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RESEARCH ARTICLE | *Sensory Processing*

The third-stimulus temporal discrimination threshold: focusing on the temporal processing of sensory input within primary somatosensory cortex

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Leodori G, Formica A, Zhu X, Conte A, Belvisi D, Cruccu G, Hallett M, Berardelli A. The third-stimulus temporal discrimination threshold: focusing on the temporal processing of sensory input within primary somatosensory cortex. *J Neurophysiol* 118: 2311–2317, 2017. First published July 26, 2017; doi:10.1152/jn.00947.2016.—The somatosensory temporal discrimination threshold (STDT) has been used in recent years to investigate time processing of sensory information, but little is known about the physiological correlates of somatosensory temporal discrimination. The objective of this study was to investigate whether the time interval required to discriminate between two stimuli varies according to the number of stimuli in the task. We used the third-stimulus temporal discrimination threshold (ThirdDT), defined as the shortest time interval at which an individual distinguishes a third stimulus following a pair of stimuli delivered at the STDT. The STDT and ThirdDT were assessed in 31 healthy subjects. In a subgroup of 10 subjects, we evaluated the effects of the stimuli intensity on the ThirdDT. In a subgroup of 16 subjects, we evaluated the effects of S1 continuous theta-burst stimulation (S1-cTBS) on the STDT and ThirdDT. Results show that ThirdDT is shorter than STDT. We found a positive correlation between STDT and ThirdDT values. As long as the stimulus intensity was within the perceivable and painless range, it did not affect ThirdDT values. S1-cTBS significantly affected both STDT and ThirdDT, although the latter was affected to a greater extent and for a longer period of time. We conclude that the interval needed to discriminate between time-separated tactile stimuli is related to the number of stimuli used in the task. STDT and ThirdDT are encoded in S1, probably by a shared tactile temporal encoding mechanism whose performance rapidly changes during the perception process. ThirdDT is a new method to measure somatosensory temporal discrimination.

NEW & NOTEWORTHY To investigate whether the time interval required to discriminate between stimuli varies according to changes in the stimulation pattern, we used the third-stimulus temporal discrimination threshold (ThirdDT). We found that the somatosensory temporal discrimination acuity varies according to the number of stimuli in the task. The ThirdDT is a new method to measure somatosensory temporal discrimination and a possible index of inhibitory activity at the S1 level.

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somatosensory temporal discrimination; primary somatosensory cortex; theta-burst stimulation

TEMPORAL RESOLUTION in the somatosensory domain is relevant for several functions requiring adequate temporal processing of multiple afferent stimuli (e.g., vibratory sense, graphesthesia) (Lacruz et al. 1991). Somatosensory temporal discrimination, defined as the ability to recognize a pair of somesthetic stimuli as clearly distinct, has been investigated in recent years by means of a reliable experimental technique known as the somatosensory temporal discrimination threshold (STDT). The STDT is defined as the shortest time interval at which an individual recognizes a pair of stimuli as separate in time (Artieda et al. 1992; Bradley et al. 2009; Conte et al. 2010; Fiorio et al. 2008; Ramos et al. 2016; Scontrini et al. 2009, 2011).

Time processing of somatosensory information has been studied in the past years with several methodological approaches, including frequency discrimination tasks, time estimation tasks, and temporal order judgment (TOJ) (Knecht et al. 2003; Mangels et al. 1998; Tommerdahl et al. 2007). Compared with STDT, frequency discrimination and time estimation tasks require higher order abilities such as attention and working memory, whereas TOJ does not differentiate the spatial component from the temporal discrimination task (Koch et al. 2009; Tommerdahl et al. 2007). On the other hand, the STDT seems to be a perception threshold uninfluenced by memory formation (Artieda et al. 1992; Lacruz et al. 1991; Tamura et al. 2008).

Previous transcranial magnetic stimulation (TMS) and somatosensory-evoked potential (SEP) studies showed that the STDT is specifically codified in primary somatosensory cortex (S1), and some evidence suggested that its acuity may rely on inhibitory interneuron activity (Bolognini et al. 2010; Conte et al. 2012, 2014; Hannula et al. 2008; Rai et al. 2012; Rocchi et al. 2016; Tamura et al. 2008).

