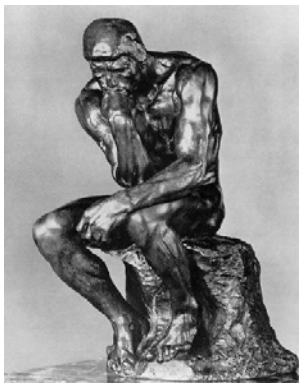
Functional and Anatomical Networks of Executive Control

A thesis submitted to the University of Oxford for the degree of Master of Science



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Abstract

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Actions are selected in the context of environmental demands and internal goals. Both change constantly and dynamically and several studies have addressed the issue of how information about these is represented, updated and integrated in the brain to form appropriate decisions and actions. The reprogramming of actions requires inhibition of movements or movement plans, resolution of response conflict and initiation of alternative actions. The right inferior frontal cortex (rIFC) and the pre-supplementary motor areas (pre-SMA) have been suggested to play a major role in response inhibition and action reprogramming. The degree to which inhibition of actions at a behavioural level can be related to physiological inhibition is unknown.

Using single and paired-pulse transcranial magnetic stimulation (TMS), we investigated M1 excitability and M1 internal inhibitory mechanisms during action reprogramming (see Chapter 2.1.1). The temporal pattern of M1 excitability and M1 internal inhibitory mechanisms differed from those during normal action execution and could therefore play a causal role in action reprogramming. These findings are important as M1 is likely to be the site of convergence from different influences exerted by regions in the frontal lobes.

In a second experiment we used paired-pulse TMS over rIFC and M1 to investigate functional rIFC-M1 interactions (see 2.1.2). We found that rIFC inhibited M1 excitability 175 ms after cue onset only in trials when actions needed to be reprogrammed and responses had to be inhibited, but not during normal action selection.

In a third experiment we used a combined paired-pulse TMS – diffusion tensor imaging (DTI) approach to elucidate anatomical pathways of functional pre-SMA-M1 and rIFC-M1 connectivity (see 2.2). We found extended networks of executive control and action reprogramming. These results suggest that both, pre-SMA and rIFC influence motor output via premotor areas and fronto-basal ganglia loops. Different latency periods of rIFC-M1 and pre-SMA-M1 interactions were mediated by different white matter paths and networks.

In a fourth experiment we tried to delineate the roles of rIFC and pre-SMA during action reprogramming using a combined paired-pulse TMS– repetitive TMS paradigm (see 2.3). The inhibitory influence exerted by rIFC over M1 during action reprogramming disappeared after mild and transient disruption of pre-SMA activity.

Besides elucidating a network of brain areas associated with action reprogramming and movement inhibition these experiments have interesting methodological implications.

This thesis contains approximately 40,000 words.

Outline

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Abstract II

Functional and Anatomical Networks of Executive Control

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Actions are selected in the context of environmental demands and internal goals. Predictions about future internal and environmental states, action outcomes and their effort guide the selection of cognitive and behavioural strategies. External and internal states change constantly and dynamically. Sometimes predictions are violated and action strategies need to be readjusted in the context of ongoing movement plans. Updating response strategies and reprogramming actions requires inhibition of actions or action plans, resolution of response conflict and initiation of alternative actions. Several regions in the frontal and parietal cortex and in the basal ganglia have been suggested to be involved in executive control and the flexible adjustment of behaviour. Especially the right inferior frontal cortex (rIFC) and the pre-supplementary motor areas (pre-SMA) have been proposed to play a major role in the resolution of response conflict, inhibition and action reprogramming. The concept of "inhibition" is widely used in the field of cognitive and molecular neuroscience, psychology and even psychotherapy. However the question whether processes of executive control, action reprogramming and task switching rely at least to some extent on inhibitory processes still needs to be answered. The degree to which inhibition of actions at a behavioural level can be related to physiological inhibition is unknown. Some theories even claim a specialized "network" of response inhibition

and action reprogramming. But whether such a specific network exists still needs to be ascertained.

