1	Action processing in the motor system: Transcranial Magnetic Stimulation (TMS) evidence of
2	shared mechanisms in the visual and linguistic modalities.
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2 Abstract

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In two experiments, we compared the dynamics of corticospinal excitability when processing
visually or linguistically presented tool-oriented hand actions in native speakers and sequential
bilinguals. In a third experiment we used the same procedure to test non-motor, low-level stimuli,
i.e. scrambled images and pseudo-words.

8 Stimuli were presented in sequence: pictures (tool + tool-oriented hand action or their scrambled 9 counterpart) and words (tool noun + tool-action verb or pseudo-words). Experiment 1 presented 10 German linguistic stimuli to native speakers, while Experiment 2 presented English stimuli to non-11 natives. Experiment 3 tested Italian native speakers. Single-pulse trascranial brain stimulation 12 (spTMS) was applied to the left motor cortex at five different timings: baseline, 200ms after 13 tool/noun onset, 150, 350 and 500ms after hand/verb onset with motor-evoked potentials (MEPs) 14 recorded from the *first dorsal interosseous* (FDI) and *abductor digiti minimi* (ADM) muscles.

We report strong similarities in the dynamics of corticospinal excitability across the visual and linguistic modalities. MEPs' suppression started as early as 150ms and lasted for the duration of stimulus presentation (500ms). Moreover, we show that this modulation is absent for stimuli with no motor content. Overall, our study supports the notion of a core, overarching system of action semantics shared by different modalities.

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1 1. Introduction

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3 It is well established that the observation of bodily actions, as well as their execution, engages the motor system in a phenomenon known as *motor resonance*. Metabolic brain imaging 4 5 (PET) and Transcranial Magnetic Stimulation (TMS) first revealed a modulation of motor cortex 6 activity induced by the observation of transitive hand actions (Fadiga et al., 1995; Grafton et al., 7 1996; Rizzolatti et al., 1996). Successively, several studies confirmed and extended these findings 8 (for review, see Rizzolatti and Sinigaglia, 2016) and it has been suggested that a system matching 9 observation with execution may subtend several cognitive functions. Noteworthy, by directly 10 mapping observed actions onto their motor commands this may represent a fast, unmediated 11 mechanism to access the meaning of observed actions (Gallese et al., 2004).

12 Interestingly, evidence for the involvement of the motor system in processing actions is not 13 limited to the visual domain. Several studies showed how action-related language (e.g. action verbs) 14 induced motor activations consistent with its motor content. For instance, seminal 15 electroencephalographic (EEG) studies showed how verbs related to specific bodily effectors (e.g. 16 to kick, to pick, to lick) elicited activations of sectors of the motor cortex where the corresponding 17 effectors are represented (e.g. Hauk and Pulvermüller, 2004; Pulvermüller, 1999; Pulvermüller et al., 2001). This evidence was later consolidated by other methodologies (e.g. brain imaging: Hauk 18 19 et al., 2004; Horoufchin et al., 2018; for a review see Kiefer and Pulvermüller, 2012; Pulvermüller 20 and Fadiga, 2010). In this domain, it has been suggested that the motor engagement may be crucial 21 for representing the semantic content of action-related language.

The possibility to collect temporally precise markers of motor system involvement is crucial for evaluating the functional role of motor resonance. To this aim, TMS coupled with simultaneous recordings of TMS-induced electrical activity in targeted muscles (Motor Evoked Potentials, MEPs) has proved to be among the most suitable methodologies. For what concerns action observation, the majority of TMS studies suggests that observing actions facilitates the motor system as indicated by an increase of MEP amplitude with respect to a baseline or control condition (Naish et al., 2014). However, a minority of studies also shows a modulation in the opposite direction, with a decrease of MEP amplitude (e.g. Lago and Fernandezdel-Olmo, 2015; Sartori et al., 2012). Overall, regardless of the direction of modulation, the available evidence points to an early engagement of the motor system, starting within 100-200ms after onset of the critical stimulus.

8 As to action language, evidence is more limited and diverging. Early effects were reported 9 by Buccino and colleagues (2005) and by Gianelli and Dalla Volta (2015) using single-pulse 10 stimulation of the hand motor cortex in correspondence of the second syllable of auditorily 11 presented hand-, foot-related action verbs, and abstract verbs. However, the direction of this 12 modulation diverged: while Buccino and colleagues reported a decrease of MEPs for hand-related 13 verbs compared to the other conditions, Gianelli and Dalla Volta, showed an opposite effect. 14 Similarly, Innocenti and colleagues (2014) reported increased MEPs at 300ms after action word 15 presentation, but this effect disappeared with stimulus repetition. Scorolli and colleagues (2012), 16 instead, reported no difference between abstract and hand-related verbs when TMS was delivered at 17 250ms from verb onset in a sentence sensibility task. Conversely, Papeo and colleagues (2009) 18 showed later effects: MEPs increased at 500ms for hand-related action verbs during a semantic task 19 as compared to non-action verbs, while they decreased at 500ms during a syllabic task. Recent 20 models suggest that indeed linguistic motor resonance might entail both directions, with the 21 modulation moving from an early decrease (within 200ms) to a later increase (Chersi et al., 2010; 22 García and Ibáñez, 2016).

Brain activity has shown to be modulated also by the observation of manipulable objects (Chao and Martin, 2000; Grèzes et al., 2003). In this sense, presenting objects produced a subthreshold motor activation (i.e. object affordance) consistent with the motor programs typically implemented for appropriate interaction with those objects (Cardellicchio et al., 2011; Makris et al., 2011). Several TMS studies showed a selective increase of corticospinal excitability in hand
muscles involved in the action afforded by objects requiring either a precision or a whole-hand grip
(Bartoli et al., 2014; Makris et al., 2013). In the same vein, early stimulation (150 ms after stimulus
onset) of the ventral premotor cortex after object presentation reduced the capacity to recognize an
object's shape (Uithol et al., 2015).

