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Highlights

- Motor errors observation elicits an early negativity over centro-frontal brain regions
- Centro-frontal brain regions are causally involved in the detection of action error
- The fronto-central cortex encodes motor error
- Stimulation over motor areas facilitates error detection in non-familiar actions
- Early anterior negativities may index syntactic processing in the action domain
- Syntactic processing may share neuro-functional resources across cognitive domains

Abstract

Action execution is prone to errors and, while engaged in interaction, our brain is tuned to detect deviations from what one expects from other's action. Prior research has shown that Event-Related-Potentials (ERPs) are specifically modulated by the observation of action mistakes interfering with goal achievement. However, in complex and modular actions, embedded motor errors do not necessarily produce an immediate effect on the global goal. Here we dissociate embedded motor goals from global action goals by asking subjects to observe familiar but untrained knotting actions. During knotting an embedded motor error (i.e. the rope is inserted top-down instead of bottom-up during the formation of a loop) while not producing any immediate mistake, may strongly affect the final result. We found that embedded errors elicit in the observer specific early fronto-central negativity (120-180 ms). In a second experiment, we online administered **exicitatory** transcranial magnetic stimulation (TMS) over central (C3) or occipital (Oz) scalp locations, at the timing of the ERP components observed in the first experiment. C3 stimulation produced a significant **improvement** in embedded error discrimination performance. These results show that sensorimotor areas are instrumental in the early detection of embedded motor errors. We conclude that others' embedded errors provide fundamental cues which, inserted within a complex hierarchical action plan, might be used by the observer to anticipate whether an action will eventually fail.

Keywords: action processing, early negativity, ERPs, TMS, observation of motor errors

1. Introduction

Starting early on in development, understanding the actions and the intentions of others is essential for social interactions (Grèzes & de Gelder, 2008). **While actions can be understood also via auditory inputs, we refer here to visually based action understanding** (e.g., Fang, Chen, Lingnau, Han, & Bi, 2016). **In regard to action understanding**, it has been proposed that the activities of multiple neural routes are integrated in the process of action understanding (Kilner, 2011). According to this view, action understanding is achieved by the ventral route (Middle temporal gyrus – MTG - and the anterior Inferior Frontal Gyrus - aIFG), whereas the dorsal route (Inferior Parietal Lobule – IPL - and the posterior Inferior Frontal Gyrus - pIFG) support this process by reactivating the most likely action needed to achieve the predicted goal. In line with the predictive account, action discrimination could rely on internal forward models (Flanagan & Johansson, 2003; Kilner, Vargas, Duval, Blakemore, & Sirigu, 2004) to anticipate the unfolding of a given action (Schütz-Bosbach & Prinz, 2007). Thus, by employing our own motor system as an "emulator" the dorsal route may project top-down motor-based expectations and constrain inferences on other's action (Schütz-Bosbach & Prinz, 2007).

Action execution is, however, prone to errors. One can accidentally drop a glass, omit important steps of an action sequence, use an incorrect tool to perform an action or perform erroneous movements for a specific action context. Errors may occur with different degree of severity, some of them significantly altering action outcomes. At the same time, these errors are usually unexpected by the observer and it is unlikely that we can efficiently predict them (Schiffer, Krause, & Schubotz, 2014). Studies investigating other's errors observation used so far reach-to-grasp actions containing an error (e.g., Panasiti, Pavone, & Aglioti, 2016; Pezzetta, Nicolardi, Tidoni, & Aglioti, 2018; Spinelli, Tieri, Pavone, & Aglioti, 2018), everyday actions ending either correctly or incorrectly (de Bruijn, Schubotz, & Ullsperger, 2007) or the disruption of the coherent temporal order of action steps either within an action sequence (Maffongelli et al., 2015; Maffongelli, Antognini, & Daum, 2018; Maffongelli, D'Ausilio, Fadiga, & Daum, 2019) or at the action outcome (Balconi & Canavesio, 2015). The common denominator of the studies discussed so far is given by the fact that they used action observation paradigms showing errors that immediately interfere with the achievement of action goal. However, while performing a complex action, *embedded* errors can be

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temporally dissociated from the final action goal. In fact, embedded errors, occurring during the unfolding of an action sequence, may not necessarily be erroneous actions *per se*, rather they become errors only after having considered the whole action sequence. Here we set out to investigate whether an error-driven reaction could still be elicited by the observation of these embedded errors. To this purpose, we used a familiar but untrained knotting action because it allows to investigate observer's reactions to embedded motor error during loops formation (such that the rope is inserted top-down instead of bottom-up; this action step is an essential means to reach the knotting, that is the higher-action goal. See methods for more details and Figure 1a; Cardellicchio et al., 2018). Although passing a rope over or below itself does not produce any immediate effect, in terms of the final goal (knotting) it may or may not have significant effects.

In Experiment I, we used EEG to assess the ERPs correlates of the experimental manipulation (Error condition) as compared to the canonical condition (i.e. No Error) and to a further control condition consisting of a visual change of the appearance of the rope (Color-change condition, to control for unspecific attentional effects). In Experiment II, during a similar experimental paradigm, participants were administered with online dual-pulse TMS (over central or occipital locations) at the time of the latency of the ERPs modulation observed in Experiment I. Left central (C3) or occipital regions (Oz) were investigated to verify the role of sensorimotor and visual areas on the discrimination of embedded errors.

Based on previous results on the observation of action sequence violations (Maffongelli et al., 2015, 2018) we predict that embedded motor errors would elicit an early negativity effect within a fronto-central ROI (region of interests). As far as the TMS experiment is concerned, we hypothesize that TMS stimulation to left central scalp location will perturb motor error discrimination performance**. Given the essential role played by state-dependency, it is very difficult to predict the direction of effects for online TMS protocols. Indeed, in order to infer causality the direction of the effect is of relative importance. Behavioral change in any of the two directions,** would suggest that sensorimotor brain regions activities play a causal role in driving our ability to detect errors **embedded** in everyday actions.

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2. Experiment I

2.1. Materials and Methods

2.1.1. Participants

Twenty-five subjects participated in Experiment I (mean age: 27 years; SD: 2.4, 11 females) and gave informed consent to the experimental procedures, which were approved by the local ethics committee ASL-3 ("Azienda Sanitaria Locale", Local Health Unit, Genoa) and were in accordance with the Declaration of Helsinki of 1975 (as revised in 1983). All participants had normal or corrected-to-normal vision and were right-handed (Oldfield, 1971). Four subjects were excluded from the subsequent analyses due to excessive EEG artifacts or outlier behavior (see below for details). We selected participants without specific knot expertise (i.e., climbing, sailing, etc.).