To address some of these questions we aimed to investigate (1) general excitability of M1 and M1 internal inhibitory processes during action execution and action reprogramming, (2) functional connectivity between rIFC and M1 during action execution and action reprogramming, (3) anatomical pathways mediating functional rIFC-M1 and Pre-SMA-M1 connectivity and (4) rIFC-M1 functional connectivity after transient and mild disruption of pre-SMA activity.

Using single and paired-pulse transcranial magnetic stimulation (TMS), we investigated M1 excitability and M1 internal inhibitory mechanisms during action reprogramming (see Chapter 2.1.1). We hypothesised that excitability and internal inhibitory processes in M1 would change during the time course of action reprogramming and would differ from situations when only action execution was required. We found that during action reprogramming the excitability of the motor system changes according to the necessity of inhibiting a prepared response and select an alternative action between 75 ms and 175 ms after the cue onset. In switch trials 75 ms after cue onset excitability of the motor system of the prepared but incorrect response is still bigger than the excitability of the motor system of the unprepared but appropriate response. This effect is reversed 225 ms after cue onset suggesting that action reprogramming on the level of the motor system takes place between 75 ms and 225 ms after the onset of the instructive cue. This reversion could be achieved by GABA-ergic M1 internal inhibitory interneurons. The inhibitory influence of these interneurons can be measured as short-interval intracortical inhibition (SICI). We observed a release of SICI for the unprepared but correct hand in action reprogramming trials. This could suggest that one mechanism achieving successful

action reprogramming is an even stronger release of SICI to overcome the prepared but incorrect action plan. These findings are important as M1 is likely to be the site of convergence from different influences exerted by regions in the frontal lobes.

To investigate the source of the inhibitory control of the motor system we studied functional connectivity between rIFC and M1 during normal action execution and action reprogramming (see Chapter 2.1.2). Again a paired-pulse TMS paradigm was used with a conditioning pulse over the posterior rIFC and the test pulse over the left M1. Our results indicate that rIFC influences M1 corticospinal excitability in both, action execution and action reprogramming situations at about 175 ms after instruction cue onset. Whereas rIFC facilitates the correct movement in action execution trials, it inhibits the wrong movement in action reprogramming trials.

In a third experiment we used a paired-pulse TMS paradigm to investigate different pathways of functional pre-SMA-M1 connectivity and rIFC-M1 connectivity during action reprogramming and response inhibition (see 2.2). The aim of this experiment was to show different pathways of functional pre-SMA-M1 and rIFC-M1 connectivity during action reprogramming and to localise these pathways within the brain's white matter. To elucidate whether these pre-SMA-M1 and rIFC-M1 pathways belong to a bigger network involved in action reprogramming, conflict resolution and response inhibition we carried out probabilistic diffusion tractography based on the white matter clusters that showed FA values significantly correlated with the paired-pulse TMS measurements of functional connectivity. We found clusters that showed FA values significantly correlated with the TMS effects of functional pre-SMA-M1 and rIFC-M1 connectivity. Probabilistic diffusion tractography derived from these clusters of significant correlation revealed complex networks of executive control and action reprogramming in the dorsal white matter (superior longitudinal fascicle), in the white

matter underlying rIFC and in the vicinity of the basal ganglia. These results suggest that both pre-SMA and rIFC influence motor output via premotor areas and fronto-basal ganglia loops. Different latency periods of rIFC-M1 and pre-SMA-M1 influence were mediated by different white matter paths and networks.

In a fourth experiment we tried to delineate the roles of rIFC and pre-SMA during action reprogramming using a combined paired-pulse TMS – repetitive TMS (rTMS) paradigm (see 2.3). The inhibitory influence exerted by rIFC over M1 during action reprogramming disappeared after mild and transient disruption of pre-SMA activity.

Despite elucidating a network of brain areas associated with action reprogramming and movement inhibition these experiments have interesting methodological implications. Combining paired-pulse TMS with DTI could help to elucidate anatomical white matter pathways mediating functional connectivity between distant brain areas. A combined rTMS – paired-pulse TMS paradigm can be sued to elucidate the effects of the transient disruption of an area on functional connectivity between other areas.

