm-PAS affects tactile acuity when there is a longer ISI (150 ms) between the two paired stimulations, being ineffective with the ISI of 20 ms effective for the fixed-frequency version. This longer time interval is in line with the time-course of S1 recruitment during the observation of visual stimuli conveying tactile information, as shown by a previous paired-pulse TMS experiment (Bolognini et al., 2014).

In our previous work (Zazio et al., 2019) we hypothesized the involvement of predictive coding-like mechanisms in the cm-PAS efficacy, and the results of the present study are consistent with such account, highlighting the effect of the anticipatory simulation of the incoming visual-touch

on the cm-PAS effectiveness. Indeed, in the fixed frequency version of the cm-PAS, the time-course of a visual-cortical paired stimulation **is locked and has the same regular occurrence among trials, thus generating – through its statistical regularities - an implicit anticipation of the visual-touch stimulus before its occurrence. In this way participants may have extracted the regularities between trials, anticipating the upcoming (visual) touch. This sort of anticipatory simulation could have induced an early S1 activation by touch observation, allowing its interaction with the TMS pulse at an ISI of 20 ms.** This proposal is in line with paired-association studies in the anterior temporal cortex of macaques showing that that ‘predictive’ activity requires a representation-specific activity prior to sensory input. After paired-association learning, neurons fire more strongly when the first stimulus of a pair predicts that the second stimulus will be their preferred stimulus, than when the first stimulus predicts a non-preferred stimulus; the increased firing to preferred stimuli is present already before stimulus presentation (Meyer and Olson, 2011).

On the other hand, the jittered version of the cm-PAS, **by preventing the anticipation of the visual-touch stimulus, and in turn the pre-activation of S1, allows the interaction of the TMS pulse and the visual-touch stimuli is S1 at longer latency (150 ms). Such latency is more in line with the mirror activity of S1, at least as documented by single-pulse TMS investigations** (Bolognini et al., 2014). Due to the less predictability of the upcoming paired stimulations **given by the jittered frequency of the PAS protocol,** the cortical network mediating the induction of Hebbian associative plasticity cannot be activated in an anticipatory manner, and consequently, the ISI of 20 ms between the visual-touch stimulus and the TMS pulse **becomes** too short to allow their neural interaction.

There is an extensive empirical evidence, both in animals and in humans, showing how prior expectations can modulate sensory processing, by inhibiting or enhancing sensory representations, at both early and late stages (Hindy et al., 2016; Kandula et al., 2015; Kok et al., 2012; O’Neill and Schultz, 2018; Okada et al., 2018; Rao and Ballard, 1999; Rauss et al., 2011). For example, in the visual domain, Kok and co-workers (2017) found stimulus-specific patterns of activation in the

primary visual cortex induced by expectations, which emerge before the appearance of the visual stimulus. These patterns resemble the neural signal evoked by an actually presented sensory stimulus, and are associated to behavioral improvements of a valid expectation. This evidence indicates that expectations allow the sensory cortex to prepare for upcoming sensory signals, in turn facilitating perception (de Lange et al., 2018; Kok et al., 2017). Importantly, with respect to the cm-PAS, these **anticipatory activations** operate either within and between sensory modalities (Arnal et al., 2011, 2009).

Anticipatory simulation plays a key role also in the mirror neuron systems, in particular in the action-observation network (Rizzolatti and Craighero, 2004). Cortical motor areas are indeed activated before the observation of others' actions when sufficient contextual cues about the action itself are available (e.g., Aglioti et al., 2008; Avenanti et al., 2013; Kilner, 2011; Kilner et al., 2007, 2004; Southgate et al., 2009). For instance, Maranesi and colleagues (2014) showed that when the context is highly predictable, also in term of when an event will occur, mirror neurons of monkeys' ventral premotor area are activated a long time in advance before the beginning of the observed action (Maranesi et al., 2014). **Thus, it is possible that similar anticipatory activations occur also in a visuo-tactile network with mirror properties (Blakemore et al., 2005), as suggested by the cm-PAS.**

One can argue that during the *jittered* cm-PAS, the absence of **anticipatory simulation** cannot be fully ruled out: participants could make correct predictions on the time-course of a paired stimulation when the time interval between trials is the longest one (15 s): indeed, after 10 s of cm-PAS, the probability that the stimulus would appear at 15 s is of 100%. However, we suggest that in our jittered version of cm-PAS, this recruitment is not as strong as the one that happens during the standard cm-PAS, because only 1/3 of trials are actually predictable.

Besides clarifying the mechanisms recruited by the cm-PAS, the present findings are also of relevance since they corroborate neural models claiming that **predictive coding-like** mechanisms and Hebbian associative plasticity may cooperate in association learning (Bastos et al., 2012; Friston, 2010;

Spriggs et al., 2018). Indeed, **from a broader perspective**, the *fixed-frequency* cm-PAS represents a protocol for the induction of Hebbian associative plasticity taking advantage of predictive coding-**like** mechanisms. Hence, PAS protocol could be used either to consolidate, or optimize, sensory predictions by encoding causal regularities (Friston, 2010), or to reduce prediction errors, in both cases with the aim of shaping, in a non-invasive way, the activity of predictive coding mechanisms. **However, it has to be noted that, in a predictive coding account, prediction accuracy has to be in the order of milliseconds to induce Hebbian plasticity (Friston, 2005; Friston et al., 2011) and from our results we have no direct evidence (i.e., neurophysiological or neurofunctional measures, but also behavioral ones as reaction times) that this happens during the *fixed-frequency* cm-PAS. Further studies should clarify this possibility, investigating whether S1 is actually pre-activated during the *fixed-frequency* cm-PAS and the temporal profile of such anticipatory activation.**

One limitation of the present study is the absence of neurophysiological correlates of the PAS effects. Future studies should explore the possible neurophysiological underpinnings of the *jittered* cm-PAS, for example by recording somatosensory evoked potentials before and after the administration of the protocol. This would allow exploring whether the *jittered* cm-PAS can induce neurophysiological changes in the somatosensory cortex similar to those of the frequency-fixed cm-PAS (Zazio et al., 2019). Another interesting direction of future studies could be the investigation of the role of metaplasticity (Abraham, 2008) in cross-modal versions of the PAS, as recently shown in the human visual cortex (Bocci et al., 2014).

In conclusion, the present study highlights, for the first time, the recruitment of **anticipatory simulation of sensory stimuli** by PAS protocols designed on the properties of LTP plasticity, **so far largely neglected in PAS studies. Instead, anticipatory process** represents a factor to take into account during the application of PAS protocols, either for optimizing the efficacy of the PAS itself, but even for developing novel protocols that could be used to modulate predictive coding-**like**

mechanisms, whose failures have been implicated in a vast range of neuropsychiatric disorders (Blakemore et al., 2002, 2000; Corlett et al., 2011; Fletcher and Frith, 2009; Fotopoulou, 2014).

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AUTHORS CONTRIBUTION

O. Maddaluno, G. Guidali, N. Bolognini: study concept and design, interpretation of the data, draft and revision of the manuscript. O. Maddaluno, G. Guidali: data collection and statistical analysis. C. Miniussi, A. Zazio: study design, interpretation of the data, revising the manuscript.

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