2.1.2. Stimuli

For the experimental stimuli, we used picture sequences depicting the composition of 5 knots (Overhand knot, Franciscan monk's knot, Slip knot, Figure-eight knot, Overhand knot double rope). Only knots that included a step with a looping operation (full circle) were chosen. Pictures (taken with a Canon 60D digital camera and edited with Photoshop CS 6; final resolution 1920x1280 pixels) were shot from a first-person perspective and showed the hands of an actor (1 male and 1 female, randomly intermingled during the experiment) handling the rope. Picture sequences for each knot were composed by a different number of frames depending on knot complexity (17-27 frames). Each frame encompassed a unique spatial and point-of-contact relationship between the hands and rope, so as to convey new critical information (see supplementary materials).

Sequences were manipulated by introducing an execution error (Error condition). This error concerned the loop formation and arose if the rope (working end) was inserted top-down (Figure 1, panel 1) finally causing the knot to untighten and thus, the failure in goal achievement. The second condition was a sequence in which no error was introduced (No Error) and the third condition was a control condition (Color-change condition) in which the rope's color was changed (from white to red) during one single frame of the sequence. This control condition was implemented to account for visual arousals only. This change occurred in the same frame as the one manipulated in the Error condition (Figure 1, panel 2).

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Insert Figure 1 here

2.1.3. Procedure and trial structure

Participants were comfortably seated in a dimly illuminated room, facing a 17-inch computer screen located 70 cm from their frontal plane. They were asked to avoid body movements during the recording session, and to blink, if necessary, only between trials. A short training session of 5 trials (taken from the experimental set of stimuli and showing all conditions) preceded the main experiment to familiarize subjects with the experimental situation. In the experimental blocks, each of the five knots was knotted by one of two actors (female and male actor, counterbalanced order across sequences). The ten sequences (5 knots x 2 actors) were randomly repeated in each of the three experimental conditions (Error, Color-change and No Error) and with two different frame durations (see below for details), leading to a set of 60 trials (10 knots x 3 conditions x 2 frame durations), which was repeated three times for a total of 180 trials. After 45 trials short breaks occurred where subjects could rest, and also allowed the experimenter to check electrodes impedance. The whole experiment lasted about 2 hours, including the EEG preparation.

Each trial began with the presentation of a first frame depicting the to-be-realized knot that remained on screen until participants pressed a button on the keypad to give them a cue on the final goal. Once the participant pressed the button, the frame sequence presentation started. Participants were instructed to carefully observe the individual frames composing the sequence and to press the button (task) on a keypad only once, under two possible circumstances: (i) if they detected an execution error in the sequence (Error condition), or (ii) if they perceived a change of the color of the rope (Color-change condition). Because of the different length of the sequences, the error occurred after a different number of frames over the knot sequences. Reaction times (RTs) were collected for both button presses.

Single frame duration was either 0.2 s followed by an inter-stimulus-interval (ISI) of 0.8 s or 0.6 s with an ISI of 0.4 s. This manipulation was introduced to verify whether the duration of

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stimuli presentation affected the ERP responses. In both conditions, however, the rhythm of presentation was kept constant (1 s per frame). The inter-trial interval was 5 seconds. Timing, presentation, randomization and button response recordings were controlled by the Psychtoolbox functions (Brainard, 1997), running in Matlab (Mathworks, Inc.).

2.1.4 EEG recording and pre-processing

EEG was recorded by means of a 32 Ag/AgCl active electrodes cap (ActiCAP, Brain Products, Munich, Germany) fixed on the scalp according to the international 10-20 system and by the acquisition software Brain Vision Recorder. The continuous EEG signal was amplified by Brain Amp MR plus amplifier (Brain Products, Munich, Germany) with 500 Hz sampling rate, 10s time constant and 250 Hz high-cutoff frequency, referenced to AFz. Electrode impedance was kept below 5 kΩ. The electro-oculogram (EOG) was monitored by electrodes placed at the outer canthi of the left and right eye. Data were re-referenced offline to the algebraic mean of TP9 and TP10 (Light et al., 2010), filtered offline with a 0.5-45 Hz band pass filter and then down sampled to 250 Hz. Artifacts were removed through visual inspection by Independent Component Analysis (ICA) implemented in EEGlab (Delorme & Makeig, 2004), considering time, topographic and spectral distribution of the component.

2.1.5. Analysis

2.1.5.1. Behavioral data

RTs were analyzed using a repeated measures analysis of variance (RM-ANOVA) considering the within-subject 2 levels factor CONDITION (Error condition, Color-change condition) and two levels TIMING of frame duration (t1= 0.2 s, t2= 0.6 s). The No Error condition was not included in this analysis since for this condition no button press was required. Participants were asked to respond as soon as they detected an execution error (Error condition) or a change in rope color (Color-change condition). The task was simple and accuracy high (motor error condition mean ± standard error = 88 ± 2.8%; Color-change condition mean = 99.9 ± 0.1%). Statistical analyses were performed with the R statistical package (R Development Core Team, 2011).

2.1.5.2. ERPs analysis

Data were segmented in epochs from -300 to 800 ms relative to the onset of the critical frame using a common Baseline (from -300 ms to 0 ms, where 0 represents stimulus onset). RM-ANOVA included the factors CONDITION (Error, No Error, Color-change), TIMING (t1, t2), TIME_WINDOW (Early, Late), HEMISPHERE (left, right), ANT_POST (Left & Right Anterior ROI = Anterior; Left & Right Posterior ROI = Posterior), **with subject as within factor and the amplitude of the average potential in µV as dependent variable**. The factor TIMING accounted for the two timings of frame duration: t1= 0.2 s of frame duration and 0.8 s of inter stimulus interval (ISI); t2= 0.6 s of frame duration and 0.4 s ISI. The factor TIME_WINDOW considered two separate epochs of the ERP data to focus on the early components, an Early phase from 120 to 180 ms and a Late phase from 180 to 280 ms. Analysis was done over two windows to independently capture the two negative ERP components. The factors ANT_POST and HEMISPHERE were defined based on the subdivision of the scalp into ROIs: left-anterior (F3, F7, FC1, FC5, C3) and right-anterior (F4, F8, FC2, FC6, C4) formed the anterior part of the scalp; left-posterior (CP1, CP5, P3, P7) and right-posterior (CP2, CP6, P4, P8) formed the posterior part of the scalp.