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1. Introductory Chapter

1.1. Background

1.1.1. Cognitive Processes

Flexible goal-directed behaviour requires attention to be aligned to environmental circumstances and for the direction of attention to be changed as those circumstances change, monitoring of ongoing actions and the outcomes of those actions, and, as a consequence, subsequent adjustment of performance, resolution of conflict between the different actions that might be made in a given situation and inhibition of undesired responses (Bunge and Wallis, 2007; Purves et al., 2008; Mansouri et al., 2009).

Hierarchies of Cognition, Action and Motivation

Cognitive control is argued to be necessary for flexible behaviour by choosing *actions* that are consistent with our *own goals* and appropriate for *environmental demands*. It has been proposed that the representation of our environment especially the way it guides our decisions might be structured hierarchically. For example decisions can be made based on very simple stimulus-response associations (e.g. moving the car if the traffic light turns green) or could require additional information (e.g. moving the car if the traffic light turns green *and* the front vehicle starts moving as well), memorised facts (traffic regulations) and abstract thought (route planning) (Koechlin et al., 2003;

Badre and D'Esposito, 2007; Koechlin and Summerfield, 2007; Badre, 2008; Haggard et al., 2008).

It has also been suggested that human *behaviour* might be organized hierarchically (Kennerley et al., 2004; Koechlin and Jubault, 2006). To obtain our goals it might not be enough to respond to simple or even very complex cues with one single action but to organise possible actions as subordinate elements in a more elaborate action plan or sequence (e.g. if you finally decide to move your car you have to engage the gear, push the accelerator pedal, release the brakes etc).

It has recently been discussed that motivation might be structured hierarchically as well (Rushworth et al., 2004; Kouneiher et al., 2009). Lower levels of motivational control would then represent simple effort- reward associations whereas higher levels of motivation would represent rewards that could be obtained in the remote future and their associations with more complex actions and strategies.

Cognitive control processes are often thought to perform supervisory and regulatory roles, modulating the activity of other cognitive processes and computations (Miller and Cohen, 2001; Bunge and Wallis, 2007; Purves et al., 2008). Several areas in the frontal lobes seem to conduct those processes. Patients with prefrontal damage tend to be superficially normal but faced with real-life-challenges they perform poorly (Lhermitte, 1986; Lhermitte et al., 1986; Shallice and Burgess, 1991; Anderson et al., 1999). Therefore it has proven to be difficult to develop tasks that actually show those impairments and tease apart different components of this cognitive control system.

Some paradigms turned out to be very successful in elucidating different control mechanisms and quantifying behavioural impairments in frontal lobe patients: the Wisconsin Card Sorting Test, the Stroop task, the Eriksen Flanker task, task switching

paradigms, Go/No-go tasks etc. (MacLeod, 1991; Demakis, 2003; Aron, 2007). Research employing those and many other experimental tasks has depicted some cardinal components of executive control: *Inhibition* is important to suppress automatic but inappropriate responses. Once the automatic behaviour is inhibited, other processes should be facilitated (*initiation*) and other behavioural programmes and task sets need to be retrieved (*task switching*). *Simulation* allows us to test out possible strategies and courses of action without suffering their consequences. *Evaluation* processes provide us with information about action outcomes and guide future decisions.

Inhibition

The concept of *inhibition* is widely used in neurophysiology, neuroscience and even psychology (Aron, 2007). When a GABA-containing neuron fires, it releases GABA and therefore induces an *inhibitory* postsynaptic potential (IPSP) in its target neuron. Increased firing rates of *inhibitory* striatal projection neurons reduce *inhibitory* influences of pallidal neurons on the thalamus and therefore release thalamo-cortical drive. If we want to focus our attention we might have to *inhibit* distracting stimuli. And the repression of unpleasant memories might crucially rely on active mechanisms of *inhibition* and directed forgetting (Depue et al., 2007). All those examples might refer to an underlying "inhibitory mechanism". However it seems very unlikely that these extremely diverse processes of synaptic inhibition, circuit inhibition, attentional inhibition and inhibition of emotions or memories are exerted by similar neural mechanisms. Many objections have been raised to the concept of active inhibitory mechanisms in cognitive neuroscience and psychology. And some of those processes might be much easier explained without referring to any underlying inhibitory

processes (Miller and Cohen, 2001; Miller and D'Esposito, 2005; Aron, 2007). For example selective attention to task relevant information and concurrent inhibition of irrelevant stimuli could just be two sides of the same coin: attentional competition is simply biased towards the relevant and away from the irrelevant stimulus (Desimone and Duncan, 1995; Miller and Cohen, 2001).