6 Similarly to the presentation of manipulable objects, also nouns referring to objects were 7 suggested to be effective in recruiting the motor system. Interestingly, action-related words as 8 compared to non-action-related ones facilitated hand muscle responses irrespective of the 9 grammatical class (nouns or verbs) when the hand sector of the left motor cortex was stimulated 10 250 ms after the onset of a response cue (Oliveri et al., 2004). Furthermore, early increase of 11 corticospinal excitability (150 ms) followed reading of nouns referring not only to artificial tools 12 but also to natural graspable objects (Gough et al., 2012). However, Scorolli and colleagues (2012) 13 reported no difference between graspable and non-graspable nouns in a verb-noun integration task.

14 Summing up, the large evidence of short-latency motor resonance starting as early as 150 15 ms after stimulus onset for both visually and linguistically presented action-related stimuli suggests 16 that the motor system may be a crucial node of a semantic system shared by both stimulus 17 modalities. This view is at the core of embodied accounts of language, according to which semantic 18 knowledge is functionally and neuroanatomically grounded in sensory and motor systems (Barsalou 19 et al., 2003; Gallese and Lakoff, 2005; Kiefer and Spitzer, 2001; Pulvermüller, 2005; Zwaan and 20 Taylor, 2006). However, to our knowledge very few studies directly compared the effects of the 21 same action-related material presented in both visual and linguistic modalities (Ganis et al., 1996; 22 Marino et al., 2014; Vandenberghe et al. 1996). Overall, these studies reported that pictures and 23 words are processed similarly although by, at least partially, not overlapping brain areas. In 24 addition, to our knowledge no study compared measures of corticospinal excitability collected in 25 both stimulus modalities. In this sense, it is worth noting that a direct comparison of these two 26 modalities is not straightforward, as they may convey the same motor content with different degrees

of specificity. For instance, video clips typically show the unfolding of actions over time while also
giving information about the agent and the object of the depicted action. On the contrary, isolated
verbs (e.g. infinitive verbs) do not make explicit any agent or object information, and they likely
activate only very limited temporal information (e.g. the kinematics of the most prototypical action,
Dalla Volta et al., 2009).

6 Based on these considerations, we used a set of visual (combinations of static pictures of 7 tools and tool-oriented hand actions) and linguistic stimuli (combination of nouns of tools and verbs 8 expressing tool-oriented hand actions) specifically designed to obtain a close matching of motor 9 content between the two modalities. First of all, in Experiments 1 and 2, our aim was to test whether 10 the same motor content expressed in different modalities similarly engages the motor system with 11 the similar temporal dynamics. In addition, we evaluated whether the same motor effects occur 12 regardless of the use of the native or non-native language. To this aim we tested two groups: one of 13 native German speakers, and one of sequential bilinguals (German native speakers tested in 14 English). There is evidence, in fact, suggesting that in proficient non-native speakers semantic 15 representations are rich enough for motor-related activations to develop in motor areas (Buccino et 16 al., 2017; de Grauwe et al. 2014), although possibly with different magnitude (Vukovic and 17 Shtyrov, 2014; for a review, see Kühne and Gianelli, 2019). While the presence of motor 18 activations is in keeping with the idea of a shared sensorimotor grounding in the first and second 19 language, differences in magnitude or timing might point to a different functional role. On the 20 contrary, a similar time course would point to the existence of a core sensorimotor grounding of 21 linguistic concepts that is substantially independent of additional factors (e.g. age of acquisition, or 22 proficiency) in sequential bilinguals. Importantly, TMS evidence - and specifically using single-23 pulse protocols with different stimulation timings – is currently lacking on this topic. It is thus 24 particularly relevant to compare the effects of the visual modality with the linguistic one in the first 25 and the second language.

According to the evidence reviewed above, overall we expected to observe an early modulation of corticospinal excitability for both modalities (i.e. as early as 150 ms after verb/tooloriented action). In addition, we expected to see increased MEPs as compared to baseline for both visually and linguistically presented tool and tool oriented-actions and, possibly, also increased MEPs as compared to a control condition (tool/noun).

In addition, we predicted the same pattern of modulation for the linguistic modality regardless of the language used. While we recorded from two hand muscles (FDI, ADM) in order to grasp a more complete view of stimuli-induced motor activation, we did not predict any effect to be specific to just one of the two muscles. Rather, we expected them to be similarly modulated as the set of presented actions comprised a variety of different hand grips with different muscle patterns pooled together.

12 In Experiment 3 we used control stimuli with no motor content presented with the same 13 procedure of Experiment 1 and 2. For the visual modality, we used scrambled images derived from 14 an extended version of the original set of stimuli. For the linguistic modality, we used a set of 15 pseudo-words obtained from the Italian translation of the original set of stimuli. In this experiment, 16 we tested the robustness and specificity of the results reported in Experiment 1 and 2 by 17 investigating whether this modulation is precisely dependent on the motor content and not the mere 18 visual properties of the stimuli. Furthermore we aimed at investigating whether this modulation 19 follows temporal dynamics that are dependent on the presented stimuli and not on the mere passing 20 of time, or voluntary relaxation/contraction of muscles by the participant. Overall, we expected to 21 report no MEPs' modulation in Experiment 3 for both modalities.

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- 23 2. Materials and Methods
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- 25 2.1 Participants

In Experiments 1 and 2, participants were right-handed (standard Edinburgh questionnaire 10-items, Oldfiel, 1971) native German speakers recruited at the University of Potsdam. In Experiment 3, right-handed participants were recruited at the University of Parma¹. All participants were screened for possible contraindications and gave their written informed consent. The local ethics committees at the Universities of Potsdam and Parma approved the procedure.