The main feature of the STDT is the relatively stable and reproducible time interval required to discriminate between two stimuli in individual subjects (Conte et al. 2012; Ramos et al. 2016). What is not known is whether, and how, stimulation

characteristics affect tactile temporal discrimination acuity. To gain a better understanding of the physiological mechanisms underlying temporal processing of somatosensory stimuli, in this study we investigated whether the time interval required to discriminate between two stimuli varies according to changes in the number of stimuli in the task. Evidence has shown that S1 activity in response to repetitive stimulation is not static but is modified over short periods of time, thus leading to an improvement in discrimination performance (Goble and Hollins 1993; McGlone et al. 2002; Whitsel et al. 1989). Against this backdrop, we tested whether the interval required to discriminate between two stimuli varies according to changes in the number of stimuli in the task.

To address this issue, we used an experimental paradigm, which we called the third-stimulus temporal discrimination threshold (ThirdDT) and defined as the shortest time interval at which an individual recognizes a third stimulus as clearly distinct following a pair of stimuli delivered at the STDT. Differences between STDT and ThirdDT might indicate that different processes encode the two discrimination tasks. Alternatively, STDT and ThirdDT may share a common encoding process whose acuity varies according to the number of stimuli. To better understand mechanisms underlying STDT and ThirdDT, we searched for a possible correlation between STDT and ThirdDT values and tested the effects of S1 continuous theta-burst stimulation (S1-cTBS) on the STDT and ThirdDT. A significant correlation between STDT and ThirdDT values and a similar pattern of cTBS modulation would suggest a common encoding process.

To test the reliability of ThirdDT, we tested the technique using different intensities and also compared ThirdDT values obtained across different sessions. Finally, to test whether any TMS-induced effect was due to cortical stimulation instead of being caused by possible confounding factors as drop in attention, we investigated the effect of sham S1-cTBS on ThirdDT.

MATERIALS AND METHODS

Study participants. We enrolled a total of 31 healthy subjects (mean age 30.0 ± 0.7 yr; 15 men and 16 women), all of whom were right-handed (Oldfield 1971). Written informed consent was obtained from all the participants. The experimental procedure was approved by the institutional review board at Sapienza University of Rome and conducted in accordance with the Declaration of Helsinki. Neurological examination and medical history were performed by the experimenters during an outpatient visit before recruitment. Participants were screened for peripheral sensory neuropathy by means of their medical history and clinical examination. None of the participants presented clinical signs of peripheral sensory neuropathy, reported a history of any neuropsychiatric disorders, or were taking drugs that act on the central nervous system at the time of the experiments.

STDT procedure. The STDT was investigated by administering paired-stimulus trials. Stimuli consisted of 0.1-ms square-wave electrical pulses delivered using a constant-current stimulator (Digitimer DS7AH) through surface skin electrodes with the anode located 0.5 cm distally to the cathode applied to the volar surface of the middle and distal phalanx of the left index finger. The stimulation intensity was defined for each subject by delivering a series of stimuli at an increasing intensity starting from 2 mA in 0.5-mA steps; the intensity used for the STDT procedure was 120% of the minimal intensity perceived by the subject in 10 of 10 consecutive stimuli. The STDT procedure consisted of four blocks of trials. In each block, paired-stimulus trials were delivered, starting with an interstimulus interval (ISI) of 10 ms and progressively increasing the ISI in 10-ms steps (Conte et al. 2010, 2012). The ISI corresponding to the first of three consecutive trials at which participants recognized the stimuli as temporally separate was considered the STDT value of each block. To keep the subjects' attention level constant during the test and minimize possible perseverative responses, we included "catch" trials consisting of single stimuli delivered randomly. When subjects answered "one" to a catch trial, the block simply went on. When subjects answered "two" to a catch trial, the answer was not taken into account for threshold determination, and the investigator gave verbal feedback to the participant. Paired-stimulus trials were delivered at intervals of 3–5 s. Subjects were asked to report whether they perceived a single stimulus or two temporally separate stimuli by saying "one" or "two" after each stimulation in the interval preceding the next trial. STDT of each subject was defined as the average of four STDT values, i.e., one for each block, and was entered in the data analysis (Fig. 1).

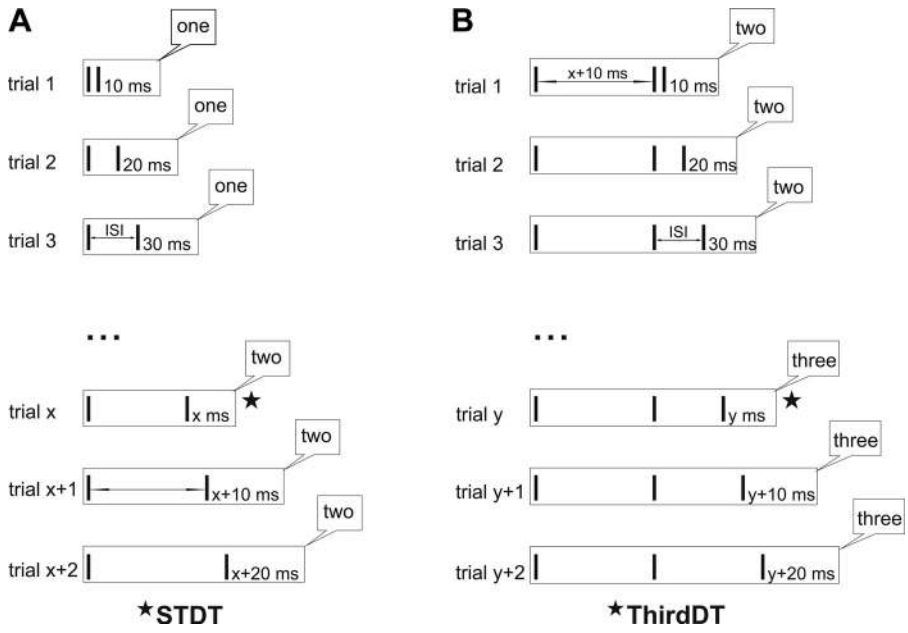

Fig. 1. Somatosensory temporal discrimination threshold (STDT) and third-stimulus temporal discrimination threshold (ThirdDT) determination procedures. *A*: the shortest interstimulus interval (ISI) at which a subject recognized a second stimulus as temporally separate from the first one was considered the STDT value of the block. *B*: the shortest ISI at which a subject recognized a third stimulus as temporally separate from the second one was considered the ThirdDT value of the block. Final STDT and ThirdDT values of each subject were calculated as the average from 4 blocks of trials.

ThirdDT procedure. When determining the ThirdDT, to ensure that the interval between the first and second stimuli was clearly perceived by the subjects, we set the ISI between the first and second stimuli 10 ms higher than the STDT value. To calculate the ThirdDT, we delivered three-stimulus trials, progressively increasing the ISI between the second and third stimuli in 10-ms steps, with an interval of 3–5 s between trials. The ThirdDT procedure consisted of four blocks of trials. The ISI corresponding to the first of three consecutive trials at which participants recognized a third stimulus as temporally separate from the first two was considered the ThirdDT value of each block. The intensity used for the ThirdDT was the same as that used to determine the STDT (i.e., 120% of the minimal intensity perceived by the subject in 10 of 10 consecutive stimuli). As in the STDT procedure, determination of the ThirdDT included "catch" trials using a randomly delivered single stimulus. The perception of "more than two stimuli" was also interpreted as meaning that three stimuli had been detected. The ThirdDT of each subject was defined as the average of four ThirdDT values, i.e., one for each block, and was entered in the data analysis (Fig. 1).

We followed the same STDT and ThirdDT procedures described above for the different sessions.

Transcranial magnetic stimulation. A Magstim Super Rapid magnetic stimulator (Magstim, Whitland, UK) connected to a figure-of-eight coil 90 mm in diameter was used to deliver cTBS over the right S1, according to the stimulation paradigm described in previous work (Conte et al. 2012; Huang et al. 2005). To determine the intensity of cTBS, the active motor threshold (AMT) was tested using single-pulse TMS by placing the coil over the right M1 area in the optimal position (hot spot) for eliciting motor-evoked potentials (MEPs) in the left first dorsal interosseous muscle (FDI). The AMT was calculated during a 20–30% maximum voluntary FDI muscle contraction and defined as the lowest intensity able to evoke an MEP of at least 200 μV in 5 of 10 consecutive trials. Visual feedback from the FDI EMG activity helped the subject to maintain a stable level of contraction during the AMT measurement.

Real S1-cTBS was performed at 80% of the intensity for the AMT and on the scalp over the right S1, defined as a point 2 cm posterior to the "motor hot spot" for the left FDI muscle (Conte et al. 2016b; Ishikawa et al. 2007; Okamoto et al. 2004; Wolters et al. 2005). For sham S1-cTBS, the coil was positioned on the same spot but held perpendicularly to the scalp surface.

Electromyographic recording. For the experiments with S1-cTBS, we recorded EMG activity from the left FDI muscle through surface electrodes placed in a belly-tendon manner. Raw signals were sampled at 5 kHz using a CED 1401 A/D laboratory interface (Cambridge Electronic Design, Cambridge, UK), amplified, and filtered (bandwidth 20 Hz–1 kHz) using a Digitimer D360 (Digitimer, Welwyn Garden City, UK). Data were stored on a computer for online visual display and offline analysis (Signal software; Cambridge Electronic Design).

Experimental sessions. The study comprised five experimental sessions that took place at least 2 weeks apart. In the main experimental session, participants underwent the STDT and ThirdDT studies ($n = 31$ subjects). In a different experimental session, we investigated changes in STDT and ThirdDT values before (T0) and 5 (T1), 15 (T2), 30 (T3), and 45 min (T4) after right real S1-cTBS ($n = 16$ subjects, mean age 31.1 ± 1.0 yr, 10 men and 6 women). In a third experimental session, the STDT and ThirdDT values were determined, and the ThirdDT was then retested by delivering the first two stimuli or the third stimulus at an intensity that was 20% below the one used in the main experiment (e.g., at 100% of the minimal intensity perceived by the subject in 10 of 10 consecutive stimuli) ($n = 11$ subjects, mean age 31.5 ± 1.5 yr, 3 men and 8 women). In a fourth experimental session, we first determined the STDT and ThirdDT and then retested the ThirdDT by delivering *1*) the first stimulus at an intensity that was 200% of that used in the main experiment (e.g., 240% of the minimal intensity perceived by the subject in 10 of 10 consecutive stimuli) and *2*) the first and the second stimuli at an intensity that was 200% of that used in the main experiment (e.g., 240% of the minimal intensity perceived by the subject in 10 of 10 consecutive stimuli) ($n = 10$ subjects, mean age 30.0 ± 1.8 yr, 5 men and 5 women). Finally, in a last experimental session, we determined the basal values of STDT and ThirdDT, and the latter was retested 5 (T1), 15 (T2), 30 (T3), and 45 min (T4) after right sham S1-cTBS ($n = 10$ subjects, mean age 31.2 ± 1.7 yr, 5 men and 5 women). The order of the sessions was randomized for each participant except for the sham S1-cTBS session, which was done as *the* last control experiment. Each session included separate basal determination of STDT and ThirdDT.

Statistical analysis. Data were analyzed using SPSS version 20.0 (IBM). We first used a paired *t*-test to evaluate any differences between STDT and ThirdDT values in the main experiment. Pearson's correlation coefficient was used to identify any correlations between STDT and ThirdDT values obtained in the first experimental session.

To analyze changes in STDT and ThirdDT values after real cTBS over S1, we ran a repeated-measures ANOVA with factors "threshold" (two levels: STDT vs. ThirdDT) and "time" (5 levels: T0, T1, T2, T3, T4). To compare the ThirdDT values delivered at different stimulation intensities, we ran a repeated-measures ANOVA with factor "stimulus intensity."

To see whether the STDT and ThirdDT tasks produce reliable values between different sessions, we calculated the intraclass correlation coefficient (ICC) for "average measures" using the basal values of subjects who took part in at least two sessions. For each threshold task, we calculated a separate ICC using two measurements in the 27 subjects who underwent 2 sessions and in the 13 subjects who underwent 3 sessions. Moreover, two separate two-way mixed ANOVA with factors "session" (3 levels: main, second, third) and "sex" (2 levels: male vs. female) was performed to evaluate the variability of STDT and ThirdDT baseline values across different sessions and to disclose a possible effect of sex on this variability ($n = 13$ subjects: those who underwent 3 sessions; 6 men and 7 women).

Finally, a mixed ANOVA with factors "session" (real cTBS, sham cTBS) and "time" (5 levels: T0, T1, T2, T3, T4) was used to analyze changes in ThirdDT values after real cTBS over S1 as opposed to sham S1-cTBS.

Before performing ANOVA procedures, we assessed data distribution by means of the Shapiro-Wilk test. $P < 0.05$ was considered to indicate statistical significance. Data were tested for nonsphericity. Greenhouse-Geisser's correction was applied when needed. Tukey's test was applied for the post hoc analysis. Data are means ± SD.

RESULTS

Shapiro-Wilk test showed that basal STDT and ThirdDT values in all sessions were normally distributed.

STDT vs. ThirdDT. Paired-sample *t*-test showed that STDT and ThirdDT values differed significantly (STDT = 53.20 ± 20.2 ms, range 20–100 ms, vs. ThirdDT = 31.26 ± 8.9 ms, range 20–50 ms; $t = 8.0$, df = 30, $P < 0.00001$; Fig. 2). Pearson's correlation coefficient showed a significant moderate correlation between STDT and ThirdDT values ($r = 0.707$, $P < 0.00001$; Fig. 3).

Changes in STDT and ThirdDT values after real S1-cTBS. Repeated-measures ANOVA, performed to detect any changes in STDT and ThirdDT values after real S1-cTBS, revealed statistical significance of the factors threshold [$F_{(1,15)} = 53.55$; $P < 0.0001$; $\eta_p^2 = 0.78$] and time [$F_{(4,60)} = 12.61$; $P < 0.0001$; $\eta_p^2 = 0.46$] and a significant "threshold" × "time" interaction [$F_{(4,60)} = 3.75$; $P = 0.009$; $\eta_p^2 = 0.20$]. The thresholds significantly increased after cTBS at T2 (STDT: $P < 0.0001$; ThirdDT: $P < 0.001$) and T3 (STDT: $P < 0.0001$; ThirdDT:

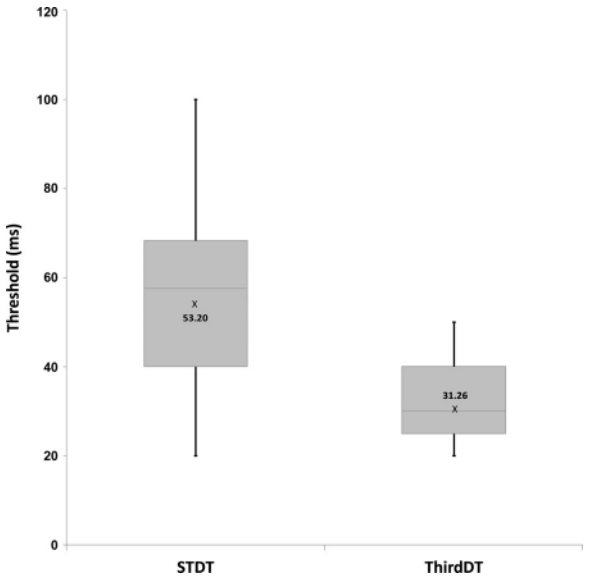

Fig. 2. Somatosensory temporal discrimination threshold (STDT) and third-stimulus temporal discrimination threshold (ThirdDT) in healthy subjects (n = 31). The whiskers represent the ranges of STDT and ThirdDT values, whereas the height of the boxes shows the interquartile ranges (STDT and ThirdDT values between the 1st and 3rd quartiles). The horizontal lines represent the median whereas X represents the mean of the STDT and ThirdDT values.

$P = 0.001$). At T4 (45 min after cTBS), only the ThirdDT was still significantly increased ($P = 0.024$), whereas the STDT returned to basal values ($P = 0.054$; all df = 15). The maximum cTBS-induced modulation in the two threshold tasks was 17.29% for the STDT and 28.54% for the ThirdDT (Fig. 4).

Effects of stimulus intensity on ThirdDT. Despite the 20% reduction in stimulus intensity, all the participants still clearly perceived a stimulus delivered at this reduced intensity, and the repeated-measures ANOVA revealed no significance of the factor intensity [$F_{(2,20)} = 0.58$; $P < 0.52$; $\eta_p^2 = 0.055$], thus showing that ThirdDT values did not change following the 20% reduction in the intensity of the first two stimuli (33.18 ± 10.55 ms) or of the third stimulus (31.36 ± 10.51 ms) compared with those used in the main experiment (e.g., 120% of the minimal intensity perceived: 34.85 ± 9.0 ms).

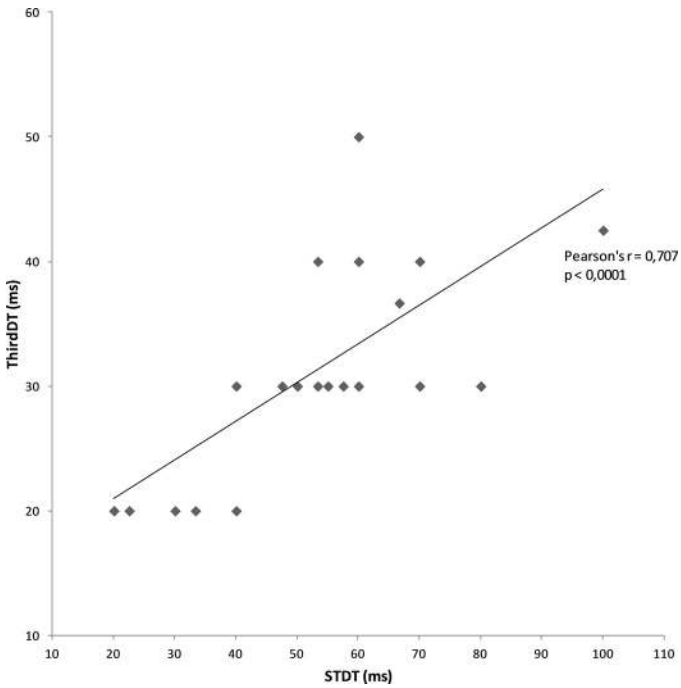

Fig. 3. Correlation between somatosensory temporal discrimination threshold (STDT) and third-stimulus temporal discrimination threshold (ThirdDT) (n = 31 subjects).

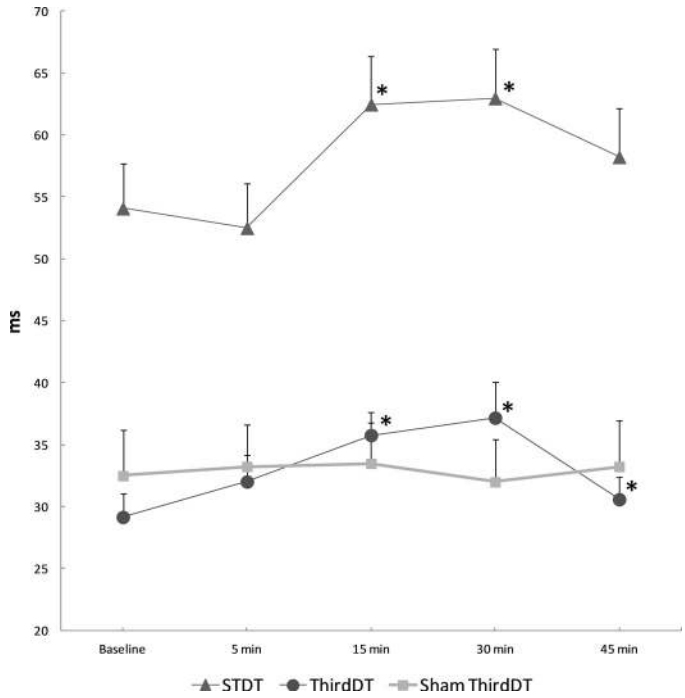

Fig. 4. Changes in somatosensory temporal discrimination threshold (STDT; triangles) and third-stimulus temporal discrimination threshold (ThirdDT; circles) induced by real continuous theta-burst stimulation (cTBS) over primary somatosensory cortex (S1-cTBS) (n = 16 subjects). Changes in ThirdDT values induced by sham S1 cTBS are represented by squares (n = 10 subjects). The y-axis indicates temporal discrimination thresholds expressed in ms; x-axis is time expressed in minutes. Error bars indicate SE. *$P < 0.05$, statistical significance following real S1-cTBS.

When the intensity of the first stimulus or of the first and second stimuli was doubled, none of the subjects described the stimulus as painful. Again, repeated-measures ANOVA revealed no significance of the factor intensity [$F_{(2,18)} = 0.62$, $P = 0.47$; $\eta_p^2 = 0.065$), thus showing that ThirdDT values did not change significantly from the basal values (same intensity used in the main experiment, 120%: 28.48 ± 8.6 ms vs. first stimulus at increased intensity: 28.36 ± 9.9 ms vs. first and second stimuli at increased intensity: 26.41 ± 7.1 ms).

STDT and ThirdDT reliability. The STDT yielded an ICC of 0.959 [IC 95%: 0.912–0.981; $F_{(26,26)} = 25.18$; $P < 0.0001$] for the two-session analysis and an ICC of 0.953 [IC 95%: 0.884–0.984; $F_{(12,24)} = 21.34$; $P < 0.0001$] for the three-session analysis, whereas the ThirdDT task yielded an ICC of 0.707 [IC 95%: 0.351–0.867; $F_{(26,26)} = 3.34$; $P = 0.002$] for the two-session analysis and an ICC of 0.815 [IC 95%: 0.546–0.938; $F_{(12,24)} = 5.82$; $P = 0.0001$] for the three-session analysis. The two separate two-way ANOVAs performed to evaluate the variability of STDT and ThirdDT baseline values showed no main effect of session [STDT: $F_{(2,22)} = 0.174$; $P = 0.842$; $\eta_p^2 = 0.016$; ThirdDT: $F_{(2,22)} = 2.013$; $P = 0.164$; $\eta_p^2 = 0.155$] and no significant session × sex interaction [STDT:

$F_{(2,22)} = 0.408$; $P = 0.670$; $\eta_p^2 = 0.036$; ThirdDT: $F_{(2,22)} = 1.472$; $P = 0.253$; $\eta_p^2 = 0.118$].

Changes in ThirdDT values after sham S1-cTBS. Mixed ANOVA revealed no significant effect of factor session [$F_{(1,24)} = 0.0002$; $P = 0.99$; $\eta_p^2 < 0.001$] but a significant effect of factor time [$F_{(4,96)} = 3.0$; $P = 0.022$; $\eta_p^2 = 0.11$] and a significant session × time interaction [$F_{(4,96)} = 3.47$; $P = 0.011$; $\eta_p^2 = 0.13$]. Post hoc comparison confirmed that values of ThirdDT were higher after real cTBS at T2, T3, and T4 (see above), whereas they were not different after sham cTBS at all time points (all P values >0.1, df = 9; Fig. 4).

DISCUSSION

The first novel finding of the study is that the interval required to discriminate the third from the second stimulus was shorter than that between the second and the first (i.e., ThirdDT was lower than STDT). STDT and ThirdDT values correlated positively, and real S1-cTBS significantly increased both the STDT and ThirdDT, although the latter was affected to a greater extent and for a longer period of time. When all the experimental sessions were considered, both the STDT and ThirdDT techniques yielded reliable results, and no sex-related differences were observed. As long as it was within the perceivable and painless range, the stimulus intensity did not affect ThirdDT values. Finally, sham S1-cTBS did not affect ThirdDT, thus confirming that the real cTBS-induced effect was due to S1 stimulation.

Because we found that the ISI required to discriminate the third stimulus is shorter than that required to recognize the first two stimuli, we may assume that the temporal acuity of sensory processing is sharpened in the ThirdDT task. Several explanations are possible for the present findings. A greater level of sustained attention required in the ThirdDT task than in the STDT task may account for the sharper time processing. Sustained attention may help S1 neurons to more accurately perform the temporal encoding of sensory stimuli (Eimer and Forster 2003; Salinas et al. 2000). However, because the reliability of results was high between sessions for both thresholds, and because ThirdDT values during the sham session were consistent in several determinations along 45 min, we tentatively exclude the hypothesis that a different level of sustained attention could be the main factor determining STDT and ThirdDT values. Alternatively, the difference between STDT and ThirdDT may rely on changes in the acuity of the time encoding system during the discrimination task We hypothesize that the "sharpened" discrimination acuity we detected in the ThirdDT may be related to the neural circuits involved in sensory processing. Closely coupled excitatory-inhibitory thalamocortical innervation allows high temporal precision in sensory information decoding (Gibson et al. 1999). A person's ability to discriminate may vary as a result of dynamic changes in the balance of excitatory-inhibitory interactions (Buonomano 2000; Gil et al. 1999). The thalamus conveys inputs to the somatosensory cortex by monosynaptically innervating glutamatergic excitatory and GABAergic inhibitory interneurons (Cruikshank et al. 2007). These inhibitory interneurons increase the signal-to-noise ratio, thereby sharpening the thalamus's temporal profile (Swadlow 2003). In keeping with this hypothesis, Tamura et al. (2008) suggested that intracortical inhibitory interneurons play an important role in somatosensory temporal discrimination capability, and recent studies have hypothesized that S1-TMS and high-frequency repetitive sensory stimulation modulate STDT via an effect on cortical inhibitory interneurons within S1 (Conte et al. 2012; Erro et al. 2016; Rai et al. 2012; Rocchi et al. 2016). It is thus possible that the enhanced temporal sensory processing acuity in the ThirdDT depends on more effective feedforward inhibition following the second stimulus in the ThirdDT task. We may speculate that the first stimulus may modulate the inhibitory tone, thereby conditioning its response to the second stimulus. This hypothesis is supported by peripheral paired-pulse SEP studies showing that a conditioning stimulus exerts an inhibitory effect on subsequent evoked cortical responses (Angel 1967; Onishi et al. 2016; Shagass and Schwartz 1964). However, our hypothesized relation between ThirdDT and inhibitory tone at the S1 level remains speculative and needs to be tested by future studies.

In keeping with the findings of previous studies, we observed that S1-cTBS modulates the STDT (Conte et al. 2012, 2014; Rocchi et al. 2016). Because real S1-cTBS also modulated ThirdDT values but sham stimulation did not, we conclude that S1 plays a prominent encoding role in both the ThirdDT and STDT. We also found that cTBS increased ThirdDT values to a greater extent than STDT values. Previous studies suggested that S1-cTBS worsens the STDT by reducing the excitability of GABAergic interneurons that produce feedforward inhibition of pyramidal neurons (Conte et al. 2012, 2014; Rocchi et al. 2016). If the inhibitory system is involved to a greater extent in the ThirdDT task, the ThirdDT may be more susceptible to cTBS than the STDT. The significant correlation we found between STDT and ThirdDT values fits well with the hypothesis of a common mechanism underlying the two thresholds.

We took numerous precautions to avoid possible confounding factors. We excluded subjects with a history or clinical features that might be indicative of peripheral nerve abnormalities. To detect any potential response bias related to changes in attention levels or perseverative responses, our experimental protocol also included catch trials consisting of a single stimulus (Scontrini et al. 2009). To ensure that subjects consistently perceived the first two stimuli in the ThirdDT task as separate, we set the ISI between the first and the second stimuli 10 ms above the STDT value and verified that subjects already perceived the two stimuli from the first ISI. In the ThirdDT task, we also considered a response of "more than two" as valid for threshold determination. We thus limited possible bias due to a process of counting encoded by cortical areas other than S1, which might be more susceptible to attention variability. We used an interval of 3–5 s to separate trials to avoid possible carryover effects of the preceding stimulation on the subsequent ThirdDT trial. Because both the ThirdDT and STDT basal values proved to be stable across different sessions on different days, a fluctuating phenomenon, such as attention, is unlikely to be able to explain the significant differences between the two thresholds. We also excluded the possibility that the differences between the ThirdDT and STDT values were due to changes in the number of peripheral fibers activated, because the different stimulus intensities we used did not have any effect on the ThirdDT values. This hypothesis is supported by a recent study showing that doubling the intensity of the stimuli has no effect on STDT values (Conte et al. 2016a).

We acknowledge that our study has some limitations. We did not investigate the effect of more than three stimuli on somatosensory temporal discrimination. However, a growing number of stimuli requires a counting process involving secondary associative areas, which are more susceptible to subjective variables and attention biases (Kansaku et al. 2006; Trick and Pylyshyn 1994). Similarly, we cannot exclude the possibility that higher stimulus intensities than those used by us modulate ThirdDT values. Using a higher intensity, however, would induce pain and consequently involve the activation of neural circuits other than those associated with the tactile discriminative function. Because we did not test the possible effects induced by changing ISIs between the first and second stimuli, the hypothesis of a conditioning effect of the first stimulus remains entirely speculative. The intraclass correlation coefficient assessed for the ThirdDT task is based on a small sample; further studies are thus needed to confirm the reliability of this testing procedure. Because our protocol did not include a neuronavigation system, the cTBS effect on the two thresholds might be due to the stimulation of areas other than S1. However, recent studies based on the same hot spot for S1-cTBS reported changes in the parietal components of somatosensory evoked potentials and in STDT values (Conte et al. 2012; Ishikawa et al. 2007), and a more recent study excluded any effects of S2-cTBS on the STDT (Rocchi et al. 2016). It is therefore likely that the changes we detected in the ThirdDT and STDT following cTBS are consequent to a direct effect of cTBS on S1.

In conclusion, somatosensory temporal discrimination acuity depends on the number of stimuli used in the task. STDT and ThirdDT are both encoded in S1. This study shows that the somatosensory temporal discrimination system is dynamically modulated with time resolution changes in a very short time during the encoding process. Investigation of both STDT and ThirdDT may provide a better understanding of the somatosensory temporal discrimination impairment reported in movement disorders (Artieda et al. 1992; Conte et al. 2010, 2014, 2016a; Rocchi et al. 2013).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

G.L., A.F., A.C., D.B., and A.B. conceived and designed research; G.L., A.F., X.Z., A.C., and D.B. performed experiments; G.L., A.F., X.Z., A.C., and D.B. analyzed data; G.L., A.F., X.Z., A.C., D.B., and A.B. interpreted results of experiments; G.L., A.F., X.Z., A.C., D.B., and A.B. prepared figures; G.L., A.F., X.Z., A.C., G.C., M.H., and A.B. drafted manuscript; G.L., A.F., A.C., D.B., G.C., M.H., and A.B. edited and revised manuscript; G.L., A.F., X.Z., A.C., D.B., G.C., M.H., and A.B. approved final version of manuscript.

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