Nonetheless inhibition is argued to be one of the cardinal components in executive control. Suppressing or stopping an already prepared but inappropriate action is important for adjusting behaviour to changing environmental demands. This process can be tested and quantified with the appropriate scientific tools, such as transcranial magnetic stimulation (TMS), (Waldvogel et al., 2000; Chambers et al., 2006; Coxon et al., 2006; Aron, 2007; Stinear et al., 2009). Such approaches have typically restricted investigation of inhibition in the primary motor cortex (M1) itself but they might also be exploited in an attempt to understand whether and how inhibition in areas such as M1 arises from the influence of higher order brain areas that are most often thought to be playing pre-eminent roles in executive control. Research into inhibition may even lead to basic insights into the nature of other inhibitory mechanisms in higher cognition and processes of self control (Aron et al., 2004a; Aron, 2007; Aron et al., 2007b).

There are various different experimental paradigms for investigating inhibition in executive control. One of the most common measures is the go/no-go task. In this task subjects are required to respond to a go-signal with a right or left hand button press. On the minority of trials a stop signal is presented after the go signal telling the subjects to withhold their action. The stop signal reaction time (SSRT) can be estimated by varying the delay of the stop signal and is thought to estimate the speed of the stopping process “in the brain” (Aron et al., 2004a; Li et al., 2006; Aron and

Verbruggen, 2008). Go and stop processes are often considered to be independent processes. But recent computational modelling has suggested that an interactive model best fits the data if the two processes are independent for most of their durations and interact strongly for a brief period in their final stages (Verbruggen and Logan, 2009). Attempts have been made to assign these processes, especially the stopping process, to particular neural circuits and mechanisms on the basis of imaging, neurophysiologic, and clinical investigations (Sasaki et al., 1989; Konishi et al., 1998b; Garavan et al., 1999; Waldvogel et al., 2000; Aron et al., 2003a; Aron and Poldrack, 2006; Li et al., 2006; Aron et al., 2007a; Aron et al., 2007b; Isoda and Hikosaka, 2007; Mars et al., 2007b; Sumner et al., 2007; Isoda and Hikosaka, 2008; Duann et al., 2009).

However global mechanisms of stopping that do not just affect the initiated action tendency but multiple action tendencies might differ from selective inhibitory mechanisms (Coxon et al., 2007; Aron and Verbruggen, 2008). Subjects are significantly faster in globally stopping all potential responses than in stopping just one response selectively indicating that those two processes might in part employ different pathways.

Attention-deficit hyperactivity disorder (ADHD) is the most commonly studied and diagnosed psychiatric disorder in children. Diagnostic manuals, for example DSM-IV, distinguish three different types of ADHD: a predominantly inattentive type, a predominantly hyperactive-impulsive type, and a combined type if criteria for both subtypes are met.

The impulsive symptom complex has been linked to failures of executive control and response inhibition (Aron et al., 2007b). Performances in some laboratory measures of executive control such as the go/no-go task, the continuous performance test and the

Stroop task were found to be impaired (Solanto, 2002). Altered brain size in the right inferior frontal cortex (rIFC), dysfunction in cortico-striatal pathways and an imbalance in ascending monoamine projections into the prefrontal cortex have all been suggested to be involved in the pathophysiology of ADHD (Solanto, 2002; Durston et al., 2004; Chamberlain et al., 2006a). Therefore ADHD has been thought of as a disease of these neural inhibition networks.