In the statistical analyses, only significant interactions or main effects ($p < 0.05$) are reported. When sphericity assumptions were violated, based on Mauchly's test, we report Huynh-Feldt-corrected p-values (Huynh & Feldt, 1970; Mauchly, 1940). Post-hoc analyses were performed by means of paired t-tests applying false discovery rate (FDR) correction for multiple comparisons. Only significant post-hoc comparisons are reported. Statistical analyses were performed with the R statistical package (R Development Core Team, 2011).

3. Results

3.1. Reaction times

Reaction times (RTs) did not differ across conditions (Error condition; Color-change condition) or timing of frame duration (t1 = 0.2 s, t2 = 0.6 s) as demonstrated by an analysis of Variance (ANOVA) showing no significant main effects for CONDITION ($F(1,79) = 2.37$, $p = 0.12$) and TIMING ($F(1,79) = 0.07$, $p = 0.79$) and no significant interaction between CONDITION and TIMING ($F(1,79) = 0.04$, $p = 0.83$; For t1, Color-change condition: Mean = 0.45 ± 0.03 s; Error condition: Mean = 0.44 ± 0.07 s. For t2, Color-change condition: Mean = 0.46 ± 0.04 s; Error condition: Mean = 0.44 ± 0.06 s). These results indicate that both

conditions induced similar response times, irrespective of experimental manipulation and presentation rate.

3.2. Event-related-potentials

The full results of the repeated measures (RM-ANOVA) concerning significant main effects and interactions are reported in Table I. The factor TIMING was not further considered in analyses, since no main effect was revealed by ANOVA. Here we describe only the post-hoc analysis of the most relevant interaction. The Error condition showed a negative deflection distributed over the left hemisphere in the early time window (120-180 ms) after stimulus onset (Figure 2). The RM-ANOVA revealed a four-way significant interaction CONDITION (Error, No Error, Color-change) x ANT_POST (anterior, posterior) X TIME_WINDOW (early, late) x HEMISPHERE (left, right) ($F(2,40) = 3.35$; $p = 0.04$). Post-hoc analysis (Figure 3) showed significant differences for the Error condition compared to the No Error condition in the left anterior ROI, in the early (120-180 ms) time window ($t(41) = 2.26$, $p = 0.04$). The comparison between Color-change condition and Error condition showed a significant effect in the late (180-280 ms) time window in both the right anterior ROI ($t(41) = 2.76$, $p = 0.01$) and the left anterior ROI ($t(41) = 3.42$, $p = 0.004$).

Insert Table 1 here

EFFECT	Dfn	Dfd	F	p value	ηp^2
CONDITION	2	40	4.10	0.02	0.170
TIME_WINDOW	1	20	8.31	0.009	0.293
CONDITION X TIME_WINDOW	2	40	13.1	0.00004	0.397
CONDITION X ANT_POST	2	40	6.01	0.005	0.231
ANTPOST X HEMISPHERE	1	20	5.07	0.03	0.202
CONDITION X TIME_WINDOW X ANT_POST	2	40	3.75	0.03	0.158
CONDITION X TIME_WINDOW X HEMISPHERE	2	40	10.2	0.0002	0.338
CONDITION X TIME_WINDOW X ANT_POST X HEMISPHERE	2	40	3.35	0.04	0.143

Table 1: Full results of the repeated measures (RM-ANOVA) for the event-related potentials, showing all main effects and interactions from the model (DFn: numerator degrees of freedom, DFd: denominator DF, F statistic, p-value and partial eta squared).

Insert Figure 2 here

Insert Figure 3 here

In posterior ROIs the comparison between Color-change and Error condition showed a significant difference in the early time window both in the left ($t(41) = 3.57$, $p = 0.003$) and in the right hemisphere ($t(41) = 2.74$, $p = 0.01$). The same effect was also found in the late time window in both hemispheres (left, ($t(41) = 3.90$, $p = 0.003$; right, $t(41) = 3.74$, $p = 0.003$).

In summary, when a motor-based error (Error condition) is compared to the No Error condition, effects are observed in left anterior regions in the early time window (120-180 ms after stimulus onset).

4. Experiment II

4.1. Materials and Methods

4.1.1. Participants

Seventeen adults participated in Experiment II (mean age: 25.2; SD: 2.7; 5 females). Five subjects were excluded from subsequent analyses because of technical problems during data acquisition. The experimental protocol was reviewed and approved by the local ethics committee ASL-3 ("Azienda Sanitaria Locale", Local Health Unit, Genoa). Participants gave informed, written consent for participation in the study according to the Declaration of Helsinki of 1975, as revised in 1983. As for the Experiment I, all subjects were naive to the purpose of the study and had no prior knot-making expertise. All had normal or corrected-to-normal vision and were right-handed, as assessed by an adapted Italian version of the Edinburgh handedness inventory (Oldfield, 1971). None of the subjects had contraindications to TMS.

4.1.2. Stimuli and Procedure

Stimulus materials were the same as Experiment I. Participants sat in a dimly illuminated room facing a 17-inch computer screen located 70 cm from their frontal plane. They were instructed to keep their hand still, as relaxed as possible and to pay attention to the displayed knotting sequences. A short training session of 5 trials preceded the main experiment to familiarize with the experimental situation. Ten sequences (5 knots x 2 actors, as in Experiment I) were randomly repeated in each of the three experimental conditions (Error, Color-change and No Error) and, differently from Experiment I, each frame was presented for 0.6 s only (as in timing t2 for Experiment I) followed by an ISI of 0.4 s.

The experiment consisted of two sessions, one per stimulation site (C3 and Oz), which took place on the same day. All participants completed the two sessions and the order was randomly counterbalanced across participants. Each experimental session was divided in 3 blocks with 2 min breaks between them. Each condition (Error, Color-change and No Error) was repeated 15 times. Each block consisted of 45 trials (30 trials with TMS pulse and 15 without), leading to a total of 90 trials. The experiment lasted about 1 hour, including the TMS mapping procedure.

Participants were asked to perform the same detection task described in the EEG study. More in detail, they were instructed to carefully observe the individual frames composing the sequence and to press a button as fast as possible on a keypad under two possible circumstances: (i) if they detected an execution error in the sequence or (ii) if they perceived a change in color of the rope. They were required to answer with their left hand (ipsilateral to the stimulated hemisphere).

4.1.3. TMS

TMS was delivered through a figure-eight coil (70 mm) using a monophasic stimulator (Magstim Company, Whitland, UK). The TMS coil was kept in position by a mechanical arm. Participant's head was restrained by a chin and forehead rest and by using a 3-points arc-shaped holder on the right parietal side of the head. TMS pulses were delivered on two different scalp sites: over the location corresponding to C3 and Oz when considering a 10-20 EEG montage. **C3 was chosen because it is located over the sensorimotor areas and as a control site, we stimulated over the occipital electrode Oz.** Targeting these scalp site would shed light on the dissociation of possible effects driven by sensorimotor (C3) versus occipital (Oz) regions.