6 The minimum required sample size (N=14) was determined by an a priori power analysis 7 using the same parameters for all experiments: alpha=0.05, power=0.8, effect size=0.03 (η_{a^2}), non-8 sphericity correction = 0.503 using Timing as the target main effect (5 measurements). The effect 9 size was chosen as a compromise between estimations available in the literature and funding 10 availability. Data collection was stopped once hit the minimum sample size, or soon thereafter 11 depending on availability, maintaining that experimenters were blind to the results till the end of 12 data collection. We tested 14 participants each (age 19-35 years old) in Experiment 1 and 2 (4 13 males in Experiment 1, 5 males in Experiment 2) and 15 (age 20-30 years old) in the third one (5 14 males). In Experiment 2, participants were German native speakers with very good proficiency in English as a second language by self-report (i.e. no formal assessment). In Experiment 3, 15 16 participants were Italian native speakers.

17 2.2 Stimuli

For Experiments 1 and 2, stimuli consisted of 24 pairs either combining the picture of a tool with the static picture of a right hand performing the appropriate action with that tool (see figure 1) or the noun of a tool with a hand-related action verb describing the appropriate action performed with that tool (e.g. "pen" – "to write"). All pictures were 440×440 pixels with the tool presented roughly at the centre of the image and the right hand coming from the right side. By observing the tool picture immediately followed by the hand-tool interaction picture, the observer was given the

¹ For logistic reasons, we carried out experiment 3 (and Experiment 4 reported in the Supplementary Materials) in the TMS laboratory of the National Research Council (CNR), hosted by the Department of Neuroscience at the University of Parma. The CNR Unit was already involved as a third hosting laboratory for the DAAD-MIUR Joint Mobility grant (project n. 57266099) to CG and RDV that funded Experiment 1 and 2.

impression to see an ongoing movement of the hand grasping and using the tool (for a similar presentation technique, see for instance Ortigue et al., 2010). The verbal labels were written in lower case and presented centrally. The same set of tools was presented as a picture or as verbal label. Linguistic items were in German in Experiment 1 and in English in Experiment 2, while pictures remained unchanged.

6 For Experiment 1, an independent sample of 60 participants was asked to rate the stimuli. 7 Verbs and nouns were matched for number of syllables and word frequency (database: dlexDB), 8 and rated for familiarity, imageability, valence and arousal. Pictures of tools and tool-related actions 9 were rated for familiarity, valence, arousal, picture naming and picture-word-association. Two-10 tailed t-test comparisons were used to test for differences between categories ($p_s > 0.05$). For 11 Experiment 2, nouns and verbs were translated in English and submitted to a new sample of 60 12 participants to check for familiarity of these words among readers with good English proficiency 13 and for any divergence under any of the other parameters. One item was replaced. A list of all items 14 is presented in Table 1.

For Experiment 3, we used a set of scrambled images in the visual modality and of pseudowords in the linguistic one. Scrambled images were produced by applying distort filters (e.g. Whirl and Pinch) available in GIMP 2.1 (<u>www.gimp.org</u>) on an extended version of the original set of stimuli. We filtered the images so that they maintained a certain degree of visual complexity (e.g. colours, contours, background/foreground) but the original stimuli were no longer recognizable.

As to pseudo-words, first we translated the original stimuli to Italian. Second, we derived from them a set of pseudo-words (see Table 2) by changing one vowel and one consonant in different syllables of the same word, while the last syllable did not vary (e.g. "pe<u>nne</u>llo" = "pe<u>rna</u>llo"; "<u>dipingere</u>" = "<u>cipongere</u>"). Pseudo-words were thus phonetically correct, recognizable forms of the Italian language, but with no meaning.

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2.3 Procedure and stimulation

Participants sat on a comfortable armchair, with their elbow flexed at 90° and their hands prone in front of a PC screen (1920×1080 pixel resolution and 60Hz refresh rate). The eye-to-screen distance was about 60cm. Each trial started with a white fixation cross, displayed at the centre of a black background (duration: 2000ms). Subsequently, a rapid sequence of two pictures or words was presented (500ms each stimulus of the pair), followed by a 4 s blank screen. Each experiment consisted of one session of 120 fully randomized trials divided into two blocks.

Participants were requested to maintain the right arm relaxed and to carefully attend to the presented stimuli. In order to maintain participant's attention, in 10% of the trials a written question randomly appeared right after the experimental stimuli disappeared. Questions were task-irrelevant, such as "Was the last letter of the word a "T"?" or "Was the object blue?" and participants responded silently. In Experiment 3, participants responded vocally and the experimenters checked that a response was given. In all experiments, the task-irrelevant response was not recorded.

14 For each trial, a single-pulse stimulation was given at one of five possible timings: 1000ms 15 after the onset of a fixation cross (baseline), 200ms after the onset of tool/noun (control condition, 16 object) and 150ms (experimental condition, t1), 350ms (experimental condition, t2) or 500ms 17 (experimental condition, t3) after the onset of the action/verb presentation (see figures 1). We chose 18 to stimulate 1000ms after the fixation cross onset to avoid possible confounds observed in a pilot 19 study (n=7) where the pulse was delivered immediately (1 ms) after the onset of the fixation cross 20 and the corticospinal activity was on average larger than in all other timings. The software Eprime 21 2.0 (Psychology Software Tools, Inc.) controlled stimulus presentation and triggering of the TMS.

TMS was delivered by a Magstim Rapid2 stimulator (Magstim Company, Whitland, UK) with a standard 70mm figure-of-eight coil placed on the skull with a medio-lateral orientation (handle pointing backwards). MEPs were recorded from the *first dorsal interosseous* (FDI) and the *abductor digiti minimi* (ADM) of the right hand (recording were performed with a Biopac MP150 + 1 EMG100C amplifier in Experiment 1 and 2, www.biopac.com; with a CED 1902 amplifier and 2 1402 interface in Experiment 3, www.ced.co.uk). Participants wore a swimming cap with a grid of 3 1-cm resolution drawn on it. Following the international 10-20 EEG system, the coordinate origin 4 was fixed at the Vertex. The Vertex was defined as the intersection of the lines joining the Nasion 5 and Inion and two pre-auricular points.

6 Moving the coil on the grid by 1-cm steps (starting from the location of the electrode C3 in 7 the 10/20 EEG system with an intensity of 55% of the stimulator output), the hand motor area was 8 localized on the left hemisphere at the beginning of each session. The resting motor threshold 9 (rMT), defined as the lowest intensity able to evoke 5 out of 10 MEPs with peak-to-peak amplitude 10 of at least 50μ V, was determined. The optimal stimulation site and the rMT were defined first on 11 FDI and subsequently used to evoke constant MEPs from both muscles at an experimental intensity 12 of 120% of rMT (mean stimulator output across all experiments = 64%). The experimental intensity 13 was set based on existing literature in the visual and linguistic domain.

14 EMG data was continuously recorded from FDI and ADM by means of surface Ag-AgCl 15 electrodes in a belly-tendon montage. EMG data was digitized (sampling frequency 2000Hz), band-16 pass filtered (20-1000Hz) and stored offline for further analyses. For each trial, the peak-to-peak 17 amplitude of MEPs was calculated (Acqknowledge, v.4.3, Signal, v.2.14 and customized Matlab 18 scripts).

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2.4 Analyses

20 EMG traces were visually inspected and individual trials showing abnormal background 21 EMG activity (e.g. showing muscle contraction) within a time window 100ms long before the TMS 22 pulse were removed from further analyses. By visual inspection, we also removed trials where no 23 detectable MEP was present. In Experiment 1, we retained an average of 118 trials for each of the 24 14 participants for both muscles. In Experiment 2, we retained an average of 118 trials for both 25 muscles for 12 participants. In Experiment 2, two additional participants were tested but removed 26 before data analyses because of incomplete datasets and/or signal loss. In Experiment 3, an average 27 of 117 trials was retained from 15 participants.

1 The peak-to-peak amplitudes of the retained MEPs were then normalized (z-scores) 2 separately for each subject and muscle and submitted to a repeated measures analysis of variance 3 (ANOVA) separately with Muscle (FDI, ADM), Modality (Linguistic, Visual) and Timing 4 (Baseline, Object, t1, t2, t3) as within-subject factors for each experiment. When required by 5 sphericity tests, a Greenhouse-Geisser correction for sphericity was applied and corrected F values are reported. As a measure of effect size, η_p^2 is reported for each significant effect. Significant 6 7 effects were further explored by means of post-hoc tests with a Holm correction for multiple 8 comparisons.

9 Besides the main, planned, statistical analyses, we ran two additional sets of exploratory 10 analyses. Specifically, we conducted between-experiments analyses to compare the three 11 experiments with each other (see below). Furthermore, in order to quantify the observed evidence in 12 terms of odds ratio between the null and the alternative hypothesis, we also report the Bayes Factors 13 (BFs) calculated by means of Bayesian paired sample t-tests on selected comparisons of interest.

14 All analyses were performed by means of the software JASP (V.0.8.1.2) and Jamovi (v.1.1.7)

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16 **3. Results**

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- 18 3.1 Experiment 1 German
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All results are reported in table 3. Analyses showed a significant main effect of Timing (F_{1.645, 21.382}= 5.676, p = 0.014; $\eta_p^2 = 0.304$). Post-hoc t-tests (all reported in Table 4) showed no significant difference between Baseline and Object stimulation. Conversely, they showed a significant MEPs decrease when t1, t2 and t3 were compared with the Object condition (*p*_{holm}=0.020, 0.014 and 0.004 respectively). When comparing these timings to Baseline stimulation, the evidence is relatively weaker. Significant differences are present only between Baseline and t3 (*p*=0.036), with the comparison between baseline and t2/t3 only approaching significance (see also the uncorrected *p* values in Table 4). The timings t1, t2 and t3 did not significantly differ from each
other.

- No other significant main effects or interactions were detected (see Table 3); the overall
 pattern in the two modalities is shown in Figure 3.
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10 3.2 Experiment 2 - English

11 Analyses (all results reported in Table 5) showed a significant main effect of Timing ($F_{4, 44}$ = 12 6.918, p <0.001; $\eta_p^2 = 0.386$). Post-hoc t-tests showed (see Table 6), no MEPs increase between 13 Baseline and Object. Comparisons of t1, t2 and t3 with Baseline showed a significant MEPs 14 decrease only at t3 (p_{holm} =0.013). Conversely, comparisons with Object showed significant MEPs 15 decreases at all timings (p_{holm} =0.008, 0.019, <0.001, respectively). The timings t1, t2 and t3 did not 16 significantly differ from each other.

17 Interestingly, and differently from Experiment 1, the analyses showed also a significant interaction between Timing and Modality (F_{4, 44}= 2.709, p =0.042; $\eta_p^2 = 0.198$) which further 18 19 clarifies the effects obtained on Timing. This interaction seems mainly driven by a significant 20 difference between the two modalities ($p_{holm}=0.048$) at t1 with MEPs more strongly modulated in 21 the visual than in the linguistic one. Finally, when testing t1, t2, and t3 against Baseline and Object 22 for the linguistic modality, analyses reveal a significant effect ($p_{holm}=0.005$) only for t3. 23 Conversely, for the visual modality MEPs are significantly reduced at t1 compared to both Baseline 24 $(p_{holm}=0.020)$ and Object $(p_{holm}=0.005)$.

As in experiment 1, no other significant main effects or interactions were detected (see Table 5); the
overall pattern in the two modalities is shown in figure 4.

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3.3 Experiment 3 – Control

Experiment 3 showed no main effect nor interaction, in particular for the target factors Timing ($F_{4,56} = 0.287$, p = 0.885; $\eta_p^2 = 0.020$) and Modality ($F_{1,14} = 0.269$, p = 0.612; $\eta_p^2 = 0.020$), see Table 7.

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3.4. Exploratory between-experiments analysis

8 Experiment 1 and 2 showed significant modulations and a qualitatively similar pattern of 9 results (both had a significant effect of the factor Timing) while Experiment 3 showed no such 10 modulation (see figure 5). In order to statistically compare the three experiments and further 11 complement our evidence, we ran three additional, exploratory, between-groups analyses. 12 Specifically, we compared Experiment 1 vs. Experiment 2, Experiment 1 vs. Experiment 3, and 13 Experiment 2 vs. Experiment 3 with Muscle (FDI, ADM), Modality (Linguistic, Visual) and 14 Timing (Baseline, Object, t1, t2, t3) as within-subject factors and Group as between-subject factor.

The aim of comparing Experiment 1 and 2 was to quantitatively support the qualitative similarity between MEPs patterns induced in the linguistic modality regardless of the use of the first or the second language in sequential bilinguals.

The aim of comparing Experiment 1 with Experiment 3, as well as Experiment 2 with Experiment 3, was to assess separately for the two experiments the specificity of the observed effects against non-motor control conditions. Results from these analyses are reported in the Supplementary Materials.

22 *3.4.1 Experiment 1 vs. Experiment 2*

The results confirm a main effect of the factor Timing ($F_{2.022, 48.537} = 12.207$, p < 0.001; ηp^2 = 0.178) strengthening the results from the two experiments for H1 in the key comparisons (Objectt1, Object-t2 and Object-t3, $p_s < 0.001$). The same applies to the comparisons Baseline-t1, Baselinet2, Baseline-t3 ($p_s < 0.05$). Interestingly, the comparison between Baseline and Object still does not 1 reach significance and the same applies to the comparisons between the t1, t2, and t3 ($p_s>0.05$). No 2 main effect or interaction with the between factor Group was detected (see Supplementary 3 Materials). Notably, the interaction Modality * Timing which was present in Experiment 2, does not 4 reach significance (p=0.082).

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3.4.2 Experiment 1 vs. Experiment 3

These analyses show again a main effect of Timing (F_{2.615, 70.598} = 3.777, p = 0.018; η_p^2 = 6 0.123) and an interaction of this factor with Group approaching significance ($F_{2.615, 70.598} = 2.808$, p 7 = 0.053; η_p^2 = 0.094) with the between-subject factor Group in itself being not statistically 8 9 significant (p=0.077). This interaction Timing * Group confirms that MEPs are significantly 10 modulated in Experiment 1, but not in Experiment 3. It is also worth mentioning a significant interaction Muscle * Modality * Group (F_{1, 27}= 5.065, p = 0.033; $\eta_p^2 = 0.158$), substantially 11 12 confirming that MEPs from the two muscles are significantly more modulated in Experiment 1 than 13 in Experiment 3 (all results are summarized in the Supplementary Materials). Interestingly, it hints 14 to the possibility that the FDI muscle might be more prone to a modulation in the linguistic than in 15 the visual modality – an effect that was not significantly detected for Experiment 1 in isolation – 16 nor does significantly interact with Group.

17 *3.4.2 Experiment 2 vs. Experiment 3*

The results of this comparison show a main effect of Timing ($F_{4, 100} = 4.537$, p = 0.002; ηp^2 = 0.154), a significant effect of Group ($F_{1, 25} = 6.096$, p = 0.021; $\eta_p^2 = 0.196$) and an interaction between the two ($F_{4, 100} = 3.123$, p = 0.018; $\eta_p^2 = 0.111$). This supports the results that MEPs are significantly modulated in Experiment 2, but not in Experiment 3 (all results are summarized in the Supplementary Materials).

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24 *3.4.2 Bayes Factors*

Experiment 1 and 2 both showed a main effect of the factor Timing. The same applies to the between-experiments comparison of the two experiments, while Experiment 3 did not show any significant effects. As a further exploration of these results, we calculated BFs in order to quantify
the observed evidence in terms of odds ratio between the null and the alternative hypothesis (the
comparison we performed are fully are reported in the Supplementary Materials).

As to Experiment 1, the comparisons between object (control) stimulation and the other timings (t1, t2, t3) suggests strong to very strong evidence in favour of H1, with BF₁₀ equal to 64.016, 8.839, and 10.484 respectively. When comparing the three timings with baseline (fixation cross) BFs suggest only anecdotal evidence for H1 at t1 and t2, BF₁₀ equal to 1.921 1.123 respectively (BF₁₀ at t1 = 0.950).

As to Experiment 2, the comparisons between object (control) stimulation and the other timings (t1, t2, t3) suggests strong, moderate and very strong evidence for H1 respectively (BF₁₀ equal to 12.924, 1.964, 21.257). Interestingly, comparisons of the same three timings with baseline produced moderate evidence for H1 at timings t1 and t3 (BF₁₀=3.457, 7.876, while at t2 evidence is inconclusive BF₁₀=0.739).

As to the comparison between Experiment 1 and 2, it is worth noting that the comparison between object stimulation and t1, t2, t3 produced $BF_{10}=1692.721$, 157.075, 1069.568 suggesting strong to extremely strong evidence in favour of H1. Furthermore, comparison of the three timings with baseline here produces moderate to strong evidence, $BF_{10}=11.846$, 3.382, 13.739 respectively. Interestingly, the evidence of a difference between baseline and object stimulation remains anecdotal ($BF_{10}=1.132$).

- Finally, as to experiment 3, BFs show moderate evidence in favour of H0 ($BF_{01}>3$) for the comparisons baseline-object, baseline-t2, object-t1, object-t2 and object-t3.
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23 4. Discussion

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- 4.1 Observation of tools and tool-oriented actions
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1 Contrary to our initial expectations, our study failed to induce MEPs facilitation when 2 observing tools with respect to a baseline. Since the TMS pulse was delivered 1000ms after the 3 fixation cross onset, it is implausible that the lack of facilitation can be attributed to alertness effects 4 induced by the fixation cross appearance. Our results are in line with a study by Buccino and 5 colleagues (2009) showing no significant differences between graspable objects (cups) and a 6 baseline (abstract symbol) and with a study by Makris and colleagues (2011) showing no difference 7 between manipulable and non-manipulable objects. Further evidence, however, supports the notion 8 of facilitatory effects after object appearance, although with diverging results as to the timing of this 9 effect (i.e. Bartoli et al., 2014, at 150ms; Cardellicchio et al., 2011, at 50ms; Makris et al. 2013, at 10 300 and 450ms). Since our study used only one stimulation timing for tools (i.e. 200ms, as in 11 Buccino et al., 2009), we cannot rule out the possibility of facilitatory effects becoming evident at 12 different stimulation timings.

On the other hand, our results about observed actions are in line with TMS studies showing an early modulation of corticospinal excitability occurring within 200ms (Naish et al., 2014). In addition, our modulation lasted until 500ms. This is in agreement with studies probing the time course of motor resonance with time-resolved techniques and suggesting a phenomenon closely following the time course of the on-going observed actions (e.g. Avanzini et al., 2012).

18 For what concerns the direction of the modulation, our data clearly showed a decrease of 19 MEPs amplitude as compared to both a baseline (fixation cross) and a control condition (object) 20 suggesting a motor inhibition. Although observed less frequently than facilitation, inhibitory effects 21 have been previously reported in the literature on action observation. It has been suggested that 22 inhibition acts on the corticospinal system to prevent the execution of movements during action 23 observation (Brass and Heyes, 2005; Keysers and Gazzola 2010; van Leeuwen et al. 2009). An 24 inhibitory mechanism would be also consistent with single-cell recordings from macaques and 25 humans (Kraskov et al., 2009; Mukamel et al., 2010) demonstrating the existence of neurons that 26 are inhibited during action observation and excited during action execution. It is worth noting that

the inhibitory effect may show either as a decrease in MEPs amplitude compared to a 1 2 baseline/control condition (e.g. Sartori et al., 2012), as a lack of evident facilitatory effects 3 compared to a baseline/control (e.g. Hardwick et al. 2012; Mattiassi et al. 2014) and/or as a MEPs 4 decrease compared to other experimental conditions (e.g. Amoruso and Urgesi, 2016; Hardwick et 5 al. 2012; Janssen et al. 2015; Montagna et al. 2005; Villiger et al. 2011). In the former case it is 6 assumed that the corticospinal output is inhibited below a resting state. In the latter ones, the 7 absence of a significant modulation or the suppression of MEPs in one condition as compared to the 8 other one suggests a combination of inhibitory and facilitatory processes.

9 In the present case, MEPs suppression may represent a kind of anticipatory inhibition such 10 as the *proactive inhibition* (Cai et al., 2011; Duque et al., 2017; Lo et al., 2009) since the sequence 11 of stimuli presentation (tool/noun followed by hand action/hand verb) did not vary across trials and 12 our participants could easily anticipate which kind of hand action would appear. In the relatively 13 few studies that showed significant inhibitory or no facilitatory effects of action observation, a 14 common trait is the absence of overt motor responses (e.g. Sartori et al. 2012; Villiger et al. 2011) 15 while explicit instructions/controls are in place to maximize participant's muscle relaxation and 16 prevent any EMG activity prior to TMS, as it was in our study. In addition, we presented a 17 combination of stimuli representing tools and bodily actions, which indeed have accumulating 18 strong motor salience. It is likely that this combination is particularly efficient in engaging the 19 motor system, thus requiring stronger inhibitory mechanisms to prevent overt movement execution.

It is worth noting, that the use of scrambled images as a non-motor control does not fully rule out that the presentation of recognizable "abstract" stimuli, e.g. geometric figures, might modulate MEPs at some level. Evidence collected in a preliminary study employing only the visual modality (see Supplementary materials) shows that this might indeed be the case when a presentation procedure explicitly producing a sensation of motion is used. In this sense, although devoid of motor content, the presentation of abstract geometric figures might be capable of modulating MEPs in a way that resembles that of stimuli with explicit motor content. This might be due to the pace of stimuli presentation or to the fact that 2D geometric figures could be mapped into their 3D counterpart thus carrying some sort of motor information (e.g. affordances) that might affect MEPs. Our preliminary data cannot rule out any of these explanations. It does, however, suggest that the identification of genuine "abstract" stimuli to be used as controls should be carefully addressed by future studies. A direct comparison of the visual and the linguistic modalities seems appropriate to also tackle this issue – i.e. the linguistic modality would at least eliminate the implied movement issue.

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9

4.2 Observation of nouns of tools and related action verbs

10 In the linguistic modality, the findings of Experiment 1 showed a close similarity with the 11 temporal dynamics of the corticospinal excitability observed in the visual one. Similarly to pictures 12 of tools, nouns referring to the same tools did not elicit a significant MEPs modulation as compared 13 to baseline. However, the fact that we cannot rule out the possibility of a sub-threshold motor 14 activation not detectable at 200ms is particularly relevant here. In fact, a qualitative trend (although 15 not statistically significant) towards facilitation of MEPs was evident when analysing Experiment 1 16 and 2 together for tools irrespective of presentation modality. A weak or absent motor facilitation 17 by nouns of tools, as well as tools, could be explained by a balance between facilitation triggered by the strong affordance offered by tools and compensatory inhibitory activity rising up to avoid the 18 19 onset of overt movement. In this way, facilitation would be masked by concomitant inhibition.

The early modulation elicited by action verbs is in line with previous reports of early motor activations during linguistic processing. In addition, in Experiment 1 the reported effect is relatively long-lasting and is present across all stimulation timings. This supports the notion of an integral role of motor activation in the semantic processing of linguistically presented actions (for a review of this issue see Meteyard et al., 2012). In the case of a passive task, i.e. no request of specific syntactic or semantic processing, this suggests both an early/automatic and a prolonged access to action semantics.

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1 As for the direction of modulation, our data are partially consistent with previous reports of 2 inhibitory effects during linguistic processing. However, our report differs from previous studies in 3 how inhibitory effects are defined, namely considering comparisons with both a baseline and a 4 static object condition (control), and not with other experimental conditions (e.g. sentences 5 involving other effectors or abstract content, Buccino et al., 2005). Interestingly, despite using three 6 different stimulation timings, our study did not provide evidence of an evolution of motor resonance 7 effects from an early inhibition to a later facilitation. On the contrary, we showed an early and long-8 lasting modulation of MEPs in the same direction. Furthermore, the similarity of effects with the 9 visual modality supports the hypothesis of the same mechanisms of action representation being 10 activated in both cases. Following the hypothesis of a compensatory inhibition, it is possible that the 11 MEPs suppression triggered by action verbs, as well as hand actions, simply reflects the need for a 12 progressively stronger inhibition to prevent overt movement when motorically salient objects are 13 followed by explicit actions upon them. Summing up, motor inhibition may be viewed as the 14 paradoxical effect of presenting motorically powerful stimuli in a setting where complete muscle 15 relaxation is requested. In this sense, motor inhibition could reflect the degree of motor 16 engagement.

Finally, Experiment 3 showed no MEPs modulation following the presentation of scrambled images and pseudo-words with no motor content. This supports the specificity of the effects obtained in Experiment 1 and 2 in the visual and linguistic modality. In addition, evidence from Experiments 1-3 as a whole suggests that MEPs inhibition is genuinely linked to the presentation of salient motor stimuli, and not to a voluntary, and increasing, active muscle inhibition (or increased relaxation) produced by the participants during the deployment of the trial.

23

24 4.3 Comparison between native and non-native speakers

In line with our predictions, Experiment 1 and 2 showed overall a similar MEPs reduction in
German and English in the linguistic modality, thus confirming the same direction of the effect.

However, Experiment 2 in itself suggests that the MEPs reduction is stronger at later stimulation timings, e.g. t3, and not statistically significant in the other timings. In addition, Experiment 2 suggests that MEPs modulation at early stimulation timings (t1) is stronger in the visual than in the linguistic modality when the latter uses a second language. This suggests – in line with existing literature – that the magnitude and timings of effects in the non-native language might differ from the native one.

7 This is particularly relevant since TMS evidence on this issue is lacking (see Kuhne and 8 Gianelli, 2019 for a summary) and supports the notion of shared semantic representations in the 9 first and the second language, at least for what concerns action-related language, in line with 10 existing models (Dijkstra and Van Heuven, 2002; Kroll et al., 2010). Regarding the timing, it is 11 worth noting that our participants all had very good English proficiency so we cannot rule out the 12 possibility that testing with the same design speakers at different levels of proficiency might elicit 13 further differences in the timing of effects. Similarly, our study reports only evidence from a 14 passive task. This is particularly crucial in the case of a L1/L2 comparison, since the use of a 15 linguistic task might produce different timings of motor activations and further research is needed 16 in this sense.

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18 **5.** Conclusions

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Overall, our data showed the same pattern of motor inhibition in response to visual and linguistic stimuli. Inhibition of the primary motor cortex may prevent the execution of the motor plans, which are resonating in premotor cortex during action observation. This mechanism could well apply also to linguistically presented actions. It is worth noting here that irrespective of the modality of presentation, both manipulable objects and actions show the potential to resonate in the motor system either as motor commands to interact with objects or motor commands simulating the observed action. Therefore, an additive effect of these stimuli is likely. We suggest that in the

1 present study the inhibition of corticospinal excitability may act to offset the augmented facilitatory 2 effects exerted by pairs of action-related visual or linguistic stimuli. While the isolated effect of 3 tools' presentation may be relatively weaker and would not require the intervention of strong 4 inhibitory inputs, the subsequent appearance of tool-oriented actions would raise the motor system 5 engagement close to the threshold for movement release, requiring a supplement of inhibition as 6 revealed by the present findings. Crucially, we report inhibitory effects developing in two 7 modalities, and in the case of the linguistic domain both in native and non-native speakers. 8 Crucially, the time course of these effects is unique to meaningful visual and linguistic stimuli 9 provided of motor content, and is absent when meaningless non-motor stimuli are presented. This 10 suggests the existence of a core, shared system of action semantics that embodies language meaning 11 in the sensorimotor systems controlling goal-directed behaviours.

12

13 Author Contributions

CG and RDV designed and prepared the study. CG, RDV, KK, SM, SLP performed the study. CG
and RDV analysed the data. CG and RDV wrote the manuscript, KK, SM, SLP revised and
approved the manuscript.

17

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24 Conflict of interest

25 The authors declare that they have no conflict of interest.

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Figure Captions:

Fig.1 Experimental procedure with stimulation timings used in Experiment 1 and 2, A) for the visual modality, B) for the linguistic modality



Fig.2 Experimental procedure with stimulation timings used in Experiment 3, A) for the visual modality, B) for the linguistic modality



Fig.3 Results of experiment 1: MEPs (z-score) plotted as a function of Timing and Modality, bars are SE



Fig.4 Results of experiment 2: MEPs (z-score) plotted as a function of Timing and Modality, bars are SE



Fig.5 Results of Experiment 1, 2 and 3: MEPs (z-score) plotted as a function of Timing and Modality, bars are SE



Tables

Table	1: Stim	uli use	d in	Experin	nent 1	and 2
rubic	1. Still	un use	л III	пирсти	nent I	

ΤοοΙ	Action	Tool	Action
brush	to paint	pinsel	malen
cloth	to wipe	wischer	wischen
knife	to chop	kochmesser	hacken
marker	to draw	filzstift	zeichnen
needle	to mend	nadel	sticken
peeler	to peel	sparschaeler	schälen
pen	to write	bleistift	schreiben
plier	to grasp	zange	greifen
scissors	to cut	schere	basteln
shovel*	to dig*	messer*	schneiden*
sponge	to wash	schwamm	waschen
spoon	to stir	löffel	rühren

Table 2: List of pseudo-words used in Experiment 3

ΤοοΙ	Action	Tool	Action
pennello	dipingere	pernallo	cipongere
panno	pulire	copesno	ripanire
coltello	affettare	moltollo	uffestare
ago	rammendare	agico	lucare
pennarello	disegnare	gnarullo	dusennare
pelapatate	pelare	lanatate	sbascare
penna	scrivere	pasnala	costrivare
pinza	afferrare	panvata	effertare
forbici	tagliare	corbica	attilliare
pala	scavare	nalotta	destavire
spugna	lavare	mastugno	silitare
cucchiaio	mescolare	cuschieio	vescilare

	Sphericity Correction	Sum of	df	Mean		F		р		η² _p
		Squares		Square						
Muscle	Greenhouse-	5.989e -5	1.000	5.989e -5		3.201		0.097		0.198
	Geisser									
Residual	Greenhouse-	2.433e -4	13.000	1.871e -5						
	Geisser									
Modality	Greenhouse-	0.085	1.000	0.085		0.443		0.517		0.033
	Geisser									
Residual	Greenhouse-	2.501	13.000	0.192						
	Geisser									
Timing	Greenhouse-	4.754	a 1.645	^a 2.890	а	5.676	а	0.014	а	0.304
-	Geisser									
Residual	Greenhouse-	10.888	21.382	0.509						
	Geisser									
Muscle * Modality	Greenhouse-	0.324	1.000	0.324		3.480		0.085		0.211
	Geisser									
Residual	Greenhouse-	1.209	13.000	0.093						
	Geisser									
Muscle * Timing	Greenhouse-	0.153	2.621	0.058		1.275		0.297		0.089
	Geisser									
Residual	Greenhouse-	1.561	34.070	0.046						
	Geisser									
Modality * Timing	Greenhouse-	0.333	2.703	0.123		0.663		0.565		0.049
	Geisser									
Residual	Greenhouse-	6.532	35.137	0.186						
	Geisser									
Muscle * Modality *	Greenhouse-	0.057	^a 2.150	° 0.027	а	0.395	а	0.692	а	0.029
Timing	Geisser									
Residual	Greenhouse-	1.885	27.953	0.067						
	Geisser									
Note. Type III Sum of Squa	res									
^a Mauchly's test of spherici	ty indicates that the assum	otion of spheric	city is violate	d (p < .05).						

Table 3: Results of repeated measures ANOVA, Experiment 1

С	omparis	son						
Timing		Timing	Mean Difference	SE	df	t	р	p _{holm}
baseline	-	object	-0.0729	0.0865	52.0	-0.843	0.403	1.000
	-	t1	0.2024	0.0865	52.0	2.340	0.023	0.116
	-	t2	0.2149	0.0865	52.0	2.486	0.016	0.097
	-	t3	0.2522	0.0865	52.0	2.917	0.005	0.036
object	-	t1	0.2752	0.0865	52.0	3.183	0.002	0.020
	-	t2	0.2878	0.0865	52.0	3.329	0.002	0.014
	-	t3	0.3251	0.0865	52.0	3.760	< .001	0.004
t1	-	t2	0.0126	0.0865	52.0	0.145	0.885	1.000
	-	t3	0.0499	0.0865	52.0	0.577	0.567	1.000
t2	-	t3	0.0373	0.0865	52.0	0.431	0.668	1.000

Table 4: Post-hoc comparisons for Experiment 1

Table 5: Results of repeated measures ANOVA, Experiment 2

	Sum of Squares	df	Mean Square	F	р	η² _p
Muscle	1.206e -5	1	1.206e -5	0.098	0.761	0.009
Residual	0.001	11	1.237e -4			
Modality	0.518	1	0.518	2.914	0.116	0.209
Residual	1.955	11	0.178			
Timing	4.662	4	1.165	6.918	< .001	0.386
Residual	7.412	44	0.168			
Muscle * Modality	0.011	1	0.011	0.147	0.709	0.013
Residual	0.785	11	0.071			
Muscle * Timing	0.167	4	0.042	1.033	0.401	0.086
Residual	1.781	44	0.040			
Modality *Timing	1.164	4	0.291	2.709	0.042	0.198
Residual	4.729	44	0.107			
Muscle * Modality * Timing	0.226	4	0.057	1.476	0.226	0.118
Residual	1.686	44	0.038			
Note. Type III Sum of Squares						

Table 6:	Post-hoc	comparisons	for ex	periment 2
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Comparison								
Timing		Timing	Mean Difference	SE	df	t	р	p _{holm}
baseline	-	object	-0.1052	0.0838	44.0	-1.255	0.216	0.648
	-	t1	0.1944	0.0838	44.0	2.321	0.025	0.150
	-	t2	0.1612	0.0838	44.0	1.925	0.061	0.304
	-	t3	0.2804	0.0838	44.0	3.347	0.002	0.013
object	-	t1	0.2996	0.0838	44.0	3.576	< .001	0.008
	-	t2	0.2664	0.0838	44.0	3.180	0.003	0.019
	-	t3	0.3856	0.0838	44.0	4.602	< .001	< .001
t1	-	t2	-0.0332	0.0838	44.0	-0.396	0.694	0.694
	-	t3	0.0859	0.0838	44.0	1.026	0.311	0.648
t2	-	t3	0.1191	0.0838	44.0	1.422	0.162	0.648

Table 7: Results of repeated measures ANOVA, Experiment 3

	Sum of Squares	df	Mean Square	F	D	n² "
Muscle	2.652e -5	1	2.652e -5	0.212	0.652	0.015
Residual	0.002	14	1.249e -4			
Modality	0.034	1	0.034	0.269	0.612	0.019
Residual	1.763	14	0.126			
Timing	0.207	4	0.052	0.287	0.885	0.020
Residual	10.093	56	0.180			
Muscle * Modality	0.055	1	0.055	1.438	0.250	0.093
Residual	0.536	14	0.038			
Muscle * Timing	0.216	4	0.054	1.130	0.352	0.075
Residual	2.670	56	0.048			
Modality * Timing	0.598	4	0.149	1.238	0.306	0.081
Residual	6.762	56	0.121			
Muscle * Modality * Timing	0.175	4	0.044	0.805	0.527	0.054
Residual	3.049	56	0.054			
Note. Type III Sum of Squares						