Although ADHD has generally been considered a disease of dopamine hypofunction a crucial role of the noradrenergic projection system has recently been proposed (Solanto, 2002; Chamberlain et al., 2006b). Atomexetine, a selective norepinephrine reuptake inhibitor approved for the treatment of attention-deficit hyperactivity disorder improves response inhibition in ADHD patients and healthy controls in a go/no-go task (shorter SSRTs) (Chamberlain et al., 2006b). In rats atomexetine reduces impulsive responding in several executive control paradigms (in a stop signal reaction time task, a five choice serial reaction time task and a delay discounting paradigm) (Robinson et al., 2008). Noradrenergic projections are also believed to exert regulatory influences on attention, arousal and vigilance (Nieuwenhuis et al., 2005a). Problems in attention are indeed integral to the diagnostic criteria of ADHD in addition to symptoms of impulsivity and hyperactivity. Damage to prefrontal cortex traditionally associated with response inhibition has been shown to impair "inhibition" of salient but irrelevant stimuli in a delayed-match-to-sample task (Chao and Knight, 1995, 1998). It could be of great importance for the research on inhibition to elucidate whether noradrenergic projections that influence redirection of attention and noradrenergic projections that affect inhibition in cognitive control share similarities. This might even lead to a better understanding of the ADHD symptom complex and its underlying pathology.

The locus coeruleus is a neuromodulatory brainstem nucleus. It is estimated to contain more than half of all noradrenergic neurons in the central nervous system and is argued to be critical for the regulation of several different cognitive mechanisms (Nieuwenhuis et al., 2005b). Its phasic increase in activity during the processing of motivationally relevant stimuli leads to a release of noradrenalin in widespread cortical projection areas, especially in prefrontal and parietal cortex. The phasic discharge of the locus coeruleus elicited by task-relevant or surprising stimuli has been linked to the P300, a broad, positive, large-amplitude potential in EEG recording with a typical peak latency between 300 and 400 ms after presentation of a stimulus (Nieuwenhuis et al., 2005a; Nieuwenhuis et al., 2005b; Corbetta et al., 2008). The amplitude of the P300 wave is inversely correlated with the probability of an event. Trial by trial fluctuations in the P300 wave have shown to be related to subjective probability and could therefore reflect adjustment of information processing (Mars et al., 2008). If P300 reflects the arrival of a phasic noradrenergic signal from the locus coeruleus which serves to increase signal transmission in the cortex we would expect a relation between the P300 and performance speed/accuracy. This is actually the case, (Nieuwenhuis et al., 2005a).

Interestingly coincident with abnormally poor performance of ADHD children in executive control paradigms, which is thought to be due to insufficient inhibition, they also show smaller amplitude and longer latency P300 waves than their peers with different psychiatric diagnoses (Klorman, 1991), possibly reflecting impaired ability to redirect attention. Administration of (unspecific) dopamine/ noradrenalin reuptake inhibitors (such as methylphenidate) improves executive control and inhibition as well as it enlarges the amplitude of the P300 wave.

It might be worth testing the effects of specific noradrenalin reuptake inhibitors (such as the above mentioned atomexetine) on the P300 wave in ADHD patients and healthy controls. Additionally investigating uncertainty and surprise related fluctuations in inhibitory influences of prefrontal areas over the motor system could prove to be fruitful. If noradrenergic projections from the locus coeruleus play a role in inhibitory control similar to the role in attentional redirection we would expect surprise related fluctuations in the amount of inhibition. Moreover changes in the inhibitory influence exerted by areas such as the right inferior frontal cortex over the motor output could then adjust motor-cortex excitability to environmental uncertainty. We might thus find inhibition a complex, graded mechanism instead of a simple, all-or-none stopping process. The correlation between surprise and the excitability of the motor cortex during action preparation has already been shown (Bestmann et al., 2008b). And in fact TMS could proof to be the most reliable tool for investigating the neural basis of inhibition in cognitive control (Waldvogel et al., 2000; Aron, 2007).

Decision Making

Decisions normally show three characteristics: (1) at least two possible options are available; (2) expectations about the outcomes of those options can be formed; (3) the (subjective) values of those outcomes can be assessