The Neurophysiology of internally-driven actions

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Abstract

Acting in the world in a way that matches our goals, overriding impulses, is one of the first abilities that we must learn while growing up. We often change the course of our actions because of external influences or because we simply “change our mind”. As John H. Patterson said, “Only fools and dead men don't change their minds. Fools won’t. Dead men can’t”. An important distinction must first be made between the impact of internal and external sources on action decisions, and the first part of the introduction will be devoted to this topic. In the second part, I will discuss the topic of inhibitory control. In the scientific literature, action inhibition is often treated as a unitary phenomenon, while the distinction among different types of inhibitions might explain the diverse results and be useful for future studies. My experimental work has been devoted to both externally-triggered and internally-driven voluntary action inhibition, in particular, in Experiment 1 I conducted a set of studies aiming at understanding the underlying cortical circuits for internally-driven action inhibition, whereas Experiment 2 focused on proactive inhibition mechanisms. While it is beyond the scope of this manuscript to cover the entire literature on inhibitory control, I would like to propose a common view to unify the different theories concerning how the brain exerts voluntary inhibitory control and provide some suggestions for future investigations to study the way we flexibly control our actions to cope with the constantly changing external, and internal, environment.

1.1 Internally-driven and Externally-triggered actions

As simple as drinking a glass of water may seem, many years of research on cognitive control, actions and decision making have not yet solved the issue of how even a simple act is implemented in the brain and which brain mechanisms make it happen. Even more intriguing is the study of how the brain interrupts a planned action, since in everyday life we must often stop a planned action due to changes in the environment. For instance, when a traffic light becomes red, we immediately must stop pressing the car accelerator; however, stopping an action can also result from an internal decision (Schüür & Haggard, 2011) when, for instance, one refrains from saying something inappropriate. Most of the time, a combination of internal and external factors drives our decisions to act or refrain from it. "Most people recognize the feeling of trying to get on with something they want to do, yet being constantly distracted by other pressing demands" (Astor-Jack & Haggard, 2005). This feeling, that everyone has experienced at least once in a lifetime, is likely caused by the constant balance we try to achieve between internally generated and externally triggered actions. I will first address the debate concerning the definition of these two types of actions, and, secondly, I will discuss whether it is possible to empirically address the hypotheses they raise.

Self-generated actions and their empirical tractability

In 1890, William James wrote the *Principles of Psychology*, in which he defined *ideomotor actions* (although Carpenter first coined the term in 1852), as actions that follow an "idea of a movement" whenever such "idea" is not inhibited by an antagonistic representation of another action. In particular, James stressed the concept

of competition among alternatives, which arises whenever multiple ideas, mental representations of possible acts, compete and inhibit each other. According to this view, an action can be performed when, in a momentary lapse of consciousness, the subject forgets about the antagonist ideas, thus the main ideomotor action can take place. Frith and colleagues later reformulated the idea of James and they defined spontaneous, or self-generated actions that are not triggered by external stimuli but that are internally driven (Frith et al., 1991). The distinction between internally and externally guided actions is intuitively accessible to everyone, since it easily relates to our subjective experience. However, Nachev & Husain (2010) and Obhi (2012) claimed this dichotomy cannot be empirically studied, because the tasks commonly used to investigate voluntary actions should be by definition "free choice" and "conflict" tasks, inevitably confounde in an experimental setting. Moreover, the authors claim that, despite the best effort researchers can do to account for all the possible confounding variables, it is impossible to control for internal factors. This "internal world" is only accessible through subjects' reports (e.g. Lau, Rogers, Ramnani, & Passingham, 2004; Libet, Gleason, Wright, & Pearl, 1983) and, there is no objective measure to test their reliability.

Passingham and colleagues (2010b) proposed an alternative view and suggested that conclusions can still be drawn from studies addressing the internal/external locus of action decision, if one is willing to refute the idea that external components play no role in voluntary actions. More recently, Schüür and Haggard (2011) divided self-generated actions into two types: operant actions (type I) and undetermined actions (type II). Type I actions are triggered by identifiable and experimentally manipulated internal inputs (cues), such as memory traces, elapsed time intervals, previous actions or (a change in) behavioural goals. This definition does not rule out the presence of external factors, but

they are not determinant for the choice of which action to perform, when to perform it and/or whether to act at all. On the other hand, type II actions are performed in absence of an external input, therefore by defined self-performing agents (or "agentic self" as defined by the authors). There is, however, scientific evidence showing that our perception of agentiveness can be misleading (Wegner, 2003; Lau et al., 2007; Banks & Isham, 2009). Thus, undetermined actions are prone to the previously exposed criticism, both because they are commonly studied using "free choice" paradigms, in which all cues for action (external and internal) are removed and, because our experience of self-generated actions is often incorrect.

Finally, Schüür and Haggard (2011) added a third type of self-generated actions (type III), described as the motor output that derives from processing and integrating a large number of qualitatively different types of input that can be experimentally manipulated directly (e.g. by controlling food intake of an organism) or indirectly (e.g. salt craving caused by hunger) (Schüür and Haggard, 2012). In their view, the degree of *self-generatedness* of an action depends on the number and quality of inputs (internal and external), in order to make a decision to move. Type III actions are different from operant (type II) actions in that type III actions can be triggered, in part, by external inputs. Thus, by manipulating the number and types of inputs in a task, one can empirically investigate self-generated actions

In this study, we investigate internally-driven actions, also termed voluntary, self-generated or endogenous actions, whose characteristics are more similar to operant actions (type I), with some features of type III actions. In fact, although external inputs are present in the experiment, they are likely not to drive the decision to act or inhibit. This matter will be described in more details in the method section of experiment 1.

1.2 The study of internally-driven actions: methods and limitations

The study of internally-driven actions allows us to investigate how we perform and implement our decisions to interact with the external world in a way that fits our goals. Setting our goals, deciding the best strategies to achieve them and performing the correct set of actions (while inhibiting irrelevant ones) is part of what makes us humans. Despite the importance of understanding how we generate internally-driven decisions, the number of studies addressing this issue is very scant. Furthermore, it has been shown that in a number of neurological pathologies internally-driven actions are impaired. For instance, the amplitude of the Bereitschaftspotential (BP), an electrical potential related to preparatory activity for self-generated movements, has been found to be reduced in Parkinson's Disease (PD) patients (Jahanshahi et al., 1995). PD patients experience, among other symptoms, akinesia (also termed "paralysis of will", Wilson 1925), a significant reduction or loss of voluntary motor activity. Movement-related potentials are also impaired before the execution of self-initiated movements in patients with Schizophrenia (Fuller et al., 1999).

A number of neurophysiological and imaging studies have investigated action selection processes and the timing of action execution, comparing "free selection" tasks with stimulus-driven conditions both in non-human primates (Lee & Assad, 2003; Thaler et al., 1995) and in humans (Lau et al., 2004; Thut, Hauert, & Viviani, 2000). One of the most common paradigms used in the literature to compare the internally-driven to externally-triggered actions, is the 'free selection paradigm'. Participants are asked to perform a right or a left button press, either following the instruction of an external stimulus (externally-driven action) or freely choosing one of the two, thus a 'free selection'. Typically, participants can perform the button press any time within a specific interval; however, in this case, the decision of when to act is intertwined with

the action selection phase. This is a problem in many studies, where it is difficult to disambiguate between these two components. For example, in an fMRI study, Lau and colleagues (2004) compared a free selection condition, with a "routine" task and a "specified" task. Participants were presented with a central cue, instructing them to either choose a target among a set of images (free selection), select the target highlighted by two white circles (routine task), or to select the image matching specific features of the cue (specified). The "routine" and "specified" tasks were examples of externally triggered actions with low and high attentional load respectively. The authors found a greater activation in the anterior cingulate cortex (ACC) (interpreted as a conflict-monitoring brain site) and the pre-Supplementary Motor Area (pre-SMA) in the free selection condition, relative to the "routine" and "specified" conditions. However, in the free selection condition, not only participants could freely decide which button to press (with no particular reason to choose one over the other), but also when to press it, within 5 seconds interval. They were therefore instructed to move to the next trial (by moving a cursor) only after they had decided which target to move, whereas the action in the "specified" and in the "routine" conditions was expected immediately after the presentation of the cue. This confound was later addressed by Mueller and colleagues (2007) with an experimental paradigm that better distinguished between the *what* component (the selection of one action among a subset of possible alternatives), and the *when* component (the precise time of the action execution). Finally, this distinction has been later formulated and expanded in the "What, When, Whether model of intentional action" – WWW model – (Brass & Haggard, 2008) that will be described in the next paragraph.

The 'WWW' model and its implication in action research

In this model, Brass and Haggard distinguish between three main components of intentional actions: the *what* component, refers to the selection of one action among a subset of possible alternatives, the *when* component, which determines the precise time of the action execution and the *whether* decision to finally perform the planned action or refrain from it. This model has been very influential and it has changed the way in which intentional actions are investigated. An effort has been made to investigate the three components separately, when comparing internally-driven and externally-generated actions. Particularly, the *what* and *when* components have been studied more extensively, while the *whether* component is more difficult to study for reasons that we will explain later. Here we will review some studies that investigated voluntary action selection and the timing of action execution.

In an interesting fMRI study, Mueller and coworkers (2007) investigated the *what* aspect of voluntary actions, while controlling for the *when* component. Two stimuli were presented separated by 1200 ms and participants were asked to perform a left or right button press 600 ms after the presentation of the first stimulus. Critically, they used two conditions: the 'externally-selected' condition where the action selection (left or right button) had to be congruent with the location of the preceding stimulus, and the 'internally-selected' condition, where the action choice would have determined the side of presentation of the upcoming stimulus. They found a greater activation of the Rostral Cingulate Zone (RCZ), a portion of the Cingulate Motor Areas, for the 'internally-selected' condition. Unexpectedly, no pre-SMA activation difference was found between the two modes of action selection, as previously found by Lau and colleagues (2004), and, since the pre-SMA showed equal activity in both conditions, the authors suggested it might be involved in the timing or initiation of actions, present in both

conditions. In another fMRI study (Soon et al., 2008) the authors measured participants' brain activity during a freely-paced motor decision task, in which subjects pressed one of two buttons when they felt the *urge* to do so, while watching letters presented on a screen. At the end of each trial, they had to report which letter was displayed when they decided on the motor action. The authors assessed, through statistical pattern recognition techniques, how much predictive information was contained in specific brain regions at various time points. The activity of the fronto-polar cortex (BA 10) and precuneus/posterior cingulate cortex was highly predictive of the subsequent outcome of the free decision (action selection), at least seven seconds before the actual action took place. Conversely, activity in the SMA/pre-SMA complex was informative with respect to the timing of action execution, supporting the hypothesis of a role of these areas in the *when* component of voluntary actions. More recent evidence supports the idea of a common circuit responsible for action selection and onset timing of voluntary actions. fMRI results (Momennejad and Haynes, 2012) and direct extracellular recordings in patients undergoing surgery for pharmacologically intractable epilepsy (Fried et al., 2011) revealed the involvement of the medial frontal cortex, including the SMA, in the *what* and *when* components of internally-driven actions. Furthermore, Hoffstaedter and colleagues (2012) reported a role of the anterior mid-cingulate cortex (aMCC) in both internal selection (what) and timing (when) of movements, but the activation was greater when these decisions were not triggered by external stimuli. In conclusion, recent scientific evidence supports the proposed distinction between, at least partially, separable circuits for internally and externally generated actions. These results are in agreement with Goldberg's (1985) view of a functional distinction between the fronto-median cortex, responsible for the intentional control of behavior and, the fronto-lateral cortex, more involved in the external control. This 'two routes hypothesis of action' includes the basal ganglia and fronto-median areas, including

the pre-supplementary (pre-SMA) and the supplementary motor area (SMA), and the cingulate motor area for internally-generated actions, while the circuit responsible for externally triggered actions includes the parietal lobes, lateral premotor areas and the cerebellum. Interestingly, Sherrington in his 1906 work "The integrative action of the nervous system" already supported the idea that both the premotor and the supplementary motor cortex project to the primary motor cortex which, in turn, provides a *final common path* to the muscles for movement (Astor-Jack & Haggard, 2005). The areas part of the internally-driven and externally-triggered action circuits have been updated over the years, but the concept of separate circuits remained valid. It is, however, less clear what are the circuits involved in action selection (what) and in the timing of the action (when).

1.3 The Whether component and inhibitory control

An important feature of cognitive control is the ability to withhold unwanted actions, in response to environmental or 'internal' changes. The term 'intentional inhibition' (Filevich, Kühn, & Haggard, 2012) refers to the *voluntary* inhibition of responses (internally-driven action inhibition). The ability to withhold or interrupt ongoing motor plans as a result of an internal decision is a distinctive feature of higher animal species (Curtis and D'Esposito, 2009), which is operationally defined in the *whether* component of intentional actions (Brass & Haggard, 2008). The study of inhibitory control is particularly relevant, since psychiatric and neuropsychological disorders often cause an impairment in the ability to inhibit compelling *urges* (addiction, Tourette syndrome, attention deficit/hyperactivity disorder, obsessive/compulsive disorder and schizophrenia). Therefore, the study of the neuronal underpinnings of internally-driven

action inhibition can help us understand how the brain exerts control over response tendencies in the normal population as well as in brain disorders, with the final aim to find possible rehabilitative strategies. However, as anticipated above, the study of internally-driven action inhibition is more complex, compared to the study of the *what* and *when* components of intentional actions.

To investigate the *whether* component, a study should compare an externally-driven condition in which the stimulus instructs participants to either execute the planned action or inhibit it, with an internally-driven condition where participants can freely decide whether to act or not. The study of the voluntary inhibition of actions is difficult because it is important to present participants with a stimulus that does not determine participants' choice. Importantly, subjects should not choose in advance whether they will inhibit the planned action or not, however at target presentation, and when they choose to inhibit the action there is, by definition, no behavioral outcome to measure. Lastly, participants should have a reason to perform an action or not. Similarly, in real life, actions or action inhibitions have consequences and an experimental paradigm should provide participants with a motivated reason to either act, or inhibit the action, on each trial. Together, all these limitations have caused the voluntary inhibition of action to be almost unexplored by cognitive neuroscientists, whereas the study of the externally-triggered inhibition of action has received much attention over the years. The majority of these studies (Aron et al., 2004, 2007) use the Stop-Signal or countermanding task paradigm (Logan, 1994), where participants are required to plan a motor response on each trial (e.g. a button press) that they will perform upon the presentation of the target signal. Critically, on some trials, a stop-signal appears with a variable delay after the presentation of the target, instructing participants to withhold the response. Another paradigm often used to investigate action inhibition is the Go/NoGo

task, in which the target instructs participants to either execute the action or not. Moreover, by manipulating the probability of occurrence of the NoGo stimulus and the allotted time to respond (speed or accuracy task), participants are more or less likely to plan the action in advance (see Swick, Ashley, & Turken, 2011 for a critical comparison between the two paradigms). Several experimental studies (e.g. Obeso et al., 2013; Neubert et al., 2010) have suggested a role of the right Inferior Frontal Cortex (IFC) in the externally-triggered inhibition of actions, probably part of a circuit that includes the pre-SMA and the subthalamic nucleus (STN) (Aron et al., 2011), as shown in Figure 1.

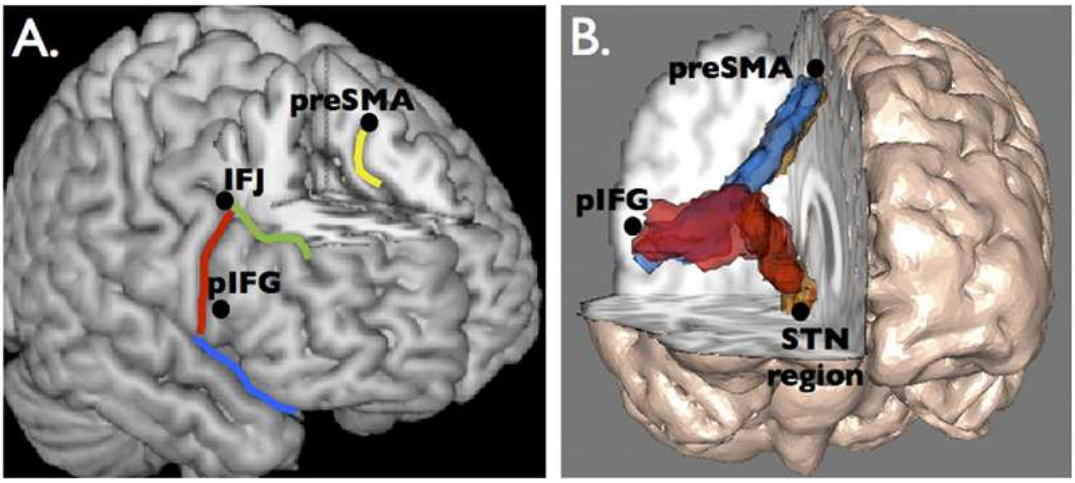

Figure 1 The brain network for reactive stopping. (A) Regions that are critical for stopping in the standard stop signal paradigm. Two regions within the inferior frontal cortex (IFC) are the inferior frontal junction (IFJ) and the posterior (p)IFG. The pre-supplementary motor area (pre-SMA) is in the medial surface. (B) White matter tractography using diffusion tensor imaging reveals a three-way network in the right hemisphere between nodes that are critical for stopping action. (modified from Aron et al., 2011)

Only few studies have used an experimental paradigm more appropriate to study internally-driven action inhibition (Brass & Haggard, 2007). In their fMRI study, Brass

and coworkers adopted a modified version of the Libet's paradigm*, in which participants could freely decide whether to act or not, and later, they were asked to report the moment in which they thought the decision took place. In this task, like in Libet's task, the time-locking event is the reported time of the decision-making process, but some studies on temporal binding have demonstrated how these subjective measures are systematically influenced by subsequent events (Banks & Isham, 2009; Herwig & Waszak, 2012; Moore & Obhi, 2012). Nevertheless, in this first attempt to investigate internally-driven action inhibition (which they call 'endogenous inhibition of intentional actions'), the authors found a greater activation of the dorsal fronto-median cortex (dFMC, anterior to the pre-SMA and dorsal to the rostral cingulate zone) for the freely inhibited trials compared to action trials. The authors interpreted the role of the dFMC as "top-down control signal gating the neural pathways linking intention to action", which is also supported by a positive correlation between individual dFMC activity on inhibitory trials and the frequency of inhibited actions. The authors further suggested that this area is likely not involved in the decision whether to act or inhibit, since this decision would be present in both action and inhibition trials, whereas they found very little activation of this area in action trials.