Stimulation sites were located on the scalp by using the 10-20 system. Since electrode C3 is in proximity of the hand primary motor cortex, we established the resting motor threshold (rMT) in that site, following standard procedures (Rossini et al., 1994). The rMT was defined as the lowest stimulation intensity capable of evoking at least 50 µV motor evoked potentials (MEPs) in at least 5 times out of 10 in the muscle of interest. MEPs from the First Dorsal Interosseous (FDI) muscle were recorded through wireless surface electromyography (Zerowire EMG, Aurion, Italy) using a belly-tendon montage. The electromyographic signal was amplified via wireless electromyography (ZeroWire EMG, Aurion), filtered with a band pass between 50-1000 Hz and digitized via an analog-to-digital acquisition board (CED Power1401, Cambridge Electronics, UK; Signal software version) with a sampling rate of 2kHz.

During the experiment, two TMS pulses separated by a 50 ms interval were applied to C3 or Oz with an intensity of 90% of the C3 rMT. This choice was made to avoid the direct activation of a descending volley and in agreement with previous investigations showing that

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TMS below threshold is more likely to produce facilitatory effects on behavior (Bartoli et al., 2015; D'Ausilio et al., 2009; D'Ausilio, Bufalari, Salmas, & Fadiga, 2012; D'Ausilio, Jarmolowska, Busan, Bufalari, & Craighero, 2011). The center of the TMS coil was placed tangentially to the position of electrode C3, 45° with respect to the midline and the handle pointing backward to generate a postero-anterior current flow. When stimulating Oz site, the coil was placed over the occipital pole centered 3 cm above the inion with the handle pointing upward, to stimulate both occipital poles at the same time.

TMS timing was selected based on the results of Experiment I. The TMS pulses were delivered at 150 and 200 ms **following the presentation of the critical frame to coincide with the occurrence of the negative deflection described in Experiment** I (**Figure 4**). The 50 ms inter-pulse interval was based on prior studies showing consistent facilitatory effects on behavior when using this protocol (Bartoli et al., 2015; D'Ausilio et al., 2009, 2012, 2011). TMS was triggered through the parallel port controlled by custom-made software in Matlab. Stimulus presentation, randomization and reaction times (RTs) recordings were controlled by the Psychtoolbox functions (Brainard, 1997), running in Matlab (Mathworks, Inc.).

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Insert Figure 4 here

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4.1.5. *Data Analysis*

Incorrect answers and response latencies 2.5 SD (Standard Deviation) above or below the mean were excluded from the analyses (on average 1.5 trials per subject on a total of 90). Normality of response latencies was verified with the Kolmogorov-Smirnov test.

First, we analyzed the response latencies recorded when no TMS stimulation occurred to ensure that no change in subjects' performance was present between the two consecutive sessions. RTs were analyzed using a repeated measures analysis of variance (RM-ANOVA) considering the within-subject 2 levels factor CONDITION (Error condition, Color-change condition) and the between-subject 2 levels factor SESSION (first, second).

Second, in a 2X2 RM-ANOVA we analyzed the ratio between RTs recorded during TMS and the NoTMS conditions, to highlight the net effect of stimulation. **In fact, it is relatively complex – and sometimes unrealistic - to match different conditions for complexity.**

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More importantly, matching for average complexity would not equalize learnability of the two tasks during the experiment. In this case, the model included the factor CONDITION (Error condition, Color-change condition) and SITE (C3/NoTMS, Oz/NoTMS). Bonferroni post-hoc tests were run on significant main effects or interactions. Overall, the accuracy was very good (Color-change condition, mean = 96.4 ± 1.7 %; Error condition, mean = 94.2 ± 2.7 %; See table 2). Statistical analyses were performed using STATISTICA 9 (StatSoft, Inc.).

Insert table 2 here

Site	Color-change condition	Error condition
Overall	M = 96.4 ± 1.7 %	M = 94.2 ± 2.7 %
C3	M = 95.8 ± 1.9 %	M = 96.7 ± 1.4 %
Oz	M = 96.7 ± 1.9 %	M = 95.8± 2.9 %
NoTMS	M = 96.7 ± 1.4 %	M = 90 ± 3.3 %

Table 2: Mean percentage of correct answers reached by participants during the detection task in the different stimulation conditions.

5. Results

5.1. Reaction Times

The first analyses were run on non-stimulated trials RTs, which showed a normal distribution (K-S d = 0.118; p > 0.20). Session order did not influence subjects performance as shown by the non-significant main effect of SESSION (F(1,11) = 0.31, p = 0.59, ηp^2 = 0.03) as well as the non-significant interaction SESSION X CONDITION (F(1,11) = 1.62, p = 0.23, ηp^2 = 0.128). There was however a main effect of CONDITION (F(1,11) = 101.76, p < 0.0001, ηp^2 = 0.902). Overall, participants were slower in the detection of the Error condition (Mean = 0.62 ± 0.027 s) than during the color change condition (Mean = 0.47 ± 0.024 s). This behavioral difference was not observed in the previous experiment. This difference between the two experiments might however be explained by the procedural and experimental adaptations needed to meet the inherent requirements of TMS and EEG. The number of trials (180 in EEG and 90 in TMS) and length of the experiment was very different (120 and 60 minutes respectively). While in EEG subjects sat comfortably and relaxed on an armchair, during the TMS experiment subjects' head was restrained. In TMS the large proportion of stimulated trials (67%) may have diverted attention away from the task and, although we used an online sub-threshold TMS protocol, TMS carry-over effects might have extended to non-stimulated trials.

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However, taking into account the differences found in the behavioral task we opted for the normalization of the data and performed an analysis of the ratio between stimulated and non-stimulated trials. **RTs values of all conditions is provided in table 3.** We then considered as dependent variable the RT ratio between stimulated and non-stimulated trials, which showed a normal distribution (K-S d = 0.111; p = 0.20). There was no main effect of CONDITION (F(1,11) = 2.06; p ≤ .179; ηp^2 = 0.158) or SITE (F(1,11) = 2.82; p ≤ .121; ηp^2 = 0.204). Instead, we found a significant CONDITION X SITE interaction (F(1,11) = 6.39; p ≤ 0.03; ηp^2 = 0.367). Post-hoc tests revealed that the interaction was driven by the different modulation of the RT ratio according to the site of stimulation **(Figure 5).** Indeed a difference between Color-change and Error conditions was present when considering the C3 stimulation, with a stronger reduction in the response time for the Error condition (mean = 0.84 ± 0.02 s vs. Color-change, mean = 0.91 ± 0.03 s; p ≤ 0.02). Such effect was not present when considering the Oz stimulation, where the RT ratios were not different between conditions (Error mean = 0.92 ± .03 s; Color-change, mean = 0.93 ± 0.03 s; p >.05). This was further confirmed by directly comparing the Error condition RT ratios between stimulation sites (p ≤ .007).

