Title of the study: Effect of stimulation of the dorsolateral prefrontal cortex on language production (study A) and motor control (study B)

Principal investigator:
Prof. Jean-Marie Annoni

Study A) Effect of stimulation of the dorsolateral prefrontal cortex on language production in healthy bilinguals – Second addendum

Background:
Language relies on two main neurocognitive components; a dedicated language network depending on perisylvian structures, and a domain-general control-executive and working memory system relying on prefrontal, particularly left cingulum and dorso-lateral prefrontal cortex (DLPFC) networks. The executive system is known to participate in language control in bilinguals, but also plays a role in many intra-languages processing, for mother language (L1) and even more in second language production (L2). For instance, the lexical access in L2 requires more left frontal activation than in L1. The integrity of language control has been associated with a parallel recovery of L1 and L2 in aphasia in bilinguals (Green et al., 2010 [1]) and increased connectedness of the control and language networks has been associated to L2 recovery after therapy (Abutalebi et al., 2009 [2]).
Repetitive transcranial magnetic stimulation (rTMS) is increasingly being used as promising therapeutic tool for psychiatric and neurological diseases as well as a valuable tool for neuroscientists investigating underlying mechanisms of cognitive functions (Machii et al., 2005 [3], Lefaucheur et al., 2014 [4]). Several studies investigating healthy or clinical populations have shown that (r)TMS applied to the left dorsolateral prefrontal cortex (DLPFC) has an effect on language-related processes such as verbal working memory (Osaka et al., 2007 [5]), sentence comprehension (Cotelli et al., 2011 [6]) and language switching (Holtzheimer et al., 2005 [7], Nardone et al., 2011 [8]).
Clinical observations and neuroimaging studies seem to confirm the role of executive functions and frontal structures in language processing in monolingual and bilingual subjects (e.g. Fabbro et al., 2000 [9], Abutalebi & Green 2007 [10]). However, there are only few studies investigating the effect of rTMS applied to the prefrontal cortex on language processes such as verbal working memory and switching, for which executive functions seem to be crucial.

Aim:
The aim of the present project is to demonstrate the impact of the modulation of executive functions, particularly subserved by DLPFC, on language production in healthy bilingual. We propose that such impact will be particularly present in non-native languages (L2).
The project will tackle the question of the role of prefrontal system and executive functioning (namely updating/working memory system, subserved by the left DLPFC) in language performance in the L1 and in L2. The core question will be whether stimulation of DLPFC will influence lexical access and language production.

The experimental task will test the impact of prefrontal stimulation by rTMS (excitatory, inhibitory, sham) applied to the DLPFC of healthy adult bilingual subjects during two tasks relying on lexical strategies: a picture naming task in L1 and L2 and a word translation task. The EEG correlates of the two tasks performed in the first and second language following rTMS will be analyzed. The EEG study will allow us to investigate precisely the spatiotemporal brain dynamics underlying the behavioral modifications induced by the modulation of left frontal areas.

The hypothesis is that:

1. Excitatory rTMS applied to the left DLPFC will increase performance and/or decrease response time in naming, particularly in L2 naming and in the translation task versus sham and inhibitory rTMS.
2. Excitatory rTMS is expected to modify the cortical activity related to the language tasks. During naming, EEG analyses will show a main effect of stimulation and an interaction between the factors Language (L1 or L2) and Stimulation (excitatory rTMS versus sham and inhibitory rTMS versus sham) at the topographic level, showing an effect of prefrontal areas larger in L2 than L1. The latency of the ERP modulation by rTMS in L2 will allow us to target the dynamics and the language planning processes) underlying improved naming performance.

Methods:

The experimental task will include 80 healthy L1-L2 unbalanced control subjects. We will analyze the influence of the DLPFC activation and inhibition on naming and complex language production task (i.e. translation ability) using rTMS as well as their EEG correlates in L1 and in their best second language (L2). The prediction is that left prefrontal stimulation will increase language performances particularly in L2. EEG analyses will unravel the spatio-temporal dynamics of executive processes involved in the two tasks.

EEG

EEG is a direct non-invasive technique for recording of the electrical activity produced by neurons over the scalp with a high temporal resolution. EEG recordings recording is obtained by placing electrodes on the scalp, with a conductive gel filling the distance between electrode and scalp. The electrodes are then connected to an amplifier to amplify and convert the signals as digital voltage values. The event-related potentials will be processed/analyzed offline. A 64 channel Biosemi ActiveTwo system will be used for the recordings.

rTMS

TMS is a painless and non-invasive method of brain stimulation which has gained considerable interest worldwide for clinical as well as neuro-scientific use. Applying TMS leads to neuronal depolarization which has an excitatory or inhibitory effect on the cortex (Rothwell, 1997 [11]). By using changes in magnetic fields to create electric currents, TMS allows modulating cortical excitability and as such stimulating focal brain areas (Walsh & Pascual-Leone, 2003 [12]). Depending on the frequency, intensity and duration of the stimulation, repetitive TMS (rTMS) can lead to temporary increases or decreases in excitability of the affected cortex, which have been shown to last for several minutes beyond the duration of the stimulation itself (Lefaucheur et al., 2014 [4]).

Sham rTMS (or placebo rTMS) refers to a control condition in which no physiological effect on the targeted cortical region should occur. For sham rTMS it is important, that the subject is not aware that he/she is receiving a placebo-stimulation. As such, placebo rTMS needs to fulfill a number of criteria (Loo et al., 2000 [13]) so that it has no effect on the targeted cortical region while at the same time appearing as similar as possible to real rTMS.

By comparing the results in subjects receiving sham rTMS with the results of subjects receiving excitatory or inhibitory rTMS, researchers can determine how much of an effect is caused by rTMS, rather than by a potential placebo effect.

Language proficiency assessment

The participants should have an intermediate level of English knowledge (based on a self-

evaluation questionnaire and DIALANG)

To assess language immersion, participants will be asked for the age of acquisition, how long they lived in a region where predominantly German and English language was spoken, the language spoken with family members, when they were child, in their current daily life, and if the language was acquired in or out of school. With regards to the self-evaluation part, participants will indicate in an analogue scale from 1 to 100% how they estimate their reading, speaking, comprehension and writing skills. As a third level of assessment, a sub-test from the computer-based DIALANG language diagnosis system (Zhang and Thompson, 2004 [14]) will be used to evaluate reading performance (see Buetler et al 2014 for a similar procedure [15]).

Procedure

Participants will be randomized to take part in either an inhibitory or excitatory rTMS session. In all sessions, the same evaluations/tasks will be used. Each session starts with sham rTMS-controlled baseline tasks.

At the beginning of the session, the individual motor threshold for the subject's relaxed small hand muscles will be determined by stimulating the right motor cortex with single pulses (e.g. Nyffeler et al., 2008 [16]). This will last about 5-10 minutes. For the rTMS session, we will be using a "theta-burst" rTMS protocol (Nyffeler et al., 2008 [16]; Huang et al., 2005 [17]), in which one of the co-applicants has extensive experience. This stimulation takes 30 seconds to 5 minutes and the resulting effects have been shown to last about 30 minutes. Each rTMS session will be conducted following suggested international rTMS guidelines (Wassermann et al., 1998 [18]).

After applying rTMS, two language tasks will be performed by the participants while an EEG will be recorded. The two language contexts (German and English) will be presented within each of the two sessions in a randomized order. EEG electrodes will be applied before the stimulation. Participants will be seated in an electrically shielded and sound attenuated booth in front of a LCD screen. Stimulus delivery and response recording are controlled using E-Prime 2.0 (Psychology Tools, Inc., Pittsburgh, PA). The stimuli will be presented in the center of the screen. Both behavioral and EEG data will be recorded.

Stimuli and tasks

This study will be conducted in parallel to the previously accepted study A. For this second addendum, there will be no translation task and a different non-verbal task.

Verbal and nonverbal switching task during EEG recording

Right after applying rTMS (inhibitory/excitatory/sham), participants will perform a language switching task during the EEG recording. The switching task will be based on a corpus of 520 pictures, the corresponding words being matched in English and German on pertinent psycholinguistic factors (Bates et al 2003 [19]). The stimuli consist of black and white line drawings (of 8.5 x 8.5cm each) representing manufactured or living objects. Right before the picture appears on the screen, a cue will be given to the participants in order to indicate the language in which they have to name the object. The subject will then have 3500 - 4500 ms to name the object.

Moreover, participants will also perform a non-verbal switching task during the EEG recording. In this task, participants have to switch frequently between high/low and odd/even classifications of a digit, based on the method of Monsell, Sumner and Waters (2003) Before each digit appears, a cue will be given to indicate to which classification criteria participants have to respond.

Translation task to L1 and L2 during EEG recording

Moreover, participants will perform a task consisting of translating 100 words (5 letters length) from L1 to their second language (L2) or vice versa (8 minutes). Both senses of translation will be mixed randomly to increase working memory load. Translation corpus consists of 50 words in each language, matched across L1 and L2 on frequency, length in syllables and phonemes. Words will be presented on the screen and the subject will be asked to translate each word in the other language. The words will be presented 1500 ms on the screen after a fixation cross of 300ms. The subject will have a maximum 2500ms to do the translation.

Verbal and non-verbal fluency tasks, without EEG recording

With the specific stimulation parameters used in our protocol, 30 minutes after applying rTMS (inhibitory/excitatory), it can be assumed that the after-effect of the stimulation will have disappeared. Participants will then perform a verbal fluency task (the subject will be asked to name as many words as he/she can which starts with a certain letter during 1 minute). Furthermore, a non-verbal fluency task (5 points test, i.e. a sheet of paper with 40 squares of fixed symmetrically arranged points (5 points) will be presented to the participant. The participant will be asked to produce as many different figures as possible with connecting the dots with at least one line connecting two dots during 2 minutes). Finally, participants will perform a 1-minute verbal fluency task in L2.

Behavioral and EEG analyses

Behavioral analysis

A first behavioral analysis will compare naming and translation performances, as well as speech onset latencies in both languages.

For statistical analyses we will compute two 2 way-ANOVAs for repeated measures with the within-subject factors *Language* (L1 vs L2) and *Stimulation* (inhibitory vs sham or excitatory vs sham). Moreover, we will compute two mixed-model ANOVAs with *Language* (L1 vs L2) as within-subject factor and *Hemisphere* (left vs. right) or *Stimulation* (inhibitory vs excitatory) as between-subject factors.

Event Related Potentials analyses

The analyses will be conducted with the same principles as in our prior works with reading and naming tasks. ERPs will be analyzed using a step-wise procedure that permits the time-wise, multifactorial statistical assessment of (1) the dynamic changes of scalp-recorded electric field configuration and their temporal segmentation into quasi-stable functional micro-states indexing modulations of the configuration of intracranial generators, (2) global electric field power indexing modulation of response strength of the intracranial generators, (3) intracranial distributed linear electrical source estimations (e.g. Brunet et al., 2011 [21]; Murray et al., 2008 [22]). These analyses will be conducted for each time frame of the whole ERP time-period; however, specific time windows are planned to be sensitive to our factors according to meta-analyses [19] and our previous results: lexical selection is thought to occurs between 170 and 270 ms after picture presentation, so there will be a first time window until 200 ms, a second time window between 200 and 300 ms, and a later time window (300-450 ms) corresponding to lexical-phonological encoding in previous studies. The main effect will be L1 and L2 patterns in term of amplitude, duration and sequences. Then, a second main effect will be the left inhibitory/excitatory/sham effect in an early time window before the vocalization will start. We will expect an interaction between language and stimulation, due to the fact that left DLPFC excitatory rTMS will modulate L2 more than L1.

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Study B: Effect of stimulation of dorsolateral prefrontal cortex on motor control

Background:

Inhibitory control (IC) refers to the ability to suppress ongoing or planned cognitive or motor processes and enables to achieve goal-directed behaviors in changing environments ([1]). While inhibitory control is involved in regulating both behavior and cognition, most of current knowledge on IC comes from the study of how motor actions are executed or suppressed depending on the presentation of go and stop signals. Investigating inhibition in the motor domain indeed enables to easily measure the effects of IC by examining the rate of inaccurate responses to stop signals (failures of inhibitory control, or 'false alarms') and/or response times in contexts where responses have sometimes to be withheld. One of the main experimental paradigms utilized to study motor IC are Go/NoGo tasks [2], in which participants have to respond as fast as possible to a set of stimuli while withholding their responses to another set of stimuli. High IC proficiency is indexed by fast reaction times coupled with a low false alarm rate (though see e.g. [3] for more sophisticated indices of IC proficiency).

Converging neuroimaging and clinical literature indicate that inhibitory control depends on a cortico-subcortical brain network comprising the inferior frontal gyrus (IFG), the presupplementary motor area (preSMA) and the basal ganglia (BG; Fig. 1a; for review: [4]). When the cancelation of an action is required, this network is engaged at around 200 ms after the onset of stop-signals [5-8]. While the right IFG seems critical for a good IC performance, the left IFG seems important but not critical [9].

Repetitive transcranial magnetic stimulation (rTMS) is increasingly being used as promising therapeutic tool for psychiatric and neurological diseases as well as a valuable tool for neuroscientists investigating underlying mechanisms of cognitive functions (Machii et al., 2005 [10], Lefaucheur et al., 2014 [11]). Neuroimaging, clinical and TMS studies show that the DLPFC plays an important role in processes involving response selection and inhibition (for review see [4]. As such, investigating the effect of rTMS applied to the DLPFC on inhibitory control would allow to further understand the neural substrates and the neural circuitry of inhibitory control.

Aim:

The aim of the present project is to demonstrate the impact of the modulation of the DLPFC on inhibitory control. The experimental task will test the impact of prefrontal stimulation by rTMS (excitatory vs inhibitory) over left and right DLPFC of 80 healthy adult subjects during a simple Go/NoGo task. The hypothesis are that excitatory rTMS of both left and right DLPFC will improve Go/NoGo proficiency, whereas inhibitory rTMS of the right but not left DLPFC will decrease Go/NoGo performance. In total, 80 healthy subjects between 18 and 45 years will be assessed for study B.

Methods:

rTMS

→ see study A

Procedure:

Each participant will take part in two sessions: 1.inhibitory rTMS left DLPFC 2. inhibitory rTMS right DLPFC or 1.excitatory rTMS left DLPFC 2. excitatory rTMS right DLPFC. There will be a two-week interval between the sessions to avoid the crossover of the effects of rTMS. In all sessions, the same evaluations/tasks will be used. The order of left DLPFC stimulation and right

DLPFC stimulation will be counter-balanced.

This study consists of 4 sessions: 1. left excitatory rTMS, 2. Left inhibitory rTMS, 3. Right excitatory rTMS, 4. Right inhibitory rTMS. There will be a two-week interval between each two sessions to avoid the crossover of the effects of rTMS. In all sessions, the same evaluations/tasks will be used. The order the sessions will be counter-balanced.

Participants are seated in an electrically shielded and sound attenuated booth in front of a LCD screen. Stimulus delivery and response recording are controlled using E-Prime 2.0 (Psychology Tools, Inc., Pittsburgh, PA). The stimuli are presented in the center of the screen. Behavioral data will be recorded.

Stimuli and tasks

In the Go/NoGo task, the stimuli are five consonants (S, T, M, H, X) and four vowels (A, E, I, O) presented in black on a white screen. Each trial starts with the presentation of a centered black cross for a duration that randomly varied between 1200 and 2200 ms (100 ms steps). Immediately after the offset of the cross, one of the nine letters is presented in a pseudo-random fashion for 500 ms. Participants have a maximum of 2200 msec to respond. Each subject performs four blocks of 100 trials each.

Participants are instructed to reply as fast as possible by a button press each time a letter is shown on the screen, except for the letter "X". When the letter "X" appears, participants have to withhold their response. To increase response prepotency, the probability of trial occurrence in each block is or 0.3 for the no go ("X") and 0.7 for the go. For the Go trials, the probability is equally distributed among the 4 consonants (S, T, M, H) and the four vowels (A, E, I, O)).
Each block lasts 3 minutes. Thirty seconds rest periods separate each block. After each rest period, the instructions are shown again to the participant, for a duration of 3 sec.

Behavioral analyses

Performance is indexed by the rate of false alarms (FA, i.e. the proportion of unsuccessfully responded NoGo trials), and RTs to Go stimuli. These indexes are analyzed according to a 2*2 design with the factors Stimulation side (Left; Right) and Stimulation type (excitatory; inhibitory). We expect an interaction between the two factors, driven by an improvement and decrease of performance after right DLPFC excitatory and inhibitory rTMS, respectively, and an improvement of performance after left DLPFC inhibitory rTMS.

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Principal Investigator Signature: Date: 06.06.2016