*In Libet's paradigm (Libet et al., 1983) participants watched a dot on the screen of an oscilloscope circulating like the hand of a clock. They were asked to perform a wrist movement at a time of their choice and later report the position of the moving dot when they were aware of the conscious decision to move.

Kuhn and coworkers devised a better experimental paradigm to study the distinction between a decision whether to act or inhibit (Kuhn et al., 2009): they compared, in the same subjects, a Go condition with a free-choice condition. In their fMRI study, the authors presented participants with a white marble on top of a tilted plane, the marble turned green on 50% of the trials (Go signal) instructing participants to press the button to stop it from falling off the plane. On the other half of the trials the marble would stay white, informing participants to freely decide whether to stop the marble (decide-Go trials) or inhibit the pre-planned action (decide-NoGo trials). In a first attempt to provide participants with a reason to either execute the action or withhold it, they used an aversive feedback (a glass breaking sound) whenever subjects decided not to inhibit the action (button press). By contrasting the two decide condition with a third control condition (externally instructed NoGo trials) they expected to find an area involved in the decision whether to act or not, an area active in both decide-Go and decide-NoGo trials. This contrast yielded the activation of the RCZ, previously associated with the *what* component. They also replicated Brass and Haggard's results, finding a greater activation of the dFMC (BA 9) in decide-NoGo trials, relative to decide-Go trials, consistent with the view that dFMC is involved in the voluntary inhibition of actions. In support of this view, they also found a significant difference in the effective connectivity with pre-SMA, suggesting that the dFMC might directly influence motor preparation.

After the WWW model of intentional action, researchers have been trying to isolate the areas involved in the three components, using different paradigms to study externally-triggered action generation and inhibition. A summary of the areas involved in the three components of intentional actions is shown in Figure 2.

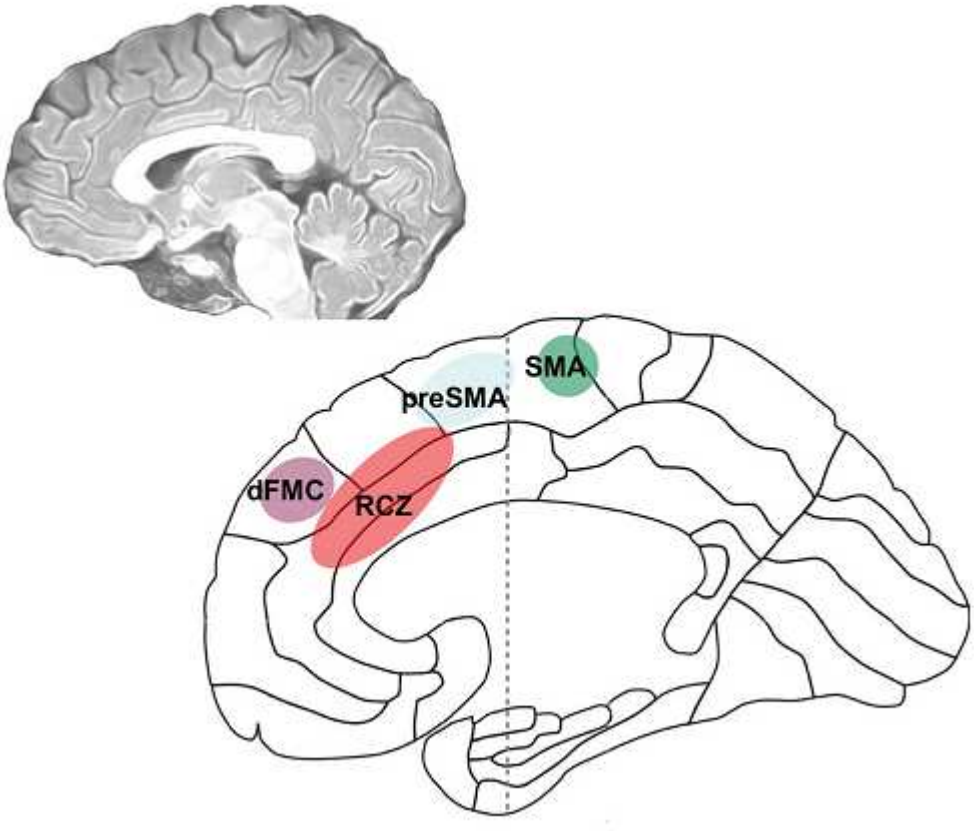

Fig 2. *Upper panel*, median view of the human cortex. *Lower panel*, schematic drawing of the frontal brain regions that have been consistently found to be involved in the *when*, *what* and *whether* components of intentional action. SMA= supplementary motor area; RCZ= rostral cingulate zone; dFMC= dorsal fronto-median cortex. (Brass & Haggard, 2008)

Despite these studies have provide some useful information regarding the brain circuits involved in internally-driven action inhibition, much still needs to be done to fully understand whether the same or different brain circuits are involved in the two types of action inhibition and whether the dFMC is causally responsible for internally-driven action inhibition. When investigating inhibitory control, as already pointed out earlier, oftentimes different names are used to refer to the same concept, like endogenous, internally-driven, intentional or self-generated action inhibition. In the next paragraph we will critically review a recent paper (Ridderinkhof et al., 2014) in which the authors

provided some dimensions along which inhibition can be distinguished and systematically investigated.

The study of the multifaceted inhibition of actions

As Ridderinkhof and collaborators correctly pointed out, action inhibition is often treated as a unitary concept, however, even within the domain of externally-triggered actions, it is easy to intuitively capture the difference between having to stop pressing the car accelerator when the traffic light turns red or refraining from smoking cigarettes because the packages says "it causes cancer". Also among internally-driven action inhibitions there are differences, for instance between avoiding asking inappropriate questions or stop eating a delicious slice of cake.

Just like the 'WWW' model tried to disentangle between different components of voluntary action inhibition, within the *whether* component Ridderinkhof and colleagues distinguished four relevant dimensions according to which, in the authors' view, inhibition can be categorized (Ridderinkhof et al., 2014). In the first dimension, the authors distinguish between *intentional* and *reactive* inhibition. These two types of inhibitions can be compared to internally-driven and externally-triggered inhibitions, and, since there is evidence suggesting that external cues activate inhibition automatically (Verbruggen & Logan, 2008), Ridderinkhof and coworkers used the term 'reactive' for the latter type. I will explain later why, in my opinion, the terms *intentional* and *reactive* might create some confusion. The second dimension regards the timing of the inhibition process, the moment in time in which the decision to inhibit is implemented. For externally-triggered actions, using the Stop Signal paradigm described earlier, researchers manipulate the time interval between the Go and stop

signal, called stop signal delay (SSD), to study the brain areas involved in action inhibition at different stages during action production. The third dimension differentiates between global and selective inhibition. Global inhibition refers to the interruption of all responses, performed when fast inhibition is needed. For instance, Majid et al., 2012 used transcranial magnetic stimulation (TMS) to show that reduced cortico-motor excitability of the leg could be find in tasks requiring fast inhibition of hand movements. However, when one action is selectively inhibited, while others are performed, a selective inhibition mechanism is employed. Finally, in their last dimension, Ridderinkhof and colleagues propose a distinction among different types of action to be inhibited. Some characteristics of the actions that modulate inhibition are the strength of stimulus-response association and the prepotency of the action (how much it has been preactivated). This last dimension can be, however, very difficult to measure in an experimental condition.

As previously anticipated, I believe that the first dimension proposed by Ridderinkhof and colleagues is useful but the chosen terminology might be confusing. The reason for this is that another type of action inhibition, called *proactive* inhibition is often contrasted to reactive inhibition (see the dual mechanisms of control (DMC) theory Braver et al., 2007; 2012). Proactive inhibition can be described as "how a subject prepares to stop an upcoming response tendency" (Aron, 2011) and it has been shown to be context-dependent (Wardak et al., 2012). Reactive stopping (also sometimes called 'outright stopping' e.g. Swann et al., 2012) is, instead, the ability to interrupt an action after the presentation of a target. While proactive inhibition probably depends on both internal and external factors, reactive inhibition, that is inhibition triggered (in time) by the presentation of a stimulus, is not necessarily externally-triggered. For example, while, in a Go-NoGo paradigm, the probability of presentation of a NoGo signal will

modulate the amount of proactive inhibition the subject will activate, and the target presented to participants can either instruct them to perform (Go signal) the action or inhibit it (NoGo signal) or just let them decide whether to act or not (decide Go/NoGo signal). For this reason it is reasonable to distinguish between proactive and reactive inhibition, the first is context-driven and the second is stimulus-triggered while the dimension regarding the level of intentionality proposed by Ridderinkhof and colleagues should instead be considered as a continuum from externally-triggered to internally-driven action inhibition (Filevich et al., 2012).

In this experimental project, I focused on two types of action inhibition: in a first set of experiments I addressed the comparison between externally-triggered and internally-driven actions. I specifically designed a task that asked participants on some trials to freely decide whether to perform the action or inhibit it, whereas on other trials the stimulus instructed participants to either perform the action (Go signal) or not (NoGo signal). In a second set of experiments I focused on proactive inhibition. In particular, I investigated the time course of proactive inhibition, hypothesized to be automatically activated after the presentation a warning signal. In particular I studied the mechanism that has been hypothesized to be responsible for the inhibition of automatic, early responses (Boulinguez, Ballanger, Granjon, & Benraiss, 2009; Criaud, Wardak, Ben Hamed, Ballanger, & Boulinguez, 2012).

2.1 Techniques employed in the experiments

In this section the imaging techniques Transcranial Magnetic Stimulation (TMS) and electroencephalography (EEG) will be briefly introduced along with the rationale for using them in the present studies. Methods and procedures will then be discussed in details for each experiment below.

Transcranial Magnetic Stimulation (TMS)

TMS is a tool used to stimulate the brain non-invasively. It uses of the principle of electromagnetic induction, discovered by Michael Faraday in 1831, which states that fluctuating magnetic fields can induce electric current in conductors placed nearby. With TMS, a magnetic field is generated by an electrical current generated inside the coil, as depicted in Figure 3. The magnetic field then induces an electrical current in the cortical surface underneath the portion of the scalp over which the coil is placed.

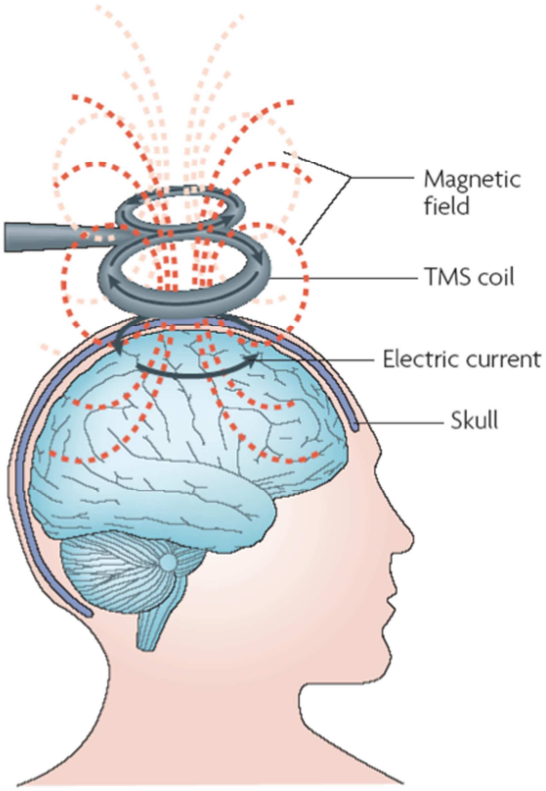

Figure 3 The figure illustrates the induction of electrical currents in the brain (black arrows in brain) through the magnetic pulses (red/pink) applied by means of the coil (grey 8-shaped figure) placed on the scalp. Adapted from Ridding and Rothwell (Ridding & Rothwell, 2007).

Through the extracellular space, the induced electrical current excites the axons of the neuronal population in the surrounding brain tissue and, with sufficient intensity, it will cause the discharge of action potentials which will propagate to local (Allen et al., 2007; Mueller et al., 2014), and probably more distant networks (Ilmoniemi et al., 1997; Paus et al., 1997). The number and type of stimulated neurons will depend on their orientation relative to the current flow (Ni et al., 2011), and this will likely affect whether the effect of TMS on the ongoing brain activity will be excitatory or inhibitory (for a review see Kobayashi & Pascual-Leone, 2003). TMS is usually more focal when delivered with a figure-of-eight coil. The magnetic pulse reaches the brain unattenuated and it has been used by cognitive neuroscientists, since 1985 (Barker et al., 1985), to

non-invasively stimulate the brain. TMS can be used to map brain functions and explore the excitability of different regions, and more recently it has also been used as a rehabilitative tool for various pathological conditions (e.g. migraine, depression, schizophrenia, stroke). The effect of TMS on the stimulated tissue varies depending on the frequency and strength of the magnetic pulses, but it is also modulated by the distance between the coil and the brain (the intensity of the magnetic field decreases in proportionately to the distance squared), the amount of extracellular liquid surround the targeted brain area (e.g. presence of fissures and deepness of the sulci) and the orientation of the coil. These factors introduce some source of variability in the responses to TMS that should be taken into account when running an experiment. However, optically tracked frameless stereotaxic navigation systems are now widely used to guide the TMS coil, and should be used to reduce variability. TMS can be delivered either single-pulse, in pairs of stimuli separated by a variable interval, called paired-pulse TMS, or in trains, hence repetitive TMS. In my studies, I used repetitive and single pulse protocols.

A single pulse of TMS delivers a magnetic field that can have an intensity of maximum 2 Tesla and lasts about 100μs. When single pulse TMS is used to stimulate the hand area within the primary motor cortex (M1), with sufficient intensity (Rossini et al., 1994), a brief, relatively synchronous muscle response can be measured with electrodes placed on the controlateral hand. The amplitude of this response, called motor evoked potential (MEP), is an indirect measure of cortico-spinal excitability, that is the susceptibility of motor areas to produce movements, due to the direct or transynaptic recruitment of cortico-spinal neurons (see Terao & Ugawa, 2002). The possibility to test, through single pulse TMS, the level of excitability of a cortical area has been later generalized to non motor brain regions. This technique can be used, in fact, to interfere

with cortical functions while participants perform a cognitive task and, due to its excellent temporal resolution (milliseconds), chronometric studies can be performed to determine the time course of the involvement of a specific brain region in a cognitive task.

During repetitive TMS (rTMS), a train of pulses is delivered at the desired frequency and it produces effects that outlast the time of stimulation. When the pulses are delivered at low frequencies (0.2-1 Hz) the net effect on the stimulated brain areas is inhibitory (Walsh & Cowey, 2000). This was originally measured by applying low frequency rTMS to M1 and measuring the induced reduction in the amplitude of MEPs (Chen et al., 1997). Low frequency magnetic stimulation is known to have inhibitory effects and can help test the functionality of a network system when the stimulated node is temporarily damped. This virtual lesion approach (Pascual-Leone et al., 1999) likely alters the entire system by either adding noise (Schwarzkopf et al., 2011), or by changing the synergies between regions that are part of the same network (Lee et al., 2006; Plow et al., 2014) likely causing homeostatic changes in other brain areas, connected to the stimulated hotspot by cortico-cortical or cortico-subcortical connections (Siebner et al., 2004).

Electroencephalography (EEG)

EEG is a widely used, neuroimaging technique to record, almost in real time, the electrical brain activity. Electrodes are placed on the surface of the scalp to record the electrical activity of the neuronal populations. This technique is quite old, and it dates back to the German physiologist and psychiatrist Hans Berger (1873–1941) who recorded the first human EEG in 1924. EEG provides a unique way to non-invasively

record the oscillations of brain electric potentials, with great temporal resolution, although with low spatial resolution. EEG records a difference in potential (voltage), measured between two electrodes, an active and a reference electrode. Each electrode registers the summed activity of different neuronal populations, and one of the drawbacks of this technique is represented by the difficulty to isolate and localize the neuronal sources that generate the signals detected on the scalp. Despite these limitations, EEG provides useful information regarding the brain activity involved in a task and, specifically, the brain's response to some events. By averaging many trials time-locked to some specific event of interest (e.g. target, motor response), it is possible to measure event-related potentials (ERPs), reducing the signal-to-noise ratio, which is another problem of EEG data. The voltage recorded on the surface of the head is, in fact, a sum of *signal*, which represents any type of brain activity the researcher is interested in, and *noise*, that is everything different than the signal. Only by averaging together the activity recorded from many trials, it possible to enhance, in the final recorded potential, the contribution of the signal and reduce the impact of the noise, assuming that the latter has a random distribution.

By averaging the signal time-locked to some event, only phase-locked activity remains visible, but EEG also contains non phase-locked rhythmic activity, that reflects neuronal oscillations. These oscillations, fluctuations in the excitability of neuronal populations are described as frequency (speed of the oscillation, measured in Hz), power (squared amplitude, measured in μV) and phase (position along the sine wave at any given time point, measured in degrees or radians). The frequency bands typically analyzed in cognitive electrophysiology are the delta (2-4 Hz), theta (4-7 Hz), alpha (8-15Hz), beta (16-31) and gamma (32-150 Hz). Different cognitive processes and neuronal functions seem to make a greater use of some frequencies instead of others (Buzsáki & Draguhn,

2004). For instance, the alpha band has been related to inhibitory control (Hwang, Ghuman, Manoach, Jones, & Luna, 2014; Klimesch, Sauseng, & Hanslmayr, 2007), whereas an increase in beta power has been associated, among other processes, to sensorimotor transmission (Kilavik, Zaepffel, Brovelli, MacKay, & Riehle, 2013). The relative power of some frequency bands changes in response to an input, or during a cognitive effort, and this difference is captured by event-related synchronizations (ERS), representing an increase in power, and desynchronizations (ERD), associated to a decrease in power. Many other types of analyses can be performed on EEG frequencies, and their description is beyond the scope of this paragraph (for a detailed description and discussion see Cohen M. X., 2014).

3.1 Experiment 1: Investigating internally-driven and externally-triggered actions

As previously reported, not many studies have investigated, in the same subjects, the brain areas involved in internally-driven and externally-triggered actions and action inhibitions. The lack of studies investigating internally-driven action inhibition can be ascribed to the difficulty to design a task using a stimulus that does not directly cause the decision to act or inhibit, and to the lack of a measurable behavior, when the action is inhibited. Previous fMRI studies reported a greater activation of the rostral cingulated zone (RCZ) for both the endogenous *what* (Mueller et al., 2007; Lau et al., 2004) and *whether* decision (Kuhn et al., 2009). However, Kuhn and coworkers gave more compelling evidence through a connectivity study, showing that the dorsomedial frontal cortex (dFMC), also named 'veto area', might be responsible for the implementation of the internally-driven decision to inhibit an action. Other studies have found the right Inferior Frontal Gyrus (rIFG) to be more involved in exogenous (externally-triggered) action inhibition (Chambers et al., 2006; Swann et al., 2012; Wessel et al., 2013).

Here for the first time we investigated the causal role of the dFMC and the rIFG in the inhibition of unwanted responses, comparing, within the same participants, externally and internally-driven action inhibition using a psychophysical paradigm. We used inhibitory transcranial magnetic stimulation (TMS) over the left dFMC and rIFG to study their role when subjects must freely decide whether to act or not. Results indicate a prevailing role of the dFMC in endogenous action inhibition. Electroencephalography (EEG) also showed distinct neuronal markers for exogenous and endogenous action inhibition.

3.2 Materials and Methods

Participants

Nineteen right-handed university students (mean age 24.7 years; SD 3.5; 10 females) with no neurological or psychiatric impairments voluntarily participated in the study. Handedness was determined via a condensed version of the Edinburgh Handedness Inventory (Oldfield, 1971). All subjects provided written informed consent, according to the ethical standards of the Declaration of Helsinki. The study was approved by the ethical committee of the University of Trento. All participants passed the TMS and EEG safety screenings.

Experimental procedure

The study comprised a behavioural session that lasted approximately one hour, followed by three TMS-EEG sessions on three separate days. The overall duration of the experiment was about 10 hours. During each TMS-EEG session, EEG was employed to record brain activity of participants while they performed the Act or Inhibit task (see below for a detailed description), before and after applying TMS over one of three brain areas (see "TMS-EEG sessions" section), during an offline procedure. Throughout the duration of each session, subjects seated on a comfortable chair at a distance of 57 cm from the computer screen and responses were collected using a low-latency USB response box (DirectIN v2012, Emprisoft, Inc.). The task was presented on a 22" Samsung 2233RZ LCD monitor running at 120Hz on a Windows 7 machine running Matlab 7.2 and Psychotoolbox 2.0 experimentation presentation software. Participants were paid a fixed amount for the behavioral session and a variable amount for each of

the stimulation sessions, depending on their performance in the cognitive task. The order and duration of each experimental session is described in Figure 4.

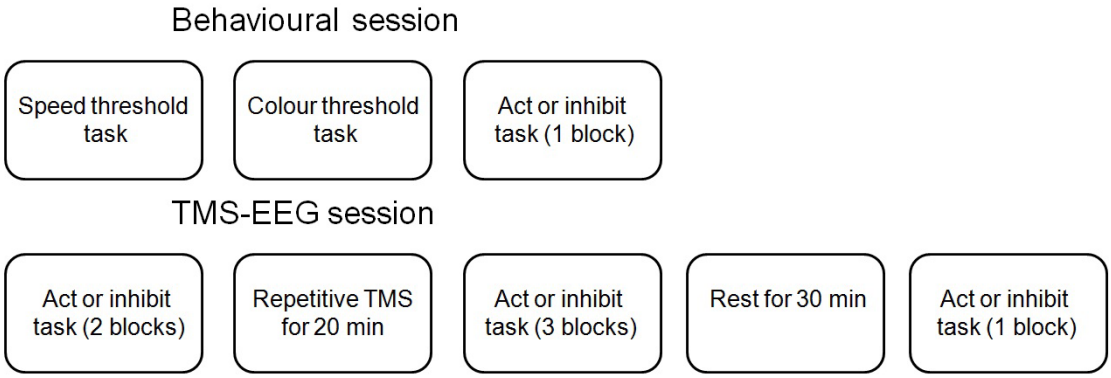

Figure 4 The figure shows the order and duration of each experimental session. During the first behavioural session subjects were tested on three tasks: the speed threshold, the colour threshold and one block of the Act or Inhibit task. This sequence was repeated three times for a total duration of about an hour. Each TMS-EEG session started with two blocks of the Act or Inhibit task (baseline), followed by 20 min TMS while subjects rested. Immediately at the end of the stimulation, subjects performed 3 blocks of the Act or Inhibit task (15 min). After 30 min of rest participants performed 1 more block of the Act or Inhibit task, for a total duration of about 3 hours for each TMS-EEG session. Brain activity of participants was recorded during every TMS-EEG session.

Behavioural session

During the first behavioural session, participants performed three tasks, repeated three times each. Two tasks had a built-in staircase procedure to measure the psychophysical thresholds for the subsequent task that participants performed during the stimulation condition. In all three tasks, participants were presented with a marble at the top of a tilted plane. The plane consisted of a white line, 0.35 thick and 27.8 long degrees of visual angle, with a luminance of 204.5 cd/m², tilted at an angle of 30 degrees, running

from the upper left to the bottom right quadrant of the screen. The marble was always presented at the top of the plane, hence on the top-left side of the monitor. All stimuli were presented on a black background (0.2 cd/m^2).

During the Speed Threshold task, on each trial, participants were presented with a static white marble 3.55 degrees in diameter, and the task was self-paced. Subjects were asked to press a key on the response box with their left index finger to start the next trial. After a variable delay (between 0 and 3 seconds) the marble started rolling down the tilted plane at a fixed velocity. Participants were asked to wait for the ball to start moving and then press another key with their right index finger as quickly as possible to stop it. A 1-up-1-down staircase procedure was employed to determine, for each participant, the threshold speed at which they were able to correctly stop the marble before falling off the plane on 50% of the trials. The task ended when the threshold speed was determined.

In the Colour Threshold task, the marble started moving after a variable time interval (same as before), after participants pressed the key to start the trial. The speed was constant as individually measured during the previous task. We used a staircase procedure, this time to measure a shade of colour that was subjectively perceived as an ambiguous colour in between two predefined colours (that is when they reported 50% of the times one of the two colours, see below for a detailed explanation). The task stopped once the ambiguous colour was found. We intermixed six staircases: one staircase started from the colour green, one from the colour magenta and four others from mid points between these two colours. The two staircases starting from green and magenta had bigger step sizes than the others. Colours were defined in the Lab Colour Space, a color-opponent space with dimension L for lightness and a and b for the color-opponent dimensions, which allowed us to modify the green/red values while keeping lightness

and yellow/blue values constant. On each trial, the marble was a different shade of colour between green and magenta and participants were asked to watch the marble rolling down the tilted plane. At the end of each trial, the word “magenta” was presented on the left side of the screen and the word “green” was displayed on the right side. Subjects were asked to press one of two keys on the response box indicating whether the marble looked “more magenta” or “more green”. The colour-related words presented on the screen were spatially congruent with the keys to help subjects remember the location of the key they meant to press. After the response, a new trial started with a new coloured marble. Once the ambiguous colour was determined, 14 other shades of colours were mathematically determined, so that 7 would be closer to magenta and 7 to green. A cumulative of normal distribution was generated with mean corresponding to the threshold value of the staircase (ambiguous colour) and standard deviation (std) of the staircase. Being the extremes of the distribution green (0) and magenta (1), two out of the seven shades of colour were randomly sampled within 1 std, for each side of the distribution. The remaining 5 shades of green were randomly sampled in the range from mean-1std. to the 20th percentile, whereas the 5 shades of magenta were randomly sampled in the range from mean+1std. to the 80th percentile.

In the third task, named Act or Inhibit task, we used a modified version of the task employed by Kuhn and colleagues (Kuhn et al., 2009). On each trial, a marble was presented on the top of a tilted plane and after a variable interval (Stimulus Onset Asynchrony: 150, 300, 500, 1200, 1500, 3000 ms) it started rolling down the plane at the threshold speed determined during the first task. On each trial, the ball could have been one of the predetermined 15 shades of colour. Colour presentation and SOAs were pseudo-randomly intermixed across trials. Participants were asked to wait for the ball to start moving and then to press a key with their right index finger “only if the colour of

the marble looked more green". Each block consisted of 120 trials: the ambiguous colour was used in half the trials, 30 trials were green trials, randomized across the 7 shades of green, and 30 were magenta, randomized across the 7 shades of magenta. Since participants were asked to perform a single button press in order to stop the marble only if its colour was perceived as being green, the green and magenta marbles are examples of "Exogenous Go" and "Exogenous NoGo" stimuli, respectively. Importantly, the ambiguous colour by definition was not easy to categorize, forcing subjects to behave randomly and, for this reason, we consider this an "Endogenous Go/NoGo " condition, since it allows participants to freely decide whether to respond or not. On Endogenous Go/NoGo trials, participants pressed the button 50% of the times (a t-test was used to confirm that the relative frequency of responses was not significantly different from chance), suggesting that the staircase procedure worked properly. We computed multiple shades of green and magenta to prevent participants from memorizing the colours and the correct stimulus-response pairing. In fact, since some shades of magenta and green looked similar to the ambiguous colour (within 1 std from threshold value), participants most likely were not aware that one specific shade of colour was presented more frequently. Furthermore, at the end of each trial, after a 300 ms interval (during which late responses were recorded), a feedback was presented for 1500 ms at the location where the next marble would appear. One of two feedbacks were provided: when the key was pressed before the marble fell off the plane the text "Blocked" appeared, whereas "Not Blocked" was displayed in case of a late response or a no key press. Additionally, if the marble was green and participants correctly pressed the button, the text "Good + 10c." was shown. Rewards were given for quick responses only, to keep participants motivated to always prepare a response and, if necessary, to inhibit it. Similarly, on Exogenous NoGo trials (magenta) the same feedback appeared if participants decided not to press the button. In case of mistake (late or no response on

green trials/key press on magenta trials) the text “No. 0c.” was presented. Critically, on ambiguous colour trials (Endogenous Go/NoGo), irrespectively of participants’ choice, one of the two feedback was randomly presented (either “Good. +10c.” or “No. 0c.” together with “Blocked” or “Not blocked”, respectively, depending on their choice). Participants were informed that they would be paid the amount of money they accumulated during this third task (except for the first behavioural session), however during the study they were unaware of the feedback manipulation (they were fully informed at the end). Subjects were also informed that due to the difficulty of the task and speed of the marble, they would probably not earn money on every trial. Since participants were rewarded (and paid) depending on their performance, they were motivated to perform a fresh decision on each trial and also to respond quickly. To avoid contamination of ocular movements at cue presentation and to have a variable inter-trial interval (ITI) in the last ten subjects, after the presentation of the feedback, a black screen was presented for a variable interval between 500 and 1500 ms, followed by a fixation cross at the location where the next marble would appear for a variable duration between 500 and 1500 ms, both in the Act or Inhibit task and the Colour Threshold task. In Figure 5 an example of exogenous Go trials is shown. Participants performed the two psychophysical tasks and one block of the Act or Inhibit task three times to obtain a stable performance and to familiarize with the Act or Inhibit task that was subsequently used for the TMS-EEG sessions.

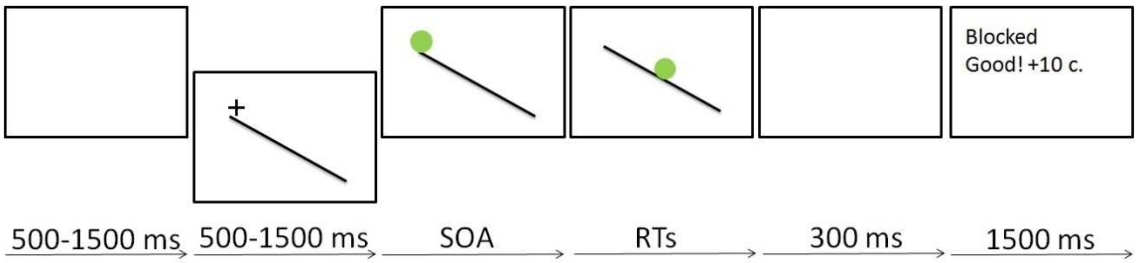

Figure 5 The trial structure is shown, using the exogenous Go condition as an example (for showing purposes colours are inverted, the background is black and the is white). The arrows at the bottom represent time intervals. In this example, a button press was performed while the marble was rolling down the tilted plane at the threshold speed. A black screen was presented subsequently for 300 ms, during which late responses are detected. On each trial, the marble was coloured in one of the 15 possible shades of colours.

TMS-EEG sessions

During each TMS-EEG session, participants wore an EEG cap where the electrodes were positioned. We first used TMS to measure subjects' resting motor threshold (rMT), defined as the lowest TMS intensity necessary to elicit visible twitches of the right index finger on five out of ten consecutive trials. A repeated measure ANOVA was used to verify that the rMT did not significantly vary across days ($p=0.3$). For the last 9 subjects, we measured the rMT again at the end of the experimental session, to check for changes in cortico-motor excitability due to the TMS stimulation, and we found no significant differences for the three cortical sites we stimulated: dFMC ($t(8)=-0.299$, $p=0.773$), rIFG ($t(8)=-0.750$, $p=0.475$) and V1 ($t(8)=-0.832$, $p=0.429$). Subsequently, participants performed two blocks (120 trials each) of the Act or Inhibit task at baseline. They then rested while we applied a 20-min train of repetitive low-frequency (1 Hz) stimulation. Right after the end of the stimulation, participants performed the Act or Inhibit task for 15 minutes (three blocks), after which they rested for half an hour before executing another block to check for long-lasting effects. For a

subset of 10 subjects, we did a final block of Act or Inhibit task at 80 minutes after the end of the stimulation.

TMS pulses were delivered using a 70 mm figure-8-coil connected to a Magstim Rapid2 (Magstim Co., UK) and the stimulation intensity was set to 110% of their rMT. On each day of stimulation session, TMS was delivered over one of three hotspots determined using MRI-based neuronavigation (Brainsight, Rogue Inc), and the hotspots averaged across subjects are shown in Figure 6. We used the brain coordinates for the left dFMC (Brodmann Areas 9) from Kuhn et al. (2009) and for the rIFG from Aron et al. (2007) to guide our individually determined hotspots. Mean x, y, z MNI coordinates were -7, 42, 21 and 57, 18, 6 for the left dFMC and rIFG respectively (Jenkinson and Smith, 2001). The control early visual area V1 was also determined using the stereotactic system (mean x, y, z coordinates: -17, -104, -5). The order of stimulation was counterbalanced across participants.

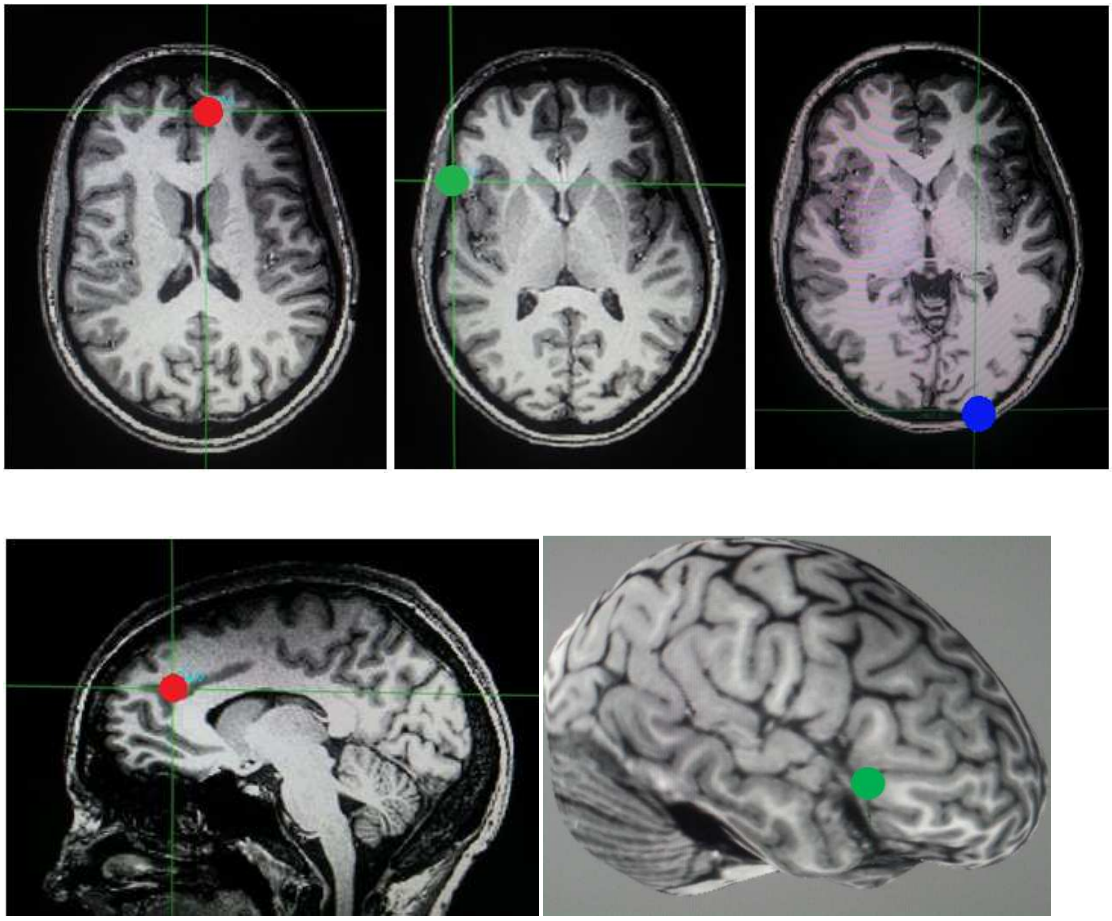

Figure 6 *Upper part,* The average stimulated areas are shown. From left to right: left dFMC, rIFG, left V1. Due to radiological conventions left is shown on the right side and viceversa. *Lower part,* on the left side the sagittal view of the left dFMC of one participants is displayed, whereas on the right side the rIFG from one subject is shown on the surface of reconstructed 3D brain. Data shown with permission.