Insert Table 3 here

Site	Color-change condition	Error condition
ALL	M = 0.44 s; ± 0.02	M = 0.57 s; ± 0.03
C3	M = 0.42 s; ± 0.02	M = 0.52 s; ± 0.03
Oz	M = 0.43 s; ± 0.03	M = 0.57 s; ± 0.03
NoTms	M = 0.47 s; ± 0.8	M = 0.62 s; ± 0.02

Table 3: Mean response latencies and standard error (±) of correct answers reached by participants during the detection task in the different stimulation conditions.

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Insert Figure 5 here

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6. Discussion

This study investigates the neurophysiological correlates of embedded errors during the observation of complex, familiar but untrained knotting actions. The aim of the current study was twofold: (1) to determine the neural markers underlying the discrimination of other's embedded error execution and (2) to verify the causal contribution played by left sensorimotor regions in the detection of such an error. To this purpose we used first an ERPs paradigm to look for brain events correlated with the processing of embedded errors and then we employed TMS stimulation to interfere with subjects' performance during the detection of these errors.

Results show that processing an embedded error elicits an early negativity effect within a left fronto-central ROI (120-180 ms). Conversely, the visual Color-change condition induced effects distributed only over posterior bilateral ROIs, pointing to a spatially dissociable modulation. **However, the fact that the effect was stronger over the left hemisphere could also account for the way we presented the action sequences. We represented errors made by the right hand because our actors as well as our participants were right-handed. Further studies should address this question focusing on the differences between right and left brain hemisphere during action error perception.**

TMS applied to central scalp sites, overlapping with the timing of these ERP components, shows a significant effect on behavioral discrimination of such embedded errors. Interestingly, we show that **stimulation over** central **(sensorimotor)** regions produces a facilitatory behavioral effect**, i.e. the stimulation lead to faster error detection**. **Moreover, accuracy remained high over conditions, also following TMS stimulation, likely because the task the participants were engaged in was relatively easy. The nature of the facilitatory effect found in reaction times** has been described as evidencing a cortical priming effect to those brain activities soon to be engaged in a particular task (Bartoli et al., 2015; D'Ausilio et al., 2009, 2012, 2011; Silvanto, Bona, Marelli, & Cattaneo, 2018). This latter result reinforces the suggestion that brain activities, with such an early latency and located in left fronto-central regions, provide a critical contribution to the discrimination of embedded motor errors, even in the absence of a clear action goal disruption.

In a similar vein, delivering a TMS pulse over M1 with respect to the participants phase of hand mental rotation, resulted in an increment of muscle specific corticospinal activity (Perruchoud, Fiorio, Cesari, & Ionta, 2018)**. The ability to predict the position of the hand with respect to the rope in order to detect possible execution errors and the capability to execute a hand mental rotation task could be conveyed by a similar action simulation process. In fact, converging evidences suggest that action observation, motor imagery and motor execution recruit the same neural network (Grèzes & Decety, 2001; see Hardwick, Caspers, Eickhoff, & Swinnen, 2018 for a meta-analysis; Jeannerod, 2001). Our results are in line with these findings and suggest a possible causal involvement of this sensory-motor network also during the monitoring of others' action-errors.**

Considering EEG, differently from the current study, prior research employing action observation was focused on action errors producing a direct perturbation of the final goal of the action. For instance, the observation of erroneous reach-to-grasp actions (e.g., Pavone et al., 2016; Pezzetta et al., 2018; Spinelli et al., 2018) elicits in the observer the typical neurophysiological markers of error detection. These are the ERN, a negativity with a midline fronto-central topography and generally reflected in an error checking monitor system (Bates, Patel, & Liddle, 2005; Coles, Scheffers, & Holroyd, 2001; Falkenstein, Hohnsbein, Hoormann, & Blanke, 1990; Gehring, Goss, Coles, Meyer, & Donchin, 1993); the positivity error (PE), often following the ERN, a more sustained positive (200-500 ms) component with a centro-parietal scalp distribution and is taken to be associated to the conscious detection of other's erroneous action (Bates et al., 2005; de Bruijn & von Rhein, 2012; Falkenstein, Hoormann, Christ, & Hohnsbein, 2000; van Schie, Mars, Coles, & Bekkering, 2004; Vocat, Pourtois, & Vuilleumier, 2008). Moreover a mid-frontal theta power modulation has been reported and has been often associated to action monitoring and cognitive control (Cavanagh, Cohen, & Allen, 2009; Pavone et al., 2016; Pezzetta et al., 2018; Spinelli et al., 2018). Additionally, the observation of complex familiar action sequences ending either correctly or incorrectly (i.e. watering the table instead of the plant; de Bruijn et al., 2007), as well as action sequences in which grip-object correctness was manipulated (van Elk, Bousardt, Bekkering, & van Schie, 2012) elicited a parietal P300, a component associated with the processing of unexpected events. Finally, the manipulation of the temporal order of action

steps within a familiar complex action sequence (i.e. making coffee) elicited an early left anterior negativity (100-250 ms; Maffongelli et al., 2015), while the perception of a mistake at the action outcome as a result of a manipulation of the coherent sequence of action steps elicited a fronto-central negativity (150-300 ms; Balconi & Canavesio, 2015).

Here, differently from previous research, we used *embedded* errors, occurring *within* a sequence of action steps, which will manifest their effect only at the end of the action. Therefore, recalling our manipulation, the observer would need to know the consequence of inserting the rope in the loop according to a top-down or a bottom-up movement, given that specific context. In fact, complex actions present a hierarchical organization (e.g., Koechlin & Jubault, 2006) and do not consist of a mere juxtaposition of individual motor constituents (Grafton & Hamilton, 2007). Rather, they imply a specific arrangement of smaller action units, according to specific rules (Bernstein, 1967). That is, each single action unit is organized according to both local dependencies with adjacent units, as well as to more distant relationship within the action hierarchy (Grafton & Hamilton, 2007; Pastra & Aloimonos, 2012).

We speculate that, due to the hierarchical organization of actions, top-down predictions allow the detection of embedded motor errors even if these action units are not intrinsically erroneous. At the same time, the detection of embedded errors can be used, via bottom-up processes, to anticipate their effects later in the unfolding of the action. For instance, the insertion of the rope in the loop (Figure 1a, panel B, circle number 2) can stand in functional conflict with the way the rope crosses itself to form the loop (Figure 1a, panel B, circle number 1). The violation of this functional relationship requires the intervention of top-down processes, containing knowledge about loop functionality and integrating information from multiple adjacent steps. At the same time, the detection of a disruption of the loop functionality can also propagate higher up in the hierarchy (bottom-up processing), providing key anticipatory cues about the possibility to achieve the action goal.

As a consequence, detecting these embedded errors and considering them within a hierarchical representation of the action, may provide a particularly powerful capability. In fact, while monitoring the emergence of these structural violations, we can project the consequences of these errors on hierarchically higher levels of action representations, thus providing a mechanism for action prediction (Friston, Mattout, & Kilner, 2011).

Current motor control theories attribute great importance to error detection during the acquisition of new motor skills. During action observation, the observer can monitor other's action covertly simulating the required motor commands and generate prediction about incoming sensory feedback (Wolpert, Diedrichsen, & Flanagan, 2011)**. In our study naïve participants observed both the correct procedure to tie the target knots and the incorrect one. It is plausible that participants during the task learned by passive observation the correct sequence of steps necessary to tie the knot. As a consequence of this learning, they became able to compare the actions made by the actor with the expected ones. In this regards, facilitation in detection of error execution induced by the stimulation of sensorimotor region can suggest its causal involvement in prediction of motor embedded errors.**

In conclusion, we can draw a parallel to other studies showing similar neural markers elicited by hierarchy-based expectancy violations in other cognitive domains, such as in language and music (Fadiga, Craighero, D'Ausilio, 2009). For example, in musical sequences the presentation of structurally regular or irregular chords elicits an early right anterior negativity (ERAN; Koelsch, Gunter, Wittfoth, & Sammler, 2005; Sammler, Novembre, Koelsch, & Keller, 2013) in analogy to the linguistic early left anterior negativity (ELAN; Koelsch & Friederici, 2003), reflecting fast syntactic parsing mechanisms (Friederici, 2002; see Kim & Gilley, 2013; Steinhauer & Drury, 2012 for a discussion on this topic). Our study then, seems to further suggest the existence of a general-purpose capacity to process hierarchical structures, rising the question whether these neuro-computational processes are dedicated or not to one specific cognitive domain (Hoen & Dominey, 2000; Jeon, 2014).

Supplementary information

The figure represents the sequences of the five knots used in the experiment in the No Error condition. The five knots included a variable amount of passages and thus the stimuli had different number of frames. Specifically, knot 1 was formed by 17 frames, knot 2 by 24 frames, knot 3 by 18 frames, knot 4 by 23 frames and knot 5 by 22 frames.

Acknowledgments

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Legends figures

Figure 1. Experimental design. Panel 1: Close-up view of the rope configuration in the No Error (A) and Error (B) conditions. The snapshots represent the critical step of the knot tying: in the No Error condition, the rope (working end) moves bottom-up under the loop knot (full circle) making the completion of the knot possible (section a) whereas, in the Error condition, the rope moved top-down, making the completion of the knot impossible (section b). The depicted circles 1 and 2 highlight the way in which the rope crosses itself to form the loop in the two conditions. In section B the functional conflict is represented, whereas in section A this conflict is not taking place. Pictures used for the experiment are shown in the supplementary materials. Panel 2: The central part of the sequence of one of the five knots is shown (overhand knot). On the left part of the figure, the first frame depicting the expected knot is shown. The No Error condition shows the correct execution of the knot. The Color-change condition is identical to the No Error condition except that in one frame the rope was colored in orange. In the Error condition an execution error is presented. The error is realized by top-down inserting the rope into the loop, causing the knot to untighten. Frames with the red outline represent conditions in which a button press was required. Figures adapted from Cardellicchio et al. (2018).

Figure 2: Event-related-potentials relative to all experimental conditions. Grand-average event-related potentials (ERP) of the Color-change condition (blue line), the Error condition (violet line) and the No Error condition (green line) for the selected regions of interest (ROIs) are reported. A significant negative deflection was found in the anterior ROIs within the 120-180 ms temporal window. In the middle of the figure, the scalp channel configuration is shown (right) with the ROIs used in the analyses (red ovals).

Figure 3: Summary ERPs results. The bar graphs show the **resulted** 4-way interaction **between the experimental factors**. ERP amplitudes (mean and SE) of Error condition (violet), the Color-change condition (blue) and the No Error condition (green) are depicted. The horizontal line highlights significant effects. **In the left anterior ROI we reported a significant difference for the comparison Error condition vs. No Error condition in the early (120-180 ms) time window. In the late time window we found a difference only**

between the Error and Color-change condition. This happens also in the right anterior ROI for the late time window (180-280 ms) as well as in the left and right posterior ROIs, in both time windows.

Figure 4. Setup for Experiment II. The upper panel shows the central part of the sequence of one of the five knots (overhand knot) in the Error and Color-change conditions. The task is the same as in Experiment I. The grey shadow represents the point in which we applied TMS. TMS pulses were delivered at 150 and 200 ms following the offset of the critical frame coinciding with the occurrence of the negative ERP deflection shown in Experiment I, as depicted in the lower part of the figure, on the right side. The scalp locations where TMS pulses were delivered (C3, session 1; Oz, session 2) are depicted on the right side of the figure.

Figure 5: Magnitude of the Ratio (stimulated trials/non-stimulated trials) in the stimulation sessions. **The ratios between TMS and NoTms trials for the Color-change and the Error condition are reported for the Sensorimotor and the Control sites. Smaller values indicate faster error detection during the TMS condition with respect to the NoTMS one.** Square brackets indicate significant comparisons (p<0.05). For definitions and abbreviations see text.

Panel 1

A
full circle   working end
bottom-up
No Error condition

B
top-down
Error condition
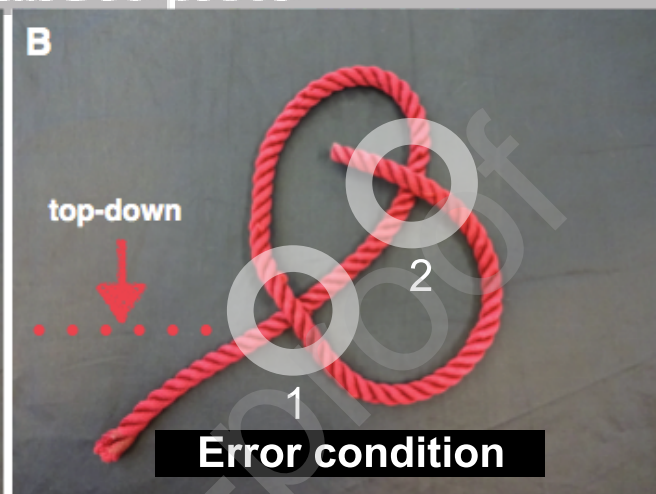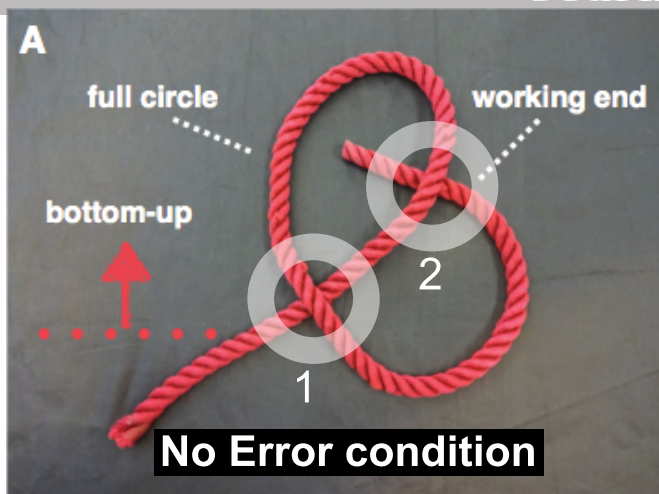
Panel 2

No Error condition

Expected Knot

Color-Change condition

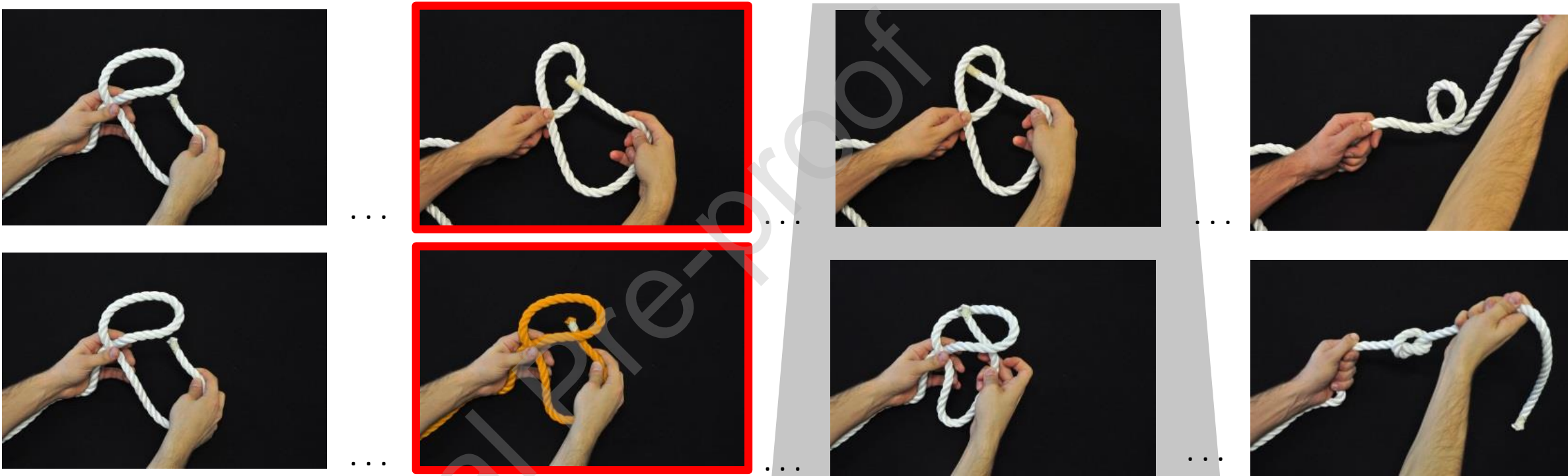Error condition

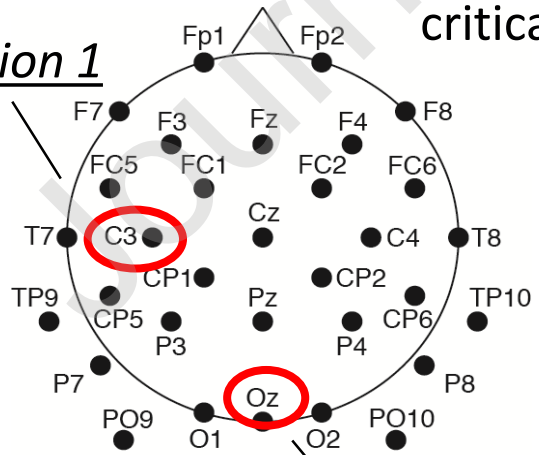Initial Frame   Final Frame
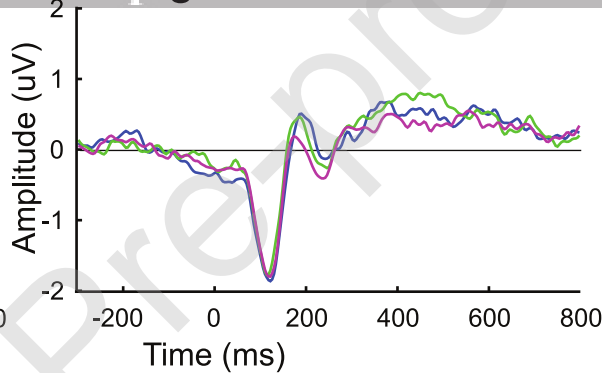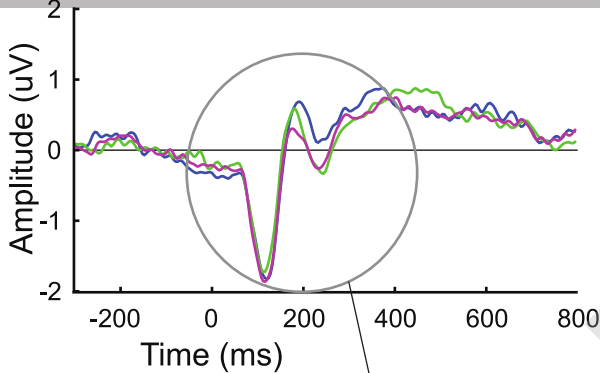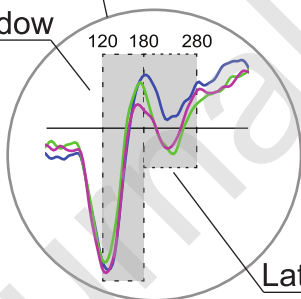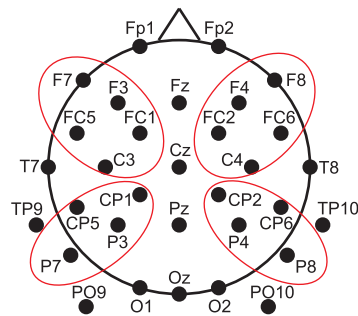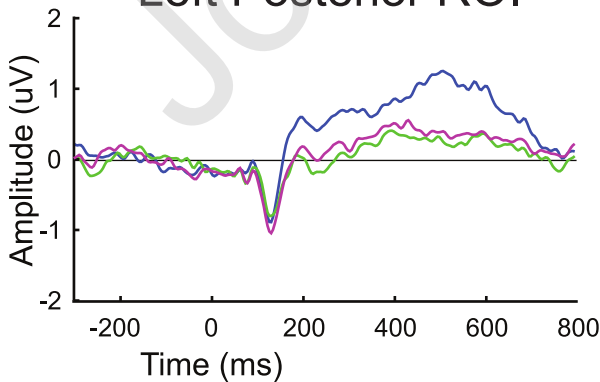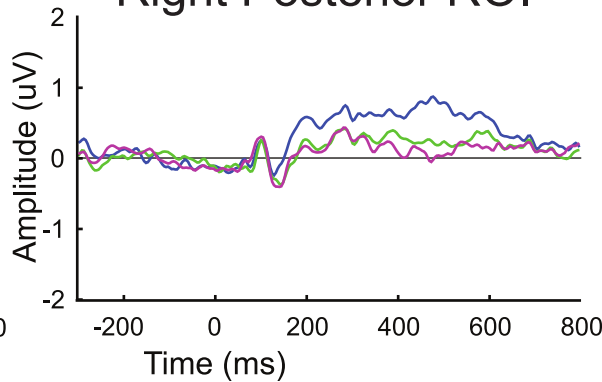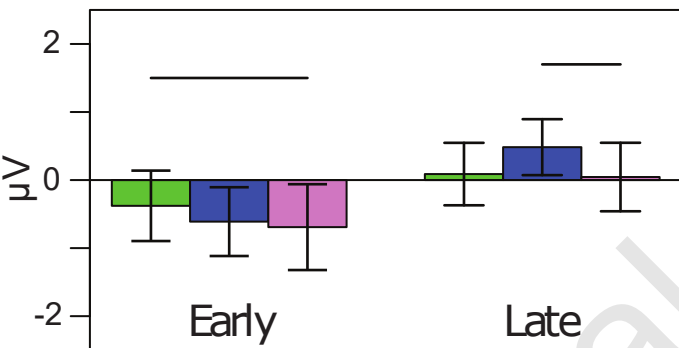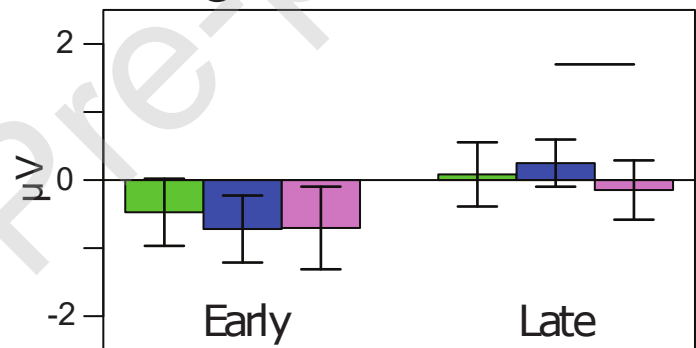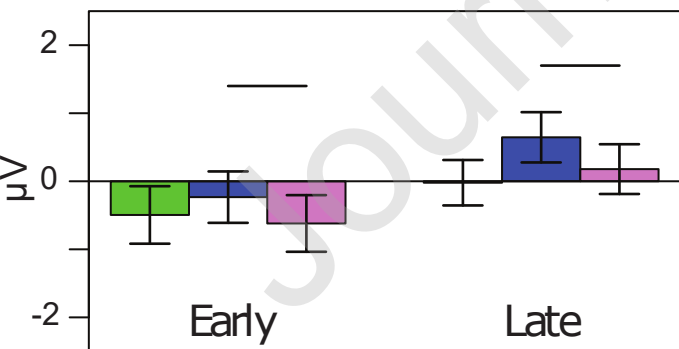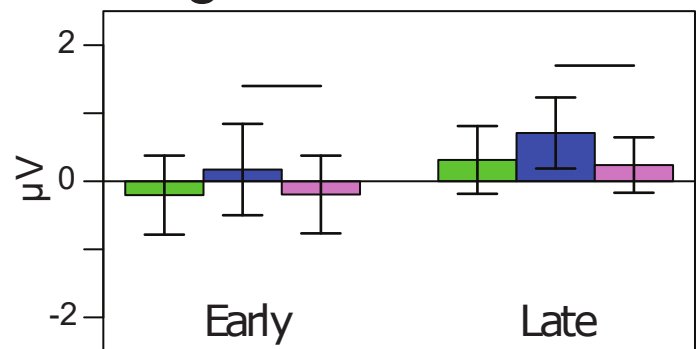
Early: 120-180 ms   Late: 180-280 ms

Error Condition

Color-change Condition

Offset critical frame

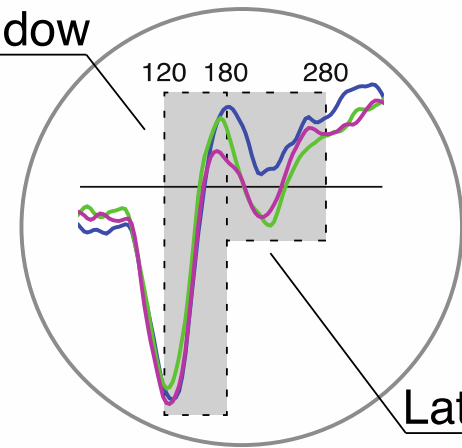TMS pulses 150/200 ms
50 ms inter-pulse-interval

Scalp locations
Experiment II

Session 1

Fp1 Fp2
F7 F3 Fz F4 F8
FC5 FC1 FC2 FC6
T7 C3 Cz C4 T8
TP9 CP5 CP1 CP2 CP6 TP10
P7 P3 Pz P4 P8
PO9 O1 Oz O2 PO10

Session 2

Early time window

120 180 280

ERPs (Exp.I) as reference for
Timing selection Exp. II

Late time window

Ratio TMS/No TMS