Continuous EEG was recorded from 27 Ag/AgCl surface ring electrodes (Fp1, Fp2, F7, F3, Fz, F4, F8, Fc5, Fc1, Fc2, Fc6, T3, C3, Cz, C4, T4, Cp5, Cp1, Cp2, Cp6, T5, P3, Pz, P4, T6, O1, and O2) mounted on a TMS-compatible elastic cap, according to the extended 10-20 international system. The ground electrode was placed between the electrodes Cz and Pz, and since the embedded reference electrode was the right mastoid, the average activity of all the electrodes was used as a reference instead, in order to

avoid lateralization effects. Eye movement EOG were recorded with two electrodes placed above and below the right eye. The layout is shown in Figure 7.

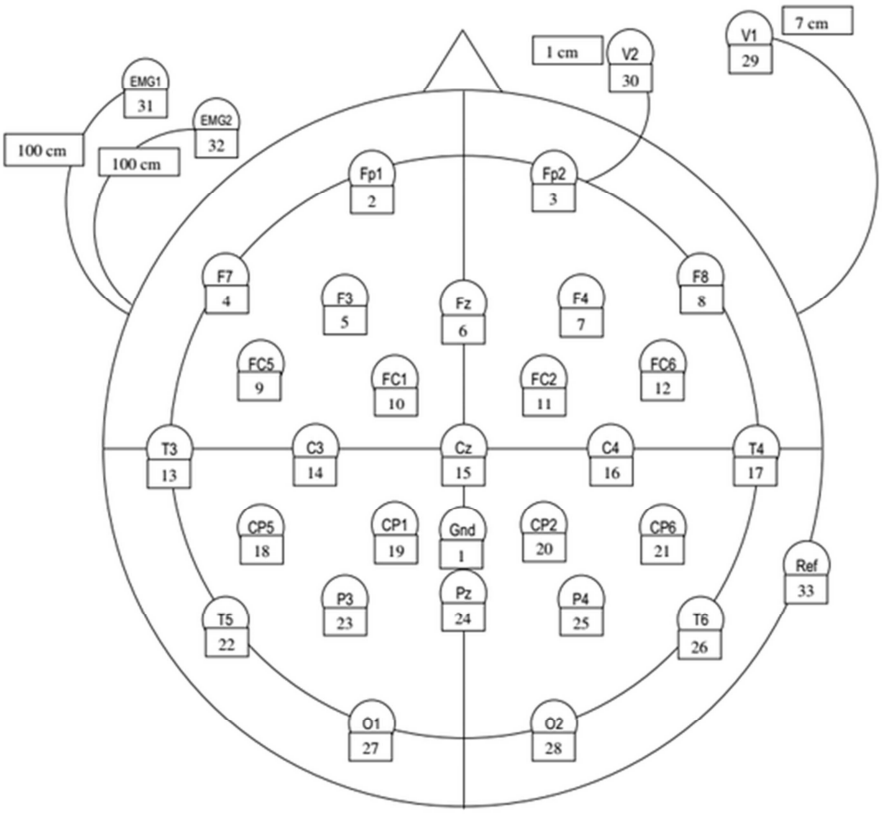

Figure 7 The figure shows the configuration of the electrodes mounted on the TMS-compatible EEG cap. EMG1 and EMG2 electrodes were not used.

The EEG was amplified and recorded with a full-band DC-EEG system (neuroConn GmbH, Llmenau, Germany) with a sampling rate of 2048 Hz. An indirect measure of impedance was calculated as a function of DC-offset which was set at less than 8µV with a standard deviation of less than 5µV over a 25 second period. A high pass filter with a frequency cut off of 0.001 Hz was applied, as well as a notch filter (band-stop 50 Hz). Continuous data were visually inspected and noisy portions of the continuous recording were removed before applying Independent Component Analysis (ICA) (Bell

& Sejnowski, 1995). Independent components related to blinks, saccades and muscle artefacts were visually detected and removed.

Behavioural analysis

On the Act or Inhibit task, we recorded whether a button press was performed in the allotted time, and whether a late or no response was given. We excluded the trials where participants pressed the button during the stimulus onset asynchrony (SOA), before the ball started to roll down the plane. Response times (RTs) shorter than 100 ms were also excluded. We used a repeated measure ANOVA to test TMS-induced effects on the RTs with Bonferroni post-hoc correction, including both on-time and late responses. The number of given button presses was recorded separately for the three conditions (Exogenous Go, Exogenous NoGo and Endogenous Go/NoGo) and an analysis on the relative frequencies of the given responses before and after each TMS stimulation condition was conducted using the nonparametric Friedman test, with Wilcoxon post-hoc analysis. Since we compared relative frequencies among conditions, the normality assumption of the ANOVA could not be tested, therefore a nonparametric test was used instead.

EEG data analysis

The software EEGLAB (Delorme & Makeig, 2004) (http://sccn.ucsd.edu/eeglab/) was used for the preprocessing and the ERP analysys of the EEG data. One subject was excluded due to excessive noise and the analysis was conducted on the remaining 18 subjects. For the statistical analyses of the ERPs a repeated measure ANOVA with Bonferroni correction was used to compare the mean peak to peak amplitude of single

trials ERPs for each subject separately. Fieldtrip and ad hoc Matlab scripts were instead employed for the analysis of the frequencies. Spectral density estimation was performed using multi-taper method based on discrete prolate spheroidal (slepian) sequences (Percival and Walden, 1993; Mitra and Pesaran, 1999). We performed time-frequency analyses of the EEG time series for all sensors over a frequency band ranging from 6 to 42 Hz (in steps of 1 Hz) using 3 orthogonal tapers 0.5s in duration and 8 Hz of frequency resolution, each stepped every 0.02s. Cluster-based permutation tests, using the Monte Carlo method, were then performed on time-frequency data at the group level (Maris et al., 2007). Subsequently, a 3-way ANOVA was conducted to test at the single subject level the effects found in the cluster-based analysis, using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995) to account for the multiple comparisons problem.

3.3 Results

EEG results

Brain activity of participants was recorded before and after the rTMS session, while they were performing the Act or Inhibit task. The pre-TMS recordings of each day of stimulation served to analyze the differences across task conditions: Exogenous-Go, Exogenous-NoGo , Endogenous-Go and Endogenous-NoGo . The last two conditions were created separating, in the Endogenous Go/NoGo condition, the trials in which participants voluntarily decided to press the button (Endogenous-Go) from the trials in which subjects decided to inhibit the planned action (Endogenous-NoGo). Since in this condition participants pressed the button half of the times at baseline, each of the four conditions has roughly the same number of trials.

In the ERP analysis, we included all the pre-TMS recordings from the three days of stimulation for a total of 180 trials per condition. We tested whether the elicited brain activity was significantly different between the endogenous and exogenous condition by comparing the stimulus-locked ERPs recorded before the TMS stimulation across the four task conditions. Stimulus-locked epochs were computed using the presentation of the static ball (cue) as time-locking event and extended from -1000 ms to +4500 ms (baseline period between -150 ms and +50 ms). For the current analysis, trials with an SOA shorter than 500 ms were excluded, thus all trials within each condition were identical until 1200ms post-stimulus. During this timeframe, participants looked at the coloured marble and decided whether to press the button or not, while waiting for it to start moving. Stimulus-locked epochs were averaged across each condition for each subject. We focused our analysis on the temporal windows of the N200 and P300 components associated with response inhibition (Greenhouse & Wessel, 2013; Ocklenburg, Gunturkun, & Beste, 2011). We computed the spectral maps of the grand averages for each of the four conditions from 250 to 550 ms post-stimulus and found a peak of negative activity during these timeframes on channels Cz, Fz, Fc1 and Fc2 in all conditions. The mean amplitude of the evoked potentials for each electrode, condition and subject was measured in three temporal windows, each 100 ms long. A 3-way ANOVA with factors channel (Fz, Cz, Fc1 and Fc2), time (250-350, 350-450, 450-550) and condition (Endogenous-Go, Endogenous-NoGo , Exogenous Go and Exogenous NoGo) was computed, applying Bonferroni correction. Results show a significant effect of time ($F(2,16)= 11.828$, $p=0.001$) and condition ($F(3,15)= 13.185$, $p=0.000$), whereas no difference across the channels was found. Moreover, the channel*time interaction ($F(6,12)= 7.373$, $p=0.002$) and time*condition ($F(6,12)= 11.596$, $p=0.000$) interactions were significant, while the channel by condition interaction did not reach significance. Since there was no main difference across channels, the mean amplitudes

of the three electrodes were averaged together, separately for each condition and for each subject. We, therefore, created a unique measure of the intensity of the ongoing activity over fronto-central areas for each of the four conditions. We performed a one way repeated measure ANOVA and results showed significant differences across conditions starting from 350 ms post-stimulus (F=15,056, p=0.000 for the temporal window between 350-450 ms post-stimulus and F=24,154, p=0.000 between 450-550 ms post-stimulus). In the 350-450 temporal window, a Fisher's LSD post-hoc test showed significant differences between the Exogenous-NoGo and all other conditions, of particular interest the comparison with the Endogenous-NoGo condition (p=0.001). We found a similar result in the temporal window 450-550 ms after stimulus presentation (p=0.000), and a significant difference between the two Endogenous conditions (p=0.002), whereas the mean amplitude of the selected electrodes between the two Go conditions did not show any significant difference for any temporal window (p=0.592 and p=0.515, for 350-450 and 450-550 intervals respectively). Data are depicted in Figure 8.

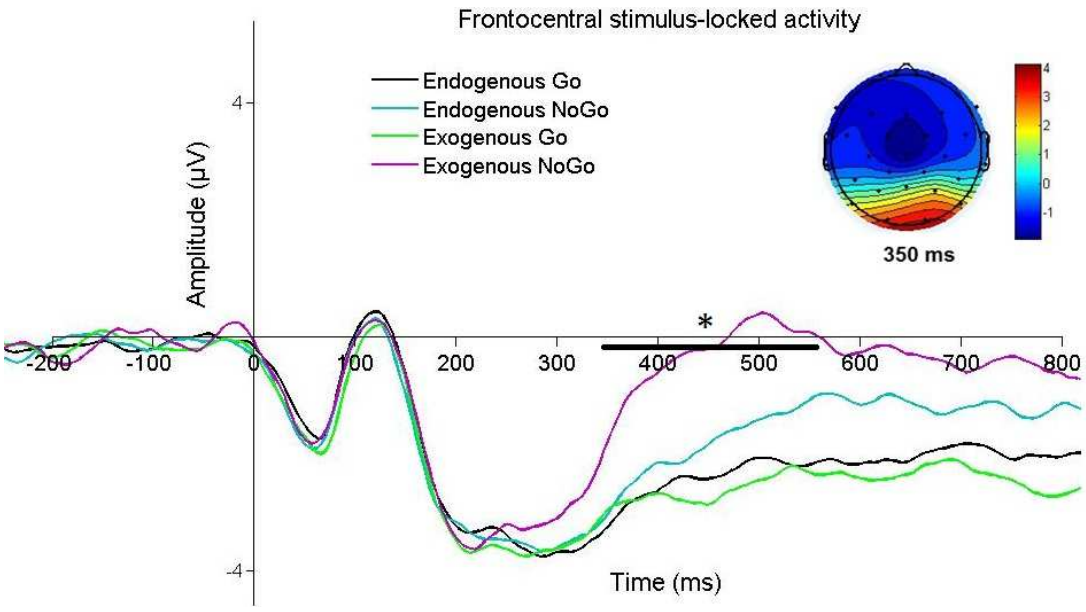

Figure 8 This figure shows the stimulus-locked ERPs from the four conditions, as recorded before the TMS stimulation. Time zero represents the time at which the marble is presented on

top of the tilted plane and participants are waiting for it to start moving. Time (ms) is shown on the X-axis, while the Y-axis represents the amplitude (in μV) of brain activity averaged from electrodes Cz, Fc1, Fc2 and Fz (positive is up). A low-pass filter with cut-off 35Hz was applied. The black line indicates the temporal window in which significant differences were found (350-550 ms). The asterisk signals a $p<0.05$. At the top right a spectral map shows the distribution of the electrical activity on the scalp at 350 ms post-stimulus with positive and negative voltages represented in red and blue, respectively.

To test whether TMS modified the amplitudes of the ERPs, the average amplitudes of the four electrodes were included in two 2-way ANOVAs, for the time intervals 350-440 and 440-550 ms, with factors task condition (Endogenous-Go, Endogenous-NoGo , Exogenous Go and Exogenous NoGo) and stimulation (preTMS, dFMC, IFG and V1). Bonferroni post-hoc correction was applied. In both cases, the effect found on pre-TMS data was replicated, with a significant effect of the task condition ($F(3,51)= 29.699$, $p=0.000$ for the 350-440 ms interval and $F(3,51)= 42.482$, $p=0.000$ for the 440-550 ms temporal window), while we found no effect of stimulation. In response-locked ERPs (where time 0 is the button press) we compared the evoked activity between Endogenous and Exogenous Go trials, but no significant differences were found.

For the time-frequency decomposition cue-locked epochs were taken from -1500 to 2000 ms, with a baseline period ranging from -500 to -100 ms. Because for the time-frequency analysis the baseline period has a greater impact on the estimated power, only the last 10 subjects were included in this analysis, since they were presented with variable ITIs. Cluster-based permutation tests (500 permutations) were then performed on the grand-averages to check for differences across task conditions, by applying paired samples t-tests. Again, trials with SOA shorter than 500 ms were excluded from the analysis. The comparison between Go and NoGo conditions revealed several significant clusters in fronto-central electrodes for both the internally-driven and the

externally-triggered condition. In Figure 9 electrode C3 is shown with significant clusters ($p<0.05$) for the Go-NoGo difference in the Endogenous (left) and Exogenous condition (right). A greater power of the slow delta frequencies is present in the Go conditions, with respect to the NoGo conditions along with a greater negativity in the alpha and beta bands, ranging from 0.5 to 1.5 seconds post-cue.

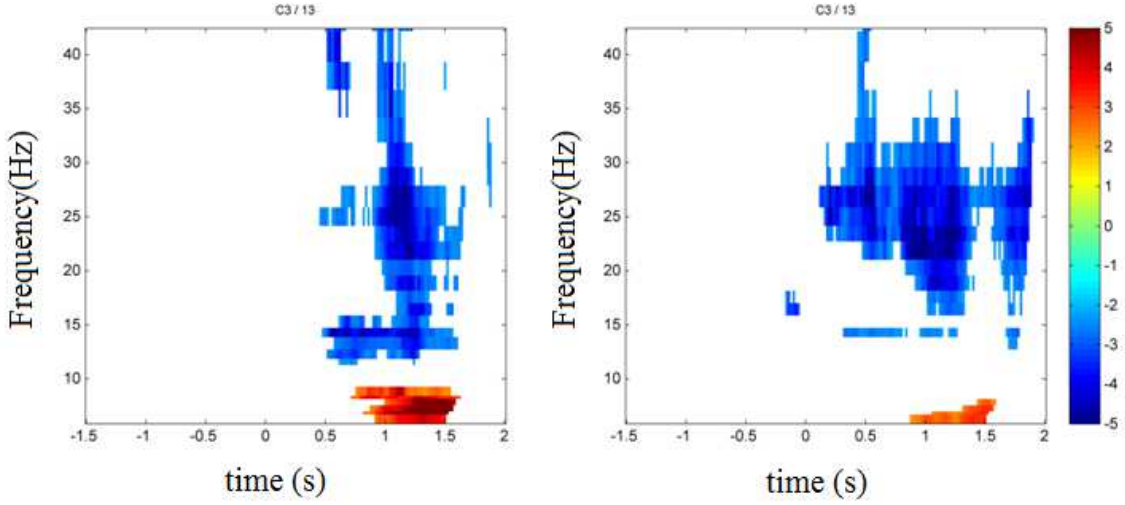

Figure 9 The difference between the time–frequency representations (TFRs) of Go and NoGo trials, masked by the spectral–temporal pattern of the significant cluster. Endogenous and Exogenous trials are shown on the left and right panel, respectively. Red denotes a positive and blue denotes a negative raw effect. The TFRs are shown for the frequency range [6 Hz, 42 Hz] and the time interval [-1.5 s, 2 s].

The difference between the internally-driven and externally-triggered Go condition was significant, as displayed in Figure 10. Several electrodes show positivity in the alpha and lower beta bands, but interestingly no effect is found for electrode C3, indicating that the two conditions did not differ at the level of motor execution. Electrode Cp5 is shown as an example (Figure 10) where a relative increase in the alpha power is visible between 0.6 and 1.2 seconds after cue presentation.

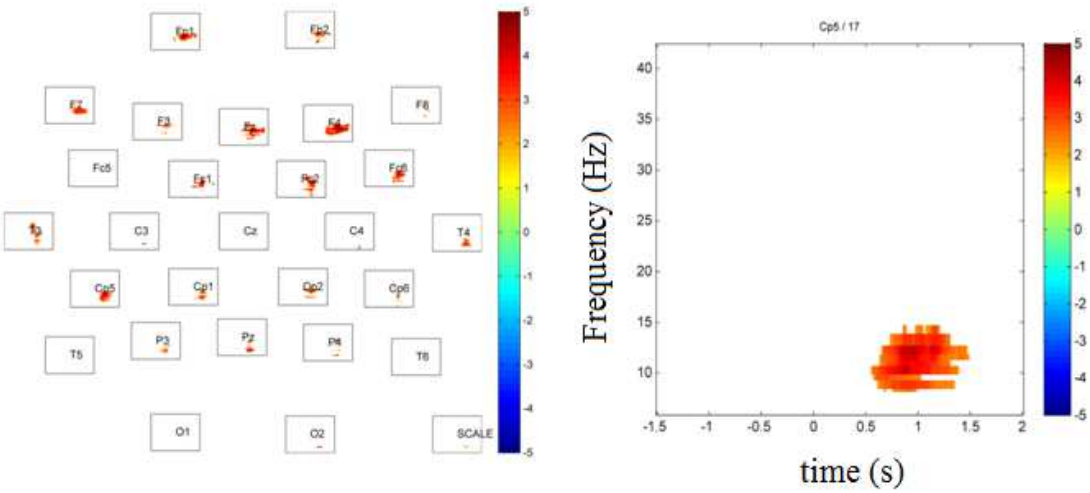

Figure 10 Difference between the time–frequency representations (TFRs) of Endogenous Go and Exogenous Go trials, masked by the spectral–temporal pattern of the significant cluster. On the left side, all the electrodes are shows, with red blobs representing significant clusters. On the right side, electrode Cp5 is displayed. Red denotes a positive and blue denotes a negative raw effect. The TFRs are shown for the frequency range [6 Hz, 42 Hz] and the time interval [-1.5 s, 2 s].

Results indicated that the time-frequency representations of the two NoGo conditions were not significantly different. To test whether these group effects were present at the single subject level, and moreover if they were modulated by the TMS, the time-frequency representations (TFRs) of each subject were Z-scored with respect to the time interval -0.5 -0.1 s. We then conducted a 3-way ANOVA with factors level of intentionality (internally-driven vs. externally-triggered), type of trial (Go vs. NoGo) and stimulation (preTMS, dFMC, IFG and V1), for the alpha and beta frequency bands. We focused our analysis on the temporal window between -0.5 to 1 s relative to cue presentation, since the *whether* decision is likely to happen within the first 600 ms from cue presentation, although effects on the frequency power might be visible a few hundred milliseconds later. The alpha band failed to show significant results, whereas a significant effect of the type of trial was found in the beta band, particularly for electrodes C3 and Cp5. In Figure 11, the difference between Go and NoGo TFRs is

shown, using electrode C3 from the exogenous condition as an example, however the same difference is also present for the Endogenous condition. No other effects were significant.

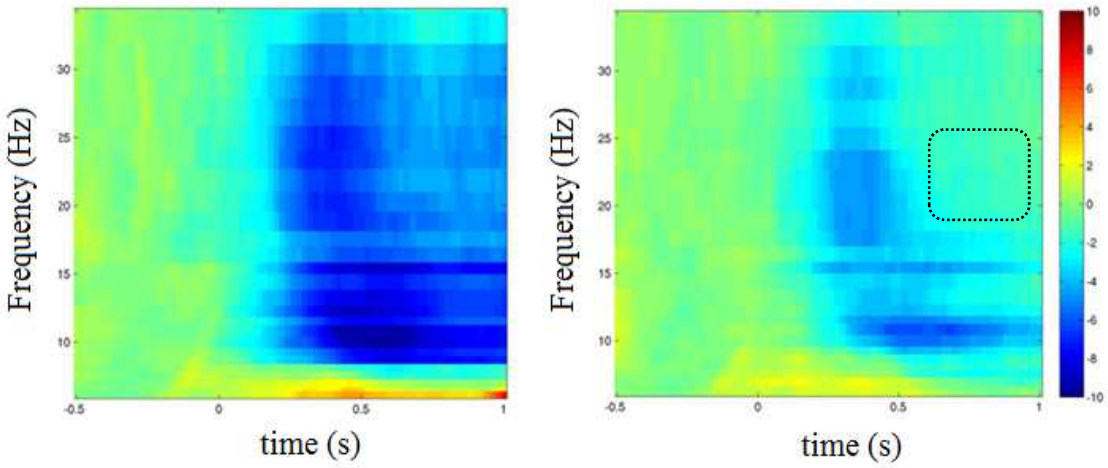

Figure 11 Grand-averages of the cue-locked TFRs for the Exogenous Go (left panel) and NoGo (right panel) conditions are displayed. On the x axis time is represented (from -0.5 to 1 second), whereas frequencies are shown on the y axis. The area surrounded by the dashed line on the right panel shows the time points and the frequencies showing a difference in power between the two conditions ($p<0.05$ after accounting for the false discovery rate). Red and blue colours represent a relative increase (ERS) and decrease (ERD) in power, respectively.

Behavioral effect of TMS

Low frequency rTMS should temporary inhibits activity of the stimulated brain areas and provides a direct mean to test whether the dFMC and rIFG are necessary to inhibit planned actions. To determine whether rTMS modified participants' ability to inhibit the urge to press the button, and therefore to interrupt the planned action, we measured the ratio between the frequency of button presses participants performed before and after rTMS for the three task conditions (Exogenous-Go, Exogenous-NoGo and Endogenous-Go/NoGo), relative to the total number of trials per condition. If, in fact, the dFMC and rIFG are causally involved in action inhibition, whether endogenous or

exogenous, we should expect participants to be less able to inhibit action plans after stimulation, hence performing more button presses relative to baseline. An additional analysis was conducted on response times (RTs) to check for difficulty differences across conditions and TMS-induced modulation of impulsivity that could explain the behavioral results.

Frequency of response

We first measured the number of button presses participants performed when the ambiguous color was presented (Endogenous Go/NoGo condition) and divided this number by the total number of trials. We hypothesized that the dFMC would be causally involved in the endogenous inhibition of actions, therefore the inhibition of the activity of this area through TMS could have temporarily disrupted and reduced the ability of participants to prevent the execution of a prepotent action plan, when they could voluntarily decide whether to perform the action or not. For this reason, we expected an increase in the number of given button presses in the Endogenous-Go/NoGo condition only. Since the role of the rIFG in the endogenous inhibition of action has never been directly investigated, we had no clear prediction for the outcome of the stimulation. To measure the after-effect of the TMS, for each rTMS session we normalized the frequency of the button presses executed in the testing phase after stimulation relative to baseline. A Friedman test was employed to check whether the six post-TMS blocks (2 blocks for each day of stimulation: immediately and 50 minutes post rTMS) were significantly different relative to baseline. Results showed a significant effect ($p=0.000$, $df=6$) of TMS on the relative frequencies of button presses. To further explore which TMS stimulation(s) was yielding the difference, we used the Wilcoxon pairwise comparisons between baseline and post-TMS blocks, across the three stimulation sites

(dFMC, rIFG and V1). Only performance after stimulation of the dFMC was significantly different from baseline (Z=-2.575, p=0.01) clearly indicating a significant increase in the number of button presses during the Endogenous condition only, as shown in Figure 12.

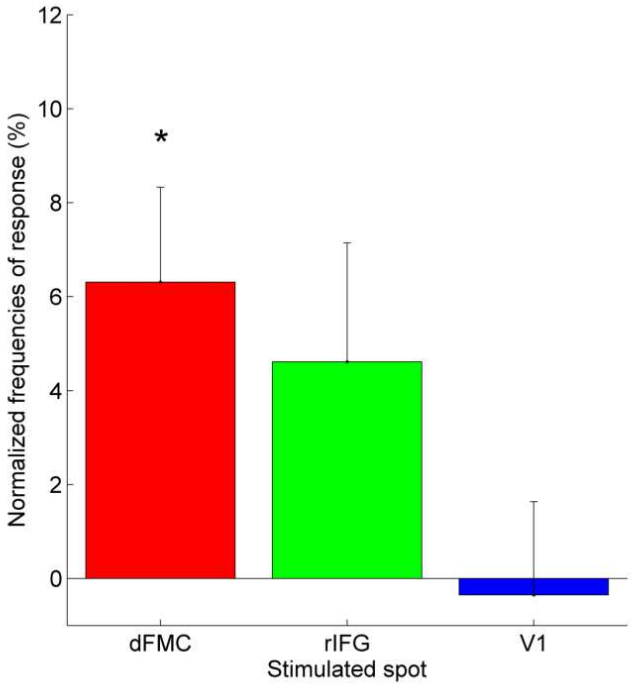

Figure 12 Response frequencies after rTMS during the Endogenous Go/NoGo condition. Normalized frequencies of button presses after rTMS over dFMC (red bar), rIFG (green bar), and V1 (blue bar). Positive numbers represents an increase in the number of given button presses, relative to baseline (0 on Y-axis), whereas negative numbers indicate a reduction in the number of button presses. Error bars represent the standard error. Significant effects ($p<0.05$) are indicated by an asterisk.

To check whether this effect was specific for the Endogenous condition, the same analysis was carried out on the Exogenous trials and, interestingly, no significant effects were found for either the Exogenous-Go (p=0.236) nor the Exogenous-NoGo trials (p=0.440). Results are shown in Figure 13.

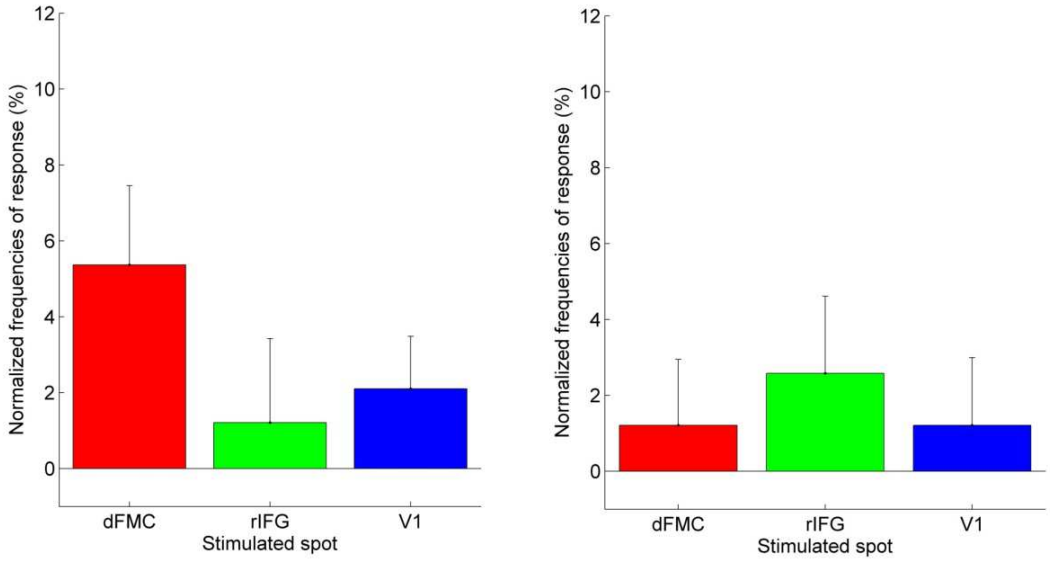

Figure 13 Response frequencies after rTMS during the Exogenous Go (left) and NoGo (right) conditions. Normalized frequencies of button presses after rTMS over dFMC (red bar), rIFG (green bar), and V1 (blue bar). Positive numbers represents an increase in the number of given button presses, relative to baseline (0 on Y-axis), whereas negative numbers indicate a reduction in the number of button presses. Error bars represent the standard error. Significant effects ($p<0.05$) are indicated by an asterisk. On the left side results from Exogenous-Go (green) trials are presented, on the right side data from Exogenous-NoGo trials are depicted .

Time Course of the Effect

To check for long lasting effects, we subsequently tested for the duration of the post rTMS effect by comparing baseline performance with the blocks recorded 50 minutes after the end of the stimulation and, surprisingly, we found significant differences for both the dFMC stimulation ($Z=-3.179$, $p=0.001$) and the rIFG ($Z=-2.374$,$p=0.018$). No significant differences were found for the control stimulation condition. Data are shown in Figure 14. For completion, data from the exogenous conditions are presented in Figure 15.

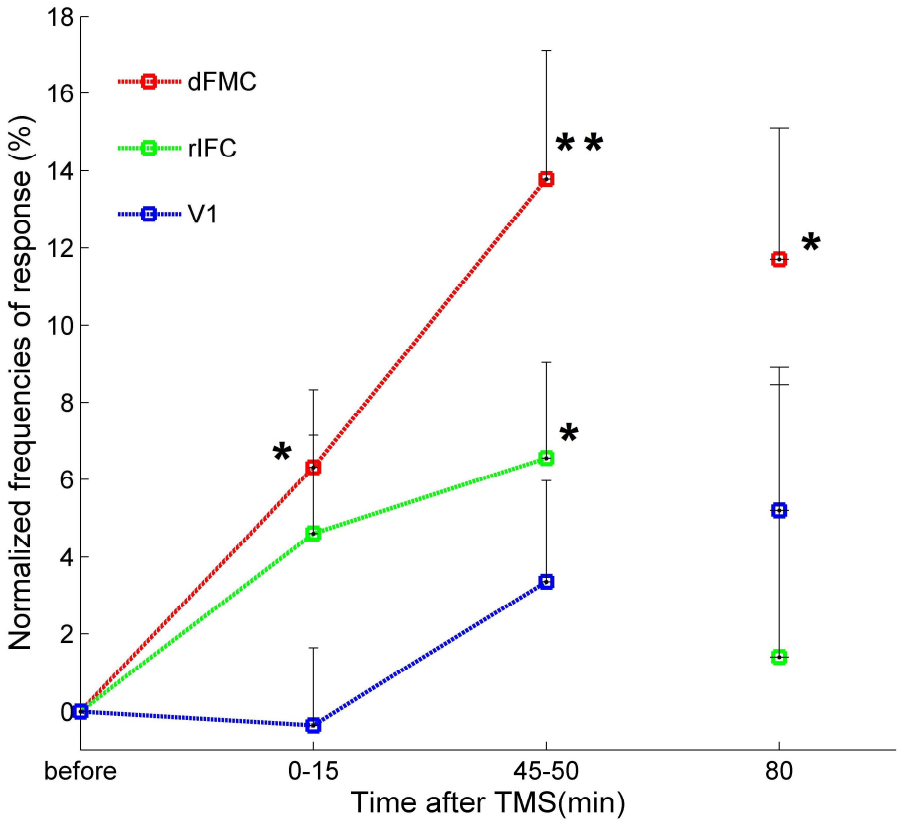

Figure 14 Response frequencies recorded at different time intervals after TMS during the Endogenous Go/NoGo condition. Normalized frequencies of button presses after rTMS over dFMC (red bar), rIFG (green bar), and V1 (blue bar). Positive numbers represents an increase in the number of given button presses, relative to baseline (0 on Y-axis), whereas negative numbers indicate a reduction in the number of button presses. Error bars represent the standard error. Significant effects ($p<0.05$) are indicated by an asterisk. Data for the 80-min time points are from the last 10 subjects.

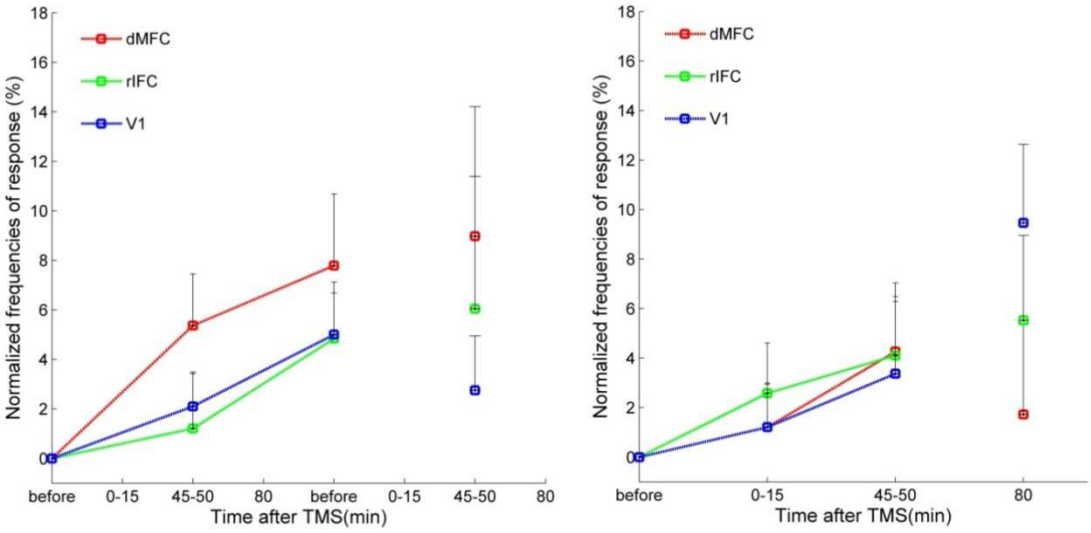

Figure 15 Response frequencies recorded at different time intervals after rTMS during the Exogenous Go (left) and NoGo (right) conditions. Normalized frequencies of button presses after rTMS over dFMC (red bar), rIFG (green bar), and V1 (blue bar). Error bars represent the standard error. Significant effects ($p<0.05$) are indicated by an asterisk.

As shown in Figure 14, the effect of the stimulation on the left dFMC led to an increase in the percentage of given button presses in the Endogenous-Go/NoGo condition, effect that was still present 50 minutes after the end of the stimulation. To better assess the duration and decay of the effect over time, in the last 10 subjects we also recorded another block of the Act or Inhibit task 80 minutes from the end of TMS. We performed the statistical analysis on these last 10 subjects including this last block. The comparison among response frequencies to the Endogenous Go/NoGo condition across the blocks yielded significant differences ($p=0.011$, $df=9$). The Wilcoxon pairwise comparisons between baseline and the fifth block for the three stimulated site showed significant differences only for the dFMC stimulation ($Z=-2.499$, $p=0.012$), whereas no effect was found for the rIFG ($Z=-0.358$, $p=0.721$) nor for V1 ($Z=1.428$, $p=0.153$) stimulation. Thus the effect of TMS over the dFMC was still present 80 minutes from

the end of the stimulation, while we found no effect for rIFG nor for V1 stimulation. In the Exogenous Go and NoGo conditions we did not find any significant differences.

Response Times

To test whether the Endogenous condition was generally more difficult than the Exogenous condition, we compared mean Response Times (RTs) at baseline between the two conditions. A paired t-test showed no significant differences ($t(18)=-0.674$, $p=0.509$). Additionally, an analysis on RTs was conducted to test whether the effect we found on the Endogenous-Go/NoGo condition was due to a general increase in "readiness" and impulsivity. For this analysis, we divided pre and post-TMS blocks, averaging together the RTs of the four post-stimulation blocks, separately for each stimulation site. A repeated measure ANOVA was employed to test the effect of the TMS on the speed of response. Results showed no significant differences ($p=0.410$). An analysis on the effect of the different SOAs on the RTs for the Exogenous Go condition is reported in the appendix.

3.4 Discussion

This study investigated the physiological basis of action inhibition using low frequency rTMS to directly interfere with cortical areas we hypothesized to be involved in action inhibition. To this aim, we compared, within the same participants, externally-triggered (exogenous) and internally-driven (endogenous) Go/NoGo conditions, the latter being a task that required participants to choose whether to perform an action or not. To our

knowledge, this is the first study to address, with a psychophysical paradigm, the causal role of the dFMC while subjects freely choose whether to act or not.

As previously anticipated in the introduction, when investigating the endogenous inhibition of actions, there are three factors that should be carefully considered and that were not addressed in previous studies: participants should not be able decide before the beginning of the trial whether they will perform the action or not; the decision should not be influenced by the stimulus or the feedback expectation; finally, subjects should have a reason to choose whether to act or not. A first attempt to provide participants with a reason to either execute the action or withhold it was made by Kuhn and colleagues (2009) using an aversive condition (a glass breaking sound) whenever subjects decided not to inhibit an action (to stop the marble rolling down a tilted plane). However, this factor did not guarantee that participants decided whether to execute the action before the trial initiated, an essential condition for a purely internally-driven condition. Other fMRI studies have attempted to design experimental paradigms to compare, in the same group, brain activations related to internally and stimulus-driven inhibitions (Schel et al., 2014; Hughes et al, 2011). In particular, the voluntary condition involved, in one case, (Hughes et al., 2011) a free choice between left and right key presses, interrupted on some trials by a stimulus instructing participants which hand to use, and in the other case (Schel et al., 2014), a modified version of the task employed by Kuhn and colleagues, which they compared to a stop signal paradigm. In our study, the endogenous condition was represented by the ambiguous colour resulting from the staircase procedure embedded in the Colour Threshold task. Aside from this colour, participants were presented with 14 other shades of coloured marbles, 7 green and 7 magenta. Some of these shades were very similar to the ambiguous colour; this way, participants were less likely to learn the correct stimulus-choice pairing, performing a

fresh decision on each trial. The presentation of the colours was randomized, so that subjects could not predict whether they were going to execute the planned action or not, therefore deciding at the time of cue onset (static colored marble). Finally, by manipulating the reward in the endogenous condition (50% chance to be rewarded), we prevented participants from being influenced, in their decision, by the feedback expectation.

Another issue that has to be considered in a Go/NoGo task is whether the subjects planned the motor response in advance or not. This is a crucial point, since the type of action inhibition under investigation would change depending on the time at which the action plan is interrupted (as described in the introduction). In the Act or Inhibit task, subjects were paid a different amount depending on their performance to the task: they received a reward after an on time response to a Go trial, or a no-response to NoGo trials, while they had 50% chance to receive money on the Endogenous Go/NoGo condition. Our preliminary psychophysical task (Speed Threshold task) warranted that participants could only press the button on time, if they were ready to respond during the Act or Inhibit task. This manipulation likely forced them to always plan the motor act and to inhibit it, if necessary. This hypothesis is also supported by the TFRs of Go and NoGo conditions previously shown in the result section. In fact, we only found significant differences between these conditions in the beta bands in the temporal window between 0.8 and 1 s after cue presentation. Alpha and beta negativity (ERD) has been associated with motor preparation (Deiber et al., 2012; Lew, Chavarriaga, Silvoni, & Millán, 2012), and as it can be seen from the TFR of the NoGo condition (right panel of Figure 11), alpha and beta ERD is present from 250 ms after cue presentation. In fact, in the first 800 ms from cue onset the power of alpha and beta frequency bands did not differ between Go and NoGo trials. The presence of an alpha-

beta power reduction early after presentation of the cue, which drove the decision to act or inhibit (endogenously or exogenously), suggests that participants always prepared a motor response every time a marble was presented, and later inhibited it on NoGo trials (reduction in ERD on NoGo trials, as shown in Figure 11).

Since the task we employed has never been used before, we wanted to characterize the brain response to the different conditions and, to this aim, we compared the evoked electrical activity across trial types. Differences in the cue-locked activity elicited by the different task conditions at baseline confirmed our expectations. The electrical activity over fronto-central electrodes showed no difference between the two Go conditions, but significantly higher amplitudes of event-related potentials elicited in the Exogenous-NoGo (externally-triggered) relative to the Endogenous-NoGo (internally-driven) condition, starting from 350 ms after stimulus onset, extending for an interval of 200 ms. The temporal window during which we found the difference is compatible with decision-making processes or motor inhibition mechanisms, which might differentially modulate the activity of fronto-central areas depending on the cortical locus of the decision to inhibit. The ERPs results contribute to demonstrate that the Exogenous/Go-NoGo trials were significantly different than the Endogenous/Go-NoGo condition. Thus our findings are in agreement with the 'common path theory' (Astor-Jack and Haggard, 2005) that predicts neurophysiological differences between endogenous and exogenous NoGo trials, without any differences between the two Go conditions. The differences we found at baseline between the ERPs elicited in the different task conditions was also replicated in post-TMS data, irrespectively of stimulation site. In the time-frequency analysis, we initially found a reduced negativity of the alpha band in the Endogenous Go condition, relative to the Exogenous Go condition (Figure 10), but this effect was not supported by the single subject analysis. The common path theory is also supported

by the lack of difference in the amplitude of response-locked evoked potentials between the two Go conditions (data not shown).

By employing low frequency rTMS, we expected to temporarily impair the activity of the stimulated brain areas. Therefore, since the dFMC has been suggested (Kuhn et al., 2009) to be the 'veto area', that is the area responsible for the implementation of the internally-driven decision to inhibit an action, we expected to elicit a disinhibition of responses, with respect to baseline, on Endogenous Go/NoGo trials only. Results showed a significant increase in the response frequency after dFMC stimulation, immediately after the end of the stimulation and up to 80 minutes, indicating a long lasting sustained effect. This result suggests that the left dFMC is involved in the implementation of the decision to inhibit, when the decision is not influenced by external sources. Despite some variability across participants, no significant differences were found in the Exogenous Go and NoGo trials (Figure 15). One might argue that the increase in button presses to the ambiguous stimulus after TMS over dFMC might be due to a potential effect on colour perception, for instance participants might have pressed the button more often after TMS because they perceived the ambiguous colour as "more green", and this might have been an external factor to influence their decision. However, the lack of an effect of the dFMC stimulation on Exogenous Go and NoGo trials rules out this hypothesis. In fact, if subjects perceived colours differently after TMS, we should have also expected an increase in the response frequencies to Exogenous trials, but this was not the case. Finally, it could be argued that TMS might have caused a general increase in impulsivity (Bari & Robbins, 2013; Lansbergen, Schutter, & Kenemans, 2007) or motor readiness (Deecke, 1996) hence the subjects pressed the button more often. However, this is unlikely for two reasons: the behavioural effect was selective for the internally-driven condition only, and we found

no effect of TMS on RTs for the Endogenous Go trials and on the rMT (as stated at the beginning of the 'TMS-EEG sessions' section). Together, these data suggest that TMS did not simply increase the level of cortico-motor excitability nor it made participants more impulsive. Finally, one could also argue that the Endogenous condition was more difficult because the stimulus was harder to categorize and, that the dFMC might have been involved in conflict detection or resolution. However, the absence of significant differences in the RTs between the Exogenous Go trials and the Endogenous Go trials does not support this interpretation (Grinband et al., 2011).

The effect of offline TMS over the dFMC peaked at around 45 minutes after the end of the stimulation. We did not predict such long-lasting effect, but in order to better characterize the peak and, eventually, the decrease of the effect, we asked our last 10 participants to perform an additional block, recorded approximately 80 minutes from the end of the stimulation. At this time point we started to see a decrease in the number of responses to the Endogenous condition and, the effect was likely returning to baseline (Figure 14). The time course of the effect is particularly interesting and relevant, since, while it is well established that TMS can induce excitatory or inhibitory effects, depending on the frequency and intensity of the stimulation, it is still unclear how long the effect can last and if it changes depending on the area that has been stimulated (Berger et al., 2011). Experimental evidence from studies of the primary and secondary motor areas as well as of the parietal cortex, using 1Hz repetitive TMS have shown that the duration of the effect is approximately the same or less than the stimulation length (Robertson et al., 2003). However, it is worth noting that not many studies investigated potentially long-lasting effects of low frequency stimulation, what could be a marker of LTD and LTP effects (Nyffeler et al., 2006). Recently, there have been examples showing low frequency rTMS effects that last at least 30 minutes in

stroke patients (Agosta et al., 2014) and, some interesting effects in healthy subjects that increase exponentially as the number of minutes of stimulation are doubled (20 minutes instead of 10; for a review see Thut and Pascual Leone, 2010) or when theta burst stimulation is used (Nyffeler et al., 2006). Some recent studies have indeed found that TMS over the prefrontal cortex in one hemisphere caused an increase in BOLD response in the opposite un-stimulated hemisphere. The effect was likely compensatory as it was evident in the behavioral performance after stimulation (Lee and D'Esposito, 2012). Other TMS studies of the motor cortex have found similar compensatory effects (Strens et al., 2003). We can therefore speculate that the temporary de-activation of the dFMC might have caused changes in other areas part of the action inhibition circuit, such as the left IFG and the preSMA (Obeso et al., 2013) through neuroplasticity processes evolving across time.

The role of the rIFG has been related to exogenous action inhibition (Ditye et al., 2012) and we could expect an effect of the TMS on Exogenous NoGo trials, however we did not find any. In our experiment, TMS over rIFG only had a significant effect in the Endogenous Go/NoGo condition around 50 minutes from the end of the stimulation, and not before, a peak that immediately decreased, as tested 80 minutes post-TMS. The lack of an effect immediately after the end of the stimulation speaks against a role of the rIFG in endogenous action inhibition and one can speculate that this later effect could be attributed to rebound activations in other parts of the action inhibition circuit. It has been demonstrated, in fact, that TMS stimulation of the left IFG can generate an up-regulation of the homologous contralateral area measured through fMRI 45 to 60 minutes after the end of the TMS stimulation (Hartwigsen et al., 2013). While at the moment we don't have a clear explanation for this after effect with a delay, we could

hypothesize that different types of neurons might react differently to stimulation and show a delayed plasticity (Stefan et al., 2000).

The absence of effect of the rIFG stimulation on the Exogenous trials was rather surprising, but the role of the rIFG in action inhibition is greatly debated (Swick et al., 2011) and it is not clear whether the portion of the inferior frontal cortex that we stimulated (for a detailed functional topography see Cai & Leung, 2011) is indeed responsible for action inhibition. Another possible explanation relies in the nature of the task, which required participants to inhibit the response, either endogenously or exogenously, on around 50% of the trials (25% exogenous NoGo trials and roughly half of the endogenous trials). The rIFG has been mostly associated with reactive inhibition (Zandbelt et al., 2013) and it is possible that the features of our Go/NoGo task were not ideal to directly involve rIFG in the inhibition of planned responses.

Finally, rTMS over the control visual area V1 had no effect at any interval and conditions we tested. This null result further support the idea that the effect we found on the frequency of responses to the internally-driven condition was not caused by a general increase in arousal due to TMS and is not likely cause by a change in visual perception.

3.5 Conclusions

In this study we investigated the causal role of specific brain areas in internally-driven and externally-triggered actions. To this aim, we created a condition (Endogenous Go/NoGo) that allowed participants to decide, on each trial, whether to perform or inhibit a planned action. We manipulated reward contingencies to give participants a motivated reason to either execute or stop the action. rTMS was employed to directly

interfere with the activity of two brain areas hypothesized to be involved in action inhibition: the left dFMC and the right IFG. The former has been related to internally-driven action inhibition and the latter to the externally-triggered inhibition of actions. Therefore, by inhibiting the activity of these areas, we could expect to find a disinhibition of responses in the endogenous and exogenous condition, respectively. Instead we only found an effect of the dFMC stimulation in the Endogenous Go/NoGo condition, without an effect of the rIFG stimulation on Exogenous trials. Furthermore, the increase in the number of given button presses, after dFMC stimulation, was very long lasting, which is very intriguing because it might indicate that plastic changes caused by TMS can have potential interesting application. In particular, the results of our study might have implications for the treatment of impulse control in brain disorders (Aron et al., 2014). It will be interesting in future studies to investigate more in detail these enduring effects to also determine the physiological changes at the cortical level on the prefrontal circuits, by pairing TMS with fMRI or MEG.

Altogether, these results support the hypothesis of separate neuronal circuits for internally-driven and externally triggered actions. EEG data showed that the endogenous and exogenous conditions are processed differently by fronto-central brain areas. We demonstrated that the left dFMC is directly involved in the endogenous inhibition of actions, however future studies will be needed in order to clarify what other brain areas are part of the internally-driven action inhibition circuit.

Future studies should also further explore the role of the primary and secondary motor areas in action inhibition and, particularly, their responses for externally driven or externally triggered action inhibition.

4.1 Experiment 2: Investigating the time course of proactive inhibition

The vast majority of studies on action inhibition involve the presentation of a stimulus (e.g NoGo stimulus, stop signal) to which participants must quickly *react* by withholding the motor response. Under these conditions, subjects interrupt all motor outputs to make sure that the action is cancelled. Inevitably, the selectivity of the action inhibition is lost, a side effect of the quick reactive inhibition required by the experimental paradigm. However, as pointed out by Aron in 2011, "the number of scenarios requiring fast stopping, and especially stopping that has global effects on the motor system, is probably limited". In everyday life, we often need to selectively inhibit one action, while continuing to perform others. This selective inhibition can be triggered by some specific events, hence named *reactive selective inhibition*, or it can be proactively modulated depending on the contextual information. *Proactive control* (Cai, Oldenkamp, & Aron, 2011; Lo, Boucher, Pare, Schall, & Wang, 2009) is a top-down sustained inhibition modulated by internal and external factors, exerted by the brain while keeping goal-directed information active in working memory. This gating mechanism is supposedly activated when there is uncertainty about the identity of an upcoming stimulus (Niemi et al., 1981; Näätänen R**.**, 1970). For this reason, in experimental settings, experimental protocols usually provide carefully chosen cues or warning signals to manipulate the amount of information provided to participants in order to involve proactive inhibition to a different degree. For instance, Smittenaar and colleagues (2013) used fMRI to investigate the neuronal circuits of proactive selective inhibition, using a modified version of a task developed by Aron and Verbruggen (2008). In their task, the authors made a distinction between "informative cue" and "uninformative cue" trials. In the "informative" trials, the cue indicated the hand subjects needed to inhibit from executing a response, while in "uninformative cue" trials

subjects were not aware of which hand to inhibit on stop trials. They found a set of brain areas active both during proactive selective inhibition, which are usually found also in reactive global inhibition (right IFG, left SMA/pre-SMA). The cues or warning signals used in these paradigms modulate response times (RTs), probably by influencing either sensory/perceptual processes, action selection and/or motor processes (Fecteau and Munoz, 2007). This general state of alertness might, therefore, increase the probability to respond to an upcoming stimulus. It has been suggested that such cue-locked motor activations are automatic (Boulinguez, Jaffard, Granjon, & Benraiss, 2008). Top-down inhibition is needed to stop them and, this inhibitory activity likely originates in the prefrontal cortex in order to prevent the execution of cue-triggered unwanted motor responses (Jaffard et al., 2008). In a recent study, Boulinguez and colleagues (2009) investigated the time course of this gating mechanism, and they tested an uncertainty condition by mixing warned and unwarned trials in the same block. In warned trials, a non informative warning cue preceded the target by a variable time interval (stimulus onset asynchrony - SOA) and participants were asked not to respond to the cue, but to wait for the target to appear. While classical theories of inhibition (Näätänen et al., 1974) would always predict shorter RTs for cued vs. non cued trials, the authors found a paradoxical lengthening of RTs for cued, compared to noncued trials, when the SOA was shorter than 300 ms.

This result cannot be explained by classical theories that would always predict shorter RTs for cued trials (e.g., Posner, Cohen, & Rafal, 1982; Warner et al., 1990). In our experiment, we wanted, therefore, to test the hypothesis whether a proactive inhibition mechanism is automatically triggered when there is uncertainty about the target identity. We used single pulse TMS to record motor evoked potentials (MEPs) while participants performed warned and unwarned trials. Our aim was to compare different task

conditions (pure vs. mixed block, warned vs. unwarned trials) to determine whether they would differently elicit proactive inhibition. The warning cue should automatically trigger motor responses that, in turn, should be inhibited by proactive inhibition; however, this process is not active in pure unwarned trials. In the mixed block, the uncertainty regarding the identity of the upcoming stimulus on each trial (either warning signal or target on warned and unwarned trials, respectively) would activate proactive inhibition. We measured both RTs and MEPs to test whether the proactive inhibition mechanism modulates the levels of corticospinal excitability. We tested two SOAs (150 and 300 ms), a time interval from the beginning of each trial and the target to test how long it takes, to the proactive inhibition mechanism, to deactivate (Boulinguez et al., 2009). We expected longer RTs and smaller MEP amplitudes for the short, compared to the long SOA, in warned trials and in the mixed condition. Additionally, we expected differences between pure unwarned and mixed unwarned trials, suggesting a deployment of the proactive inhibition mechanism in the latter case.

4.2 Materials and methods

Participants

Twenty-seven right-handed university students (mean age 22.5 years; SD 3.8; 14 females) with no neurological or psychiatric impairments voluntarily participated in the study. Handedness was determined via a condensed version of the Edinburgh Handedness Inventory (Oldfield, 1971). All subjects provided written informed consent, according to the ethical standards of the Declaration of Helsinki. The study was approved by the ethical committee of the University of Trento. All participants passed the TMS safety screenings.

Task and Experimental procedure

Participants were asked to seat on a comfortable chair with their right arm relaxed on a pillow to test their resting motor threshold (rMT). Participants' motor evoked potentials (MEPs) were recorded from the *first dorsal interosseous* (FDI) and the *abductor digiti minimi* (ADM) of the right hand, using Ag/AgCl surface electrodes. TMS pulses were delivered to the controlateral primary motor area (M1) using a 70 mm Figure-8-coil connected to a Magstim Rapid2 (Magstim Co., UK) and MEPs were registered and measured using the software LabChart 7 (ADinstruments), applying a bandpass filter between 20 and 2500 Hz. The hand area was found by eliciting visible twitches on the contralateral hand muscles and the hot spot for FDI was assessed by measuring the rMT, indexed as the lowest intensity of stimulation necessary to elicit 5 MEPs of at least 50 μV out of 10 consecutive pulses. TMS stimulation intensity was then set to 110% of the rMT (mean stimulation output: 64% of the maximal intensity of the stimulator). Participants wore an elastic cap on which the position of the coil was drawn to make sure it was in same position throughout the experiment. Participants performed a *Speed Threshold task* (see below for a detailed description), during which no TMS was applied. Subsequently, they performed three blocks of *warned* and *unwarned* trials (see below for a detailed description), two of which contained the same type of trials (pure block design) and one had the two types of trials randomly mixed (mixed block design). The order of presentation of the three blocks was pseudorandomized across participants. During these blocks, single pulse TMS was employed to trigger M1 and record MEPs. Throughout the duration of each session, subjects seated on a comfortable chair at a distance of 57 cm from the computer screen, with their chin on a chinrest. The task was presented on a 22" Samsung 2233RZ LCD monitor running at 120Hz on a Windows 7 machine running Matlab 7.2 and Psychotoolbox 2.0 experimentation

presentation software. On each trial, participants were asked to abduct the right index finger to press the space bar of the keyboard placed vertically next to their arm (on the left side), with the keys facing the right hand. Participants were asked to keep the muscles relaxed until response, and to relax it again immediately after they responded. Trials in which noise (amplitude > 50 μV) was present in the temporal window from -100 ms to 0 (TMS pulse) were discarded from the analysis. The whole duration of the experiment was approximately 2 hours.

During the *Speed Threshold* task, on each trial, participants were presented with a white fixation cross at the top of a tilted plane, for 100 ms. The plane consisted of a white line, 0.35 thick and 27.8 long degrees of visual angle, with a luminance of 204.5 cd/m^2, tilted at an angle of 30 degrees, running from the upper left to the bottom right quadrant of the screen. After 100 ms, a white static marble (3.55° in diameter) appeared and started rolling down the tilted plane at a fixed velocity randomly either 50 or 200 ms from presentation. The marble was always presented at the top of the plane, at fixation, hence on the top-left side of the monitor. All stimuli were presented on a black background (0.2 cd/m^2). Participants were asked to wait for the ball to start moving and then abduct their right index finger to press the space bar as quickly as possible to stop the marble. A 3-up-1-down staircase procedure was employed to determine, for each participant, the threshold speed at which they were able to correctly stop the marble before falling off the plane on 80% of the trials. Subjects performed 5 training trials to become familiar with the task and adjust the keyboard at a comfortable distance from their hand, and a maximum of 100 valid trials. The task ended when the threshold speed was determined.

In the subsequent tasks, participants performed two types of trials, *Warned* and *Unwarned* trials, either in a pure block or mixed block design. The structure of the two trial types is shown in Figure 16.

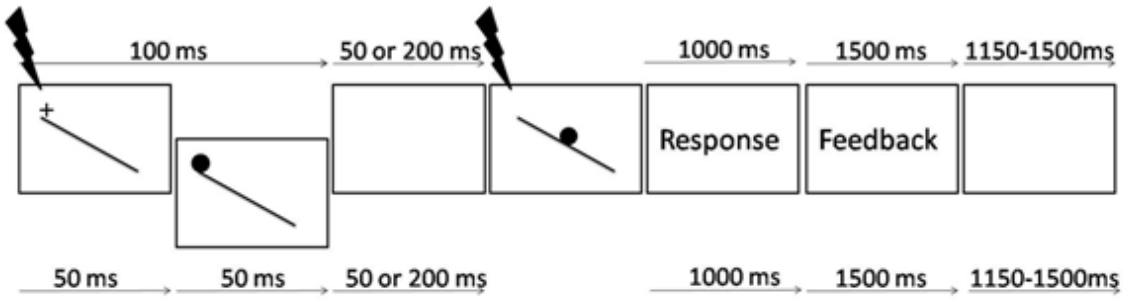

Figure 16 Trial structure (n the actual task, the background is black and the plane and marble are white). Top and bottom arrows represent unwarned and warned trials time intervals, respectively. On warned trials, the fixation cross is replaced, after 50 ms, by the cue (second image from the left), whereas on unwarned trials the fixation cross is displayed for 100 ms. Black thunder signs represent TMS pulses that could be delivered either at the presentation of the fixation cross (baseline) or at target presentation.

Each trial started with a white fixation cross on the top of the tilted plane (same as the Speed Threshold task) for either 50 ms on warned trials or 100 ms on unwarned trials. On warned trials, a white marble (same as the Speed Threshold task) was superimposed on the fixation cross for another 50 ms, representing the warn signal. After a randomly selected time interval of either 50 or 200 ms during which a black screen was presented, the marble appeared and immediately started rolling down the tilted plane at the threshold speed determined in the previous task. Participants were asked to keep the hand muscles relaxed, and then abduct the index finger as quickly as possible to press the spacebar and stop the marble. The ball started (target) after a randomly chose SOA of either 150 or 300 ms. After the target disappeared, a black background was displayed for 1000 ms during which late responses were recorded. The duration of each trial was

the same for warned and unwarned trials, and time between the appearance of the fixation cross and target presentation was always 150 or 300 ms, depending on the SOA. After the response, a feedback was shown for 1500 ms along with the text “Too early” if they responded before the target appearance, “Stopped” if they blocked the marble before falling off the plane or “Too late”, if they pressed the spacebar after the marble fell off the plane. The inter-trial interval (ITI) was randomly chosen between 1150 and 1500 ms during which a black background was presented. The total duration of each trial was variable (between 4400 and 5000 ms), depending on the SOA, the speed of the ball, the response times (RTs) and the ITI. On pure blocks, participants performed 8 training trials and 256 valid trials with a small break every 64 trials, in which subjects rested their eyes while keeping the head on the chinrest. On the mixed block, participants performed 16 training trials and 512 valid trials, with short breaks every 64 trials and a longer break halfway through the block During these trials, a TMS pulse was delivered to M1 once every two trials, in order to have enough time between two consecutive pulses (at least 8 seconds) and avoid additive effects of the pulses, and to have enough trials without TMS for RTs analysis. On trials in which the TMS pulse was delivered, the timing of the stimulation was randomly chosen between two possible time points: at the presentation of the fixation cross (baseline) or at target presentation.

Data analysis

The data in the mixed block were divided into warned and unwarned trials and analyzed separately. Therefore we compared, for each subject, RTs and MEPs across 4 task conditions: pure-warned (PW), pure-unwarned (PU), mixed-warned (MW) and mixed-unwarned (MU). Within each condition, trials were divided according to the SOA and, for the MEP analysis, according to TMS pulse time (baseline vs. target presentation).

Within the 256 trials per task condition (in the mixed block the 512 trials were divided between warned and unwarned trials), in half of the trials TMS was used with two possible SOA conditions. In total, there were 32 trials per SOA*TMS pulse condition and, for RTs, 64 trials per SOA within each task condition. For the RTs analysis, trials with TMS and with RTs shorter than 100 ms or in which a response was given during the SOA period were excluded. A 3-way ANOVA was conducted with factors SOA (150 or 300 ms), Block (pure vs. Mixed) and Cue (Warned vs. Unwarned), using Bonferroni post hoc correction. For the MEP analysis, the peak-to-peak amplitude was measured for each trial, and the number of clean trials per condition*SOA*TMS time was calculated for each subject. Due to the presence of noise in the pre-pulse recorded EMG in an excessive number of trials (leaving less than 5 clean trials per condition*SOA*TMS time), nine subjects had to be excluded and the subsequent analysis on MEPs was carried out on the remaining 18 subjects. Because of large intersubject variability, individual MEPs were Z-scored, including all MEPs during all the task conditions (see Davranche et al., 2007). MEPs amplitude from the FDI and ADM muscles were Z-scored and average amplitudes were then calculated for each subject, for each task condition. A 4-way ANOVA was conducted with factors (150 or 300 ms), Block (Pure vs. Mixed), Cue (Warned vs. Unwarned) and TMS time(baseline, target), with Bonferroni post-hoc correction. For all analyses the level of significance α was 0.05.

4.3 Results

Response Times

The 3-way ANOVA showed a significant effect of factors Cue ($F(1,26)= 151.542$, $p=0.000$) and SOA ($F(1,26)=122.004$, $p=0.000$), whereas no effect of the Block was found ($F(1,26)=1.162$, $p=0.291$). Significant results were found for the Cue by Block interaction ($F(1,26)=13.707$, $p=0.001$) and Cue by SOA interaction ($F(1,26)=16.044$, $p=0.000$), while the Block by SOA interaction was not significant ($F(1,26)=3.066$, $p=0.092$). The 3-way interaction Cue by Block by SOA was not significant ($F(1,26)=0.000$, $p=0.995$). To investigate the 2 way interaction Cue by Block, within each of the four task conditions, trials with different SOAs were collapsed and average RTs were recomputed. Two paired samples t-tests were conducted to compare unwarned trials between the pure and the mixed block and the same analysis was conducted for warned trials. A significant effect of the factor Block was only present on unwarned trials ($t(26)= -3.385$, $p=0.002$), which made RTs significantly longer for the mixed block than for the pure block, whereas no effect was found for warned trials ($t(26)= 1.049$, $p=0.304$). Results are shown in Figure 17.

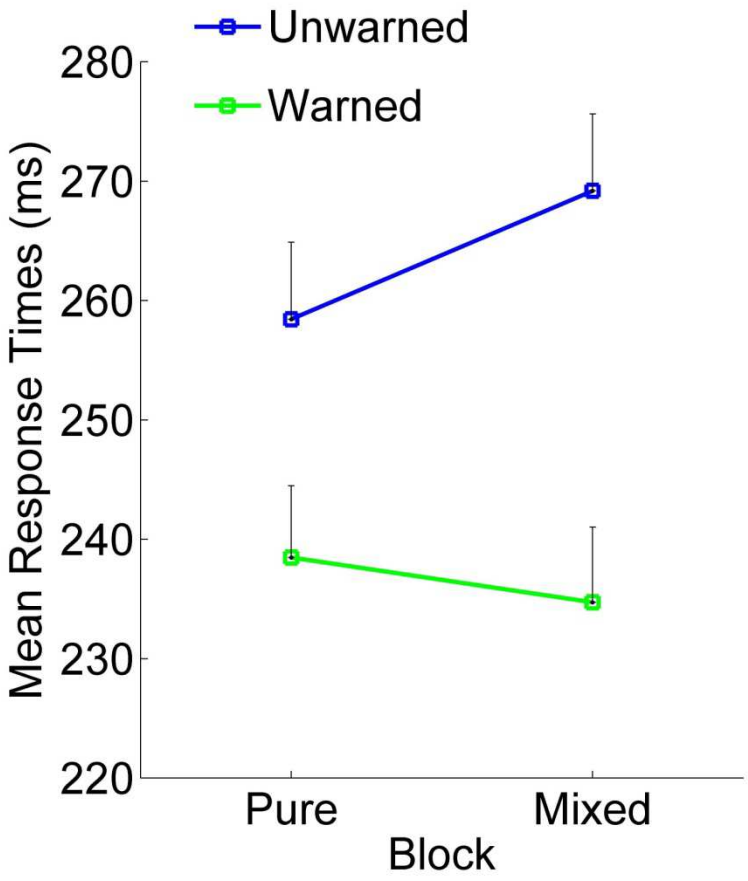

Figure 17 Mean RTs are shown for warned and unwarned trials separately. Error bars represent the standard error. A significant effect of the Block factor is present only for unwarned trials, with Pure Unwarned trials significantly slower than Mixed Unwarned trials. There was no difference across blocks for warned trials.

To explore the Cue by SOA interaction, we performed 4 separate paired samples t-tests comparing, for each Cue condition, mean RTs between short and long SOAs. Significant results were found for all the four conditions: warned pure trials ($t(26)= -7.989$, $p=0.000$), unwarned pure trials ($t(26)= -6.625$, $p=0.000$), warned mixed trials ($t(26)= -9.116$, $p=0.000$) and unwarned mixed trials ($t(26)= -6.009$, $p=0.000$). Mean RTs are always faster when the SOA is 300 ms than 150 ms. Results are displayed in Figure 18.

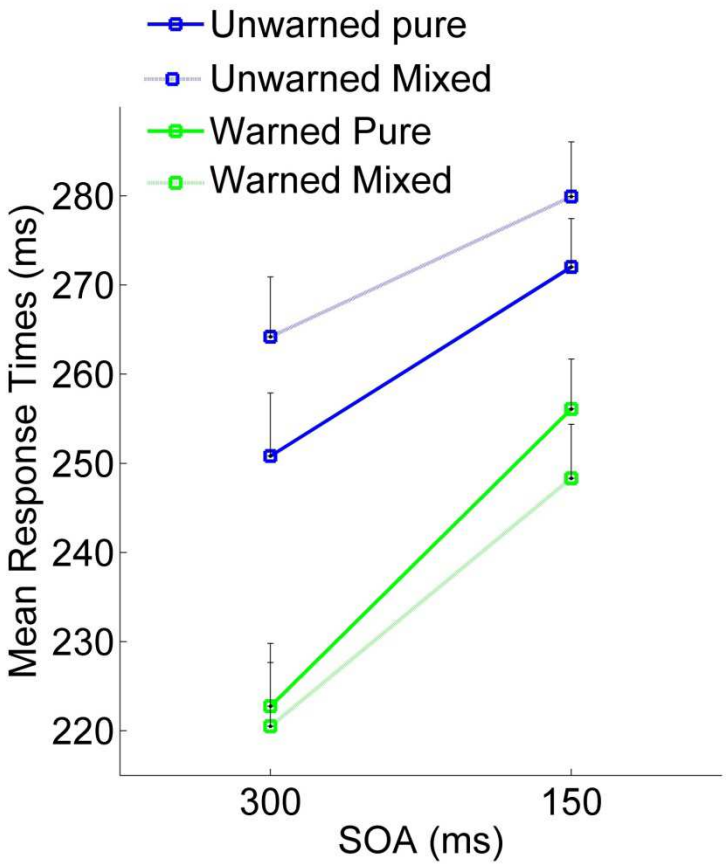

Figure 18 The figure shows mean Response Times for the four task conditions across the two SOAs. Mean RTs are faster in all conditions when the SOA is 300 ms. Error bars represent the standard error.

MEPs

The 4-way ANOVA for the control muscle ADM did not yield significant results for any of the four factors, whereas the same analysis on the mean MEP amplitudes recorded from FDI produced significant results for factors Cue ($F(1,17)=10.934$, $p=0.004$), TMS time ($F(1,17)=34.874$, $p=0.000$), SOA ($F(1,17)=8.126$, $p=0.011$), but not for factor Block ($F(1,17)=1.871$, $p=0.189$). Significant 2-way interactions were also found for Block by Cue ($F(1,17)=7.885$, $p=0.012$), Cue by TMS time ($F(1,17)=16.100$, $p=0.001$), Cue by SOA ($F(1,17)=23.030$, $p=0.000$) and a trend for TMS by SOA ($F(1,17)=4.423$, $p=0.051$). The 3-way interaction Cue*TMS time*SOA was also

significant (F(1,17)=7.824, p=0.012) and the Block*Cue*TMS (F(1,17)=3.467, p=0.080) showed a trend towards significance. The 4-way interaction was not significant. To determine the impact of TMS time on the amplitude of recorded MEPs we performed eight paired samples t-tests to compare, for each of the four task conditions, divided in two groups according to the SOA, those in which the TMS pulse was delivered at baseline vs. target appearance. Mean MEPs amplitudes are significantly higher at TMS time 2 (target) for conditions: Pure Warned SOA 300 (t(17)= -4.443, p=0.000), Mixed Warned SOA 300 (t(17)= -5.228, p=0.000) and Mixed Warned SOA 150 (t(17)= -2.849, p=0.011). All other conditions did not produce significant results.

To investigate the Cue by SOA and Block by Cue interactions, data recorded at baseline were separated from those recorded at target appearance. Two repeated measure ANOVAs were conducted (see Figure 19), one for each TMS time, to compare the eight conditions (four task conditions by 2 SOAs). Giving the high number of comparisons, Fisher's LSD post hoc test was used instead of Bonferroni correction, which would be too conservative in this case. When the TMS pulse was delivered at baseline (when the fixation cross was presented), the amplitude of the recorded MEPs did not differ across conditions (F(7,119)=0.779, p=0.503), whereas for MEPs recorded at target appearance, significant differences were found (F(7,119)=8.310, p=0.000). As shown in Figure 19 (right panel), MEPs amplitude was modulated by factor Block and SOA, only during warned trials. In particular, MEPs were significantly larger when the SOA was 300 ms with respect to 150 ms for both pure warned (p=0.002) and mixed warned (p=0.003) trials and the difference between pure warned and mixed warned trials was significant for both trials with SOA 300 ms (p=0.048) and 150 ms (p=0.024).

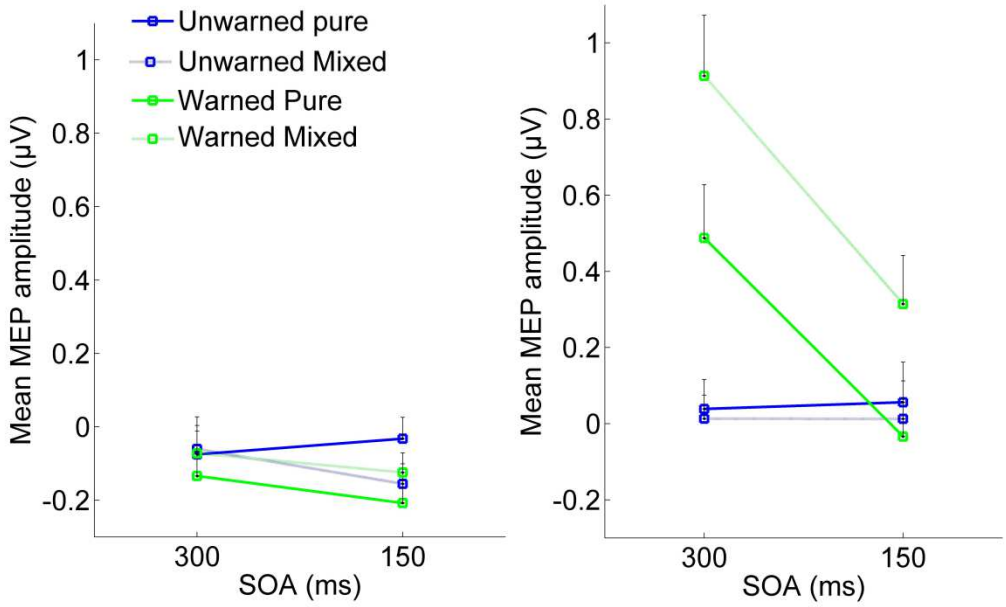

Figure 19 The Figure shows mean amplitude of MEPs recorded from the FDI muscle across the four task conditions (pure warned, pure unwarned, mixed warned, mixed unwarned), dividing trials according to the SOA. MEPs recorded at baseline (when the TMS pulse was delivered at the beginning of the trial) are shown on the left side, whereas MEPs recorded at target presentation are displayed on the right side. Error bars represent the standard error.

4.4 Discussion

In this experiment we explored the impact of warning cues on the speed of response to the presentation of a target. By comparing warned and unwarned trials, we were able to test the impact of the proactive inhibition mechanism on both RTs and MEPs. We found a significant increase in MEP amplitude for the FDI muscle at TMS time 2 (target presentation) for the mixed warned (both SOA conditions) and the pure warned (for SOA 300 ms) conditions (see figure 19). While the fixation cross (unwarned trials) had no effect on the amplitude of the MEPs recorded at target presentation, relative to baseline, cue presentation did have an effect. In fact, except from the pure warned condition with SOA=150 ms, the presentation of the cue always exerted an increase in

corticospinal excitability (higher MEPs at TMS time 2), as recorded from the FDI muscle.

According to Boulinguez and colleagues (2008, 2009), the presentation of a warning cue (static marble) should automatically activate a motor response which is then inhibited through top-down mechanisms that prevent the execution of anticipated responses (Jaffard et al., 2008). The contrast between MEPs recorded at target presentation vs. baseline allows us to test whether the presentation of the cue automatically triggers the motor response. One might say that the reason why in the pure warned condition (with SOA=150 ms) MEPs at TMS time 2 were not different than baseline is because 100 ms is not enough time to plan the response. However, in the warned mixed condition, with the same SOA, MEPs were significantly higher than those recorded at cue presentation. Given these results, we cannot conclude that the presentation of a cue automatically triggers motor response.

Irrespectively of whether the motor response is automatically triggered or not, participants still needed to prevent undesired anticipated responses that were preactivated by the cue. Proactive inhibition should be active on warned trials (irrespectively of Block condition) and for the unwarned trials of the mixed block. In this last condition, participants could not predict whether, after the presentation of the fixation cross, the cue or the target would appear. Proactive inhibition was therefore necessary to prevent unwanted responses to the cue. In their study, Bouliguez and colleagues (2009) found three main effects on the RTs to a similar task: a ‘baseline shift effect’ observed when comparing unwarned trials between the pure and mixed block conditions, where the latter induced longer RTs; the so called ‘standard warning benefit’, that is longer RTs for warned relative to unwarned trials within the mixed block (for SOA of at least 300 ms) and finally, the ‘paradoxical warning cost’, namely

longer RTs in the pure warned, relative to unwarned trials with a short SOA (100 ms). In our experiment, we replicated their baseline shift effect (Figure 17), finding longer RTs for unwarned trials in the mixed relative to pure block. This effect would support the idea that a proactive inhibition mechanism is activated when there is uncertainty about task demands (Chen, Muggleton, Tzeng, Hung, & Juan, 2009; Sharp et al., 2010). We did not find the same difference in the MEPs (Figure 19), and a possible explanation is that in the unwarned condition the motor response was not pre-activated, therefore the effect of inhibition could not be measured. Within the mixed block, a comparison of the RTs between warned and unwarned trials (Figure 17) revealed significant differences, with longer RTs for unwarned trials. This 'standard warning benefit' effect would support Boulinguez et al.'s hypothesis that the proactive inhibition mechanism, deactivated by cue presentation, would reduce the induced slowing of responses, thus allowing participants to react faster to target presentation. Finally, we could expect longer RTs for warned compared to unwarned pure trials, when the SOA was 150 ms (paradoxical warning cost). However, as Figure 18 shows, in our data warning trials always elicited shorter RTs, relative to unwarned trials, irrespectively of SOA and block condition. The paradoxical warning cost, according to Boulinguez et al. (2009), is caused by the deactivation of the proactive inhibition mechanism after cue presentation and this process should take about 300 ms. We did find a difference in RTs between the two SOA conditions, with "paradoxically" longer RTs in the short SOA trials, however this effect was also present for unwarned trials, condition that should not require (at least in the pure block condition) proactive inhibition. Moreover, we found significant differences in the MEPs as recorded at target presentation (Figure 19, right panel) for warned trials between the two SOA conditions. MEP amplitudes were, in fact, bigger for the long SOA trials relative to the short SOA trials, for both pure and mixed block conditions. The bigger MEPs recorded in the long SOA conditions are

consistent with the proactive inhibition hypothesis: if this mechanism is deactivated by the warning signal, it might take some time to fully deactivate. We could, therefore, expect bigger MEPs for long relative to short SOA trials as a sign of the disinhibition of responses. If such mechanism was fully active at short SOAs, then one would also expect to find MEPs smaller than baseline, but this is not what we found. For trials with short SOAs, MEPs in the pure warned trials are not different than baseline (Figure 19, left panel), whereas for warned mixed trials they are bigger than baseline.

Together, these results support the hypothesis of proactive inhibition mechanisms activated by specific task demands, such as uncertainty for the upcoming stimulus, which induced strategic slowing of responses. Moreover, when participants are given more time to prepare the motor response, RTs are shorter and the level of corticospinal excitability is increased (MEP amplitude), in accordance with classical theories (e.g., Näätänen 1970; Näätänen et al., 1974; Niemi and Näätänen, 1981). Our data, however, do not support the notion that the warning signal automatically deactivates this proactive inhibition mechanism in around 300 ms. We should point out that, in our paradigm, the warning signal was the static marble on top of the tilted plane, while Boulinguez and co-workers used a neutral cue (and not the stationary target, as we did). The level of information carried by the cue is known to differentially influence attention to the task (Prinzmetal, McCool, & Park, 2005; Prinzmetal, Zvinyatskovskiy, Gutierrez, & Dilem, 2009) and this could have modulated RTs differently. However, if inhibition of motor responses was activated to prevent the execution of anticipated responses, we should have found reduced MEPs, at least for the short SOA condition.

4.5 Conclusions

Under conditions of uncertainty, inhibition helps us prevent the execution of unwanted responses. This form of proactive control has been investigated by paradigms in which a warning signal (cue), to which participants are instructed not to respond, precedes the presentation of a target. By manipulating the cue-target delay (SOA), researchers have investigated the time course of the (de)activation of the proactive inhibition mechanisms. In this study we used single pulse TMS to trigger MEPs in two muscles, one involved in the motor response (FDI) and one control muscle (ADM). We found significant MEP activations induced by the warning signals for the FDI muscle only, indicating that we selectively activated the response-relevant effector. RTs were also modulated by task conditions: they were shorter in trials where a cue preceded the target. Finally, MEPs and RTs on cued trials were differently modulated depending on the SOA. This effect might indicate the build-up of a motor preparation rather than a deactivation of a proactive inhibition mechanism, as suggested by previous studies (Boulinguez, Ballanger, Granjon, & Benraiss, 2009; Jaffard, Benraiss, Longcamp, Velay, & Boulinguez, 2007). Future studies should further manipulate task demands (probability of presentation of the target, maximum time to respond) to investigate how these influence the deployment of proactive inhibition.

Final Remarks

Action inhibition has often been treated as a unitary construct, but everyday experiences and scientific results suggest that we employ different types of inhibition, depending on the context. External factors and internal urges are integrated by the brain to perform decisions, regarding which action to execute among possible alternatives, when to execute it and, ultimately, whether to perform the planned action or inhibit it.

The first set of experiments was devised to study the difference between externally-triggered and internally-driven action inhibition and we tested the direct role of two brain areas, the left dFMC and the right IFG. We used repetitive TMS to temporarily inhibit the activity of these areas, and test the effect of stimulation on the EEG activity and the performance of participants on a modified version of a Go/NoGo paradigm. In this task, we compared an Exogenous condition (externally-triggered), in which the stimulus informed participants to either perform or inhibit the planned action, to an Endogenous Go/NoGo condition (internally-driven), which required participants to freely decide whether to perform the button press or not. The activity of fronto-central electrodes showed significant differences in the amplitude of ERPs between the two NoGo conditions, suggesting that the activity of these areas is differentially modulated during action inhibition, depending on the locus of the decision (internal vs. external). Behavioural results confirmed our expectations: after inhibiting the dFMC, participants were less likely to respond, in the Endogenous Go/NoGo condition only. This result indicates that the dFMC is directly involved in the internally-driven inhibition of actions and that at least partially separated brain circuits are responsible for externally-triggered and internally-driven action inhibition.

Another condition that activates inhibitory control is when there is uncertainty regarding the appearance of a stimulus we need to respond to. For instance, while sitting in a waiting room, we wait for our name to be called before we get up. In this scenario, we might pre-activate the action while refraining from standing up too early. In this condition, a proactive inhibition mechanism is tonically active to avoid unwanted, anticipated responses. Proactive inhibition was investigated in the second set of experiments, where we compared different task conditions designed to activate proactive inhibition, whilst using single pulse TMS to elicit MEPs, a measure of cortico-motor excitability. Results indicated that the presentation of a warning signal had a beneficial effect on RTs, speeding up responses to the target, irrespectively of the time interval separating the two. MEPs recorded from the muscle involved in the motor response (FDI) showed increased amplitudes in almost all conditions in which the warning signal preceded the target, suggesting that this *standard warning benefit* involves motor preparation. Finally, the expected results on RTs of warned trials were also found for unwarned pure trials, a result that is not compatible with the previously proposed theory that proactive inhibition should be deactivated by the cue (Boulinguez et al., 2008). Moreover MEP amplitudes were not reduced on warned trials, as it would have been predicted by the proactive inhibition theory. Therefore, we can conclude that proactive inhibition causes a strategic slowing of responses, without directly modulating cortico-spinal excitability.

The way our brain exerts inhibitory control still needs further investigation, since it's a very important aspect of the executive functions that allow us to flexibly control our impulses and responses to the environment, to meet our goals. When inhibitory control is impaired, the consequence is the inability to resist to externally and internally-triggered urges, symptoms that are present in many pathological conditions such as

ADHD, Parkinson's Disease and Schizophrenia. Future studies should explore the brain circuits involved in inhibitory control, developing ad-hoc paradigms to investigate the different types of action inhibition.

Appendix

Experiment 1.

The effect of SOA on RTs

I chose to use different SOAs for this experiment based on studies on proactive inhibition and the effect of cues on visual attention (Jaffard et al., 2007; Prinzmetal et al., 2009). It has been shown how the presentation of a cue (warning signal) impacts RTs. Informative, central or peripheral cues are thought to activate voluntary and involuntary attentional processes (Prinzmetal et al., 2005) and influence the way we react to upcoming stimuli. When involuntary attention is engaged, beneficial effects (shorter RTs to spatially congruent targets) of cue presentation are already present at short SOAs (e.g., Posner, Cohen, & Rafal, 1982; Warner et al., 1990). Recently, studies on proactive inhibition (Boulinguez et al., 2009) have found prolonged RTs for short (<300 ms) relative to long SOAs. In experiment 1 the static marble was used as a cue, a stimulus that warns participants to be ready for the presentation of the target (ball motion), a condition similar to the *Warned Pure* block of experiment 2. We included 6 different SOAs: 150, 300, 500, 1200, 1500, 3000 ms. The first three SOAs were chosen in a temporal window close to 300 ms, while the longer SOAs were included so that participants could not easily predict the time of target presentation. Since in the Endogenous Go/NoGo condition participants also had to voluntarily decide whether to peform the button press or not, this analysis was conducted on Exogenous Go trials only. Trials from the pre-TMS Exogenous condition were divided according to SOA and a repeated measure ANOVA was conducted on RTs (including late responses), with Bonferroni correction. Significant differences were found across the SOAs ($F(1,18)=18.964, p=0.000$) and paired comparison showed significant differences

between trials with SOA=150ms and all the others (Figure 20). No other significant differences were found.

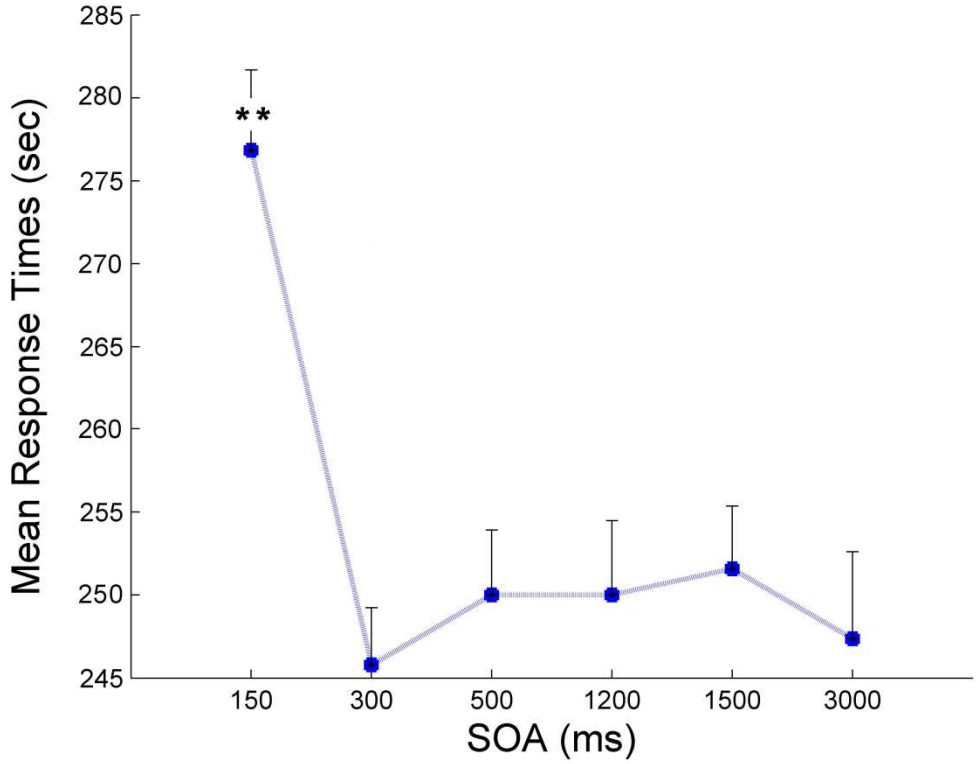

Figure 20 Mean RTs from the Exogenous Go condition, divided by the six SOAs are shown. When the SOA was 150 ms, RTs were significantly longer than all other conditions (** $p<0.001$). Error bars represent the standard error.

RTs from the Exogenous Go trials with an SOA of 150 ms were significantly longer than all the other SOA conditions. This result is consistent with the results of experiment 2, where we found longer RTs for the shortest SOA (150 ms) on warned trials. In the Act or Inhibit task, however, on roughly half of the trials participants did not have to press the button. Therefore, in addition to the uncertainty regarding the timing of target presentation, participants also had to decide (upon cue presentation)

whether to respond or not, a decision which was absent in experiment 2. In addition to the fact that the two experiments were conducted on different groups of participants, this might explain why the RTs shown in Figure 20 are generally longer than those presented in experiment 2 (Figure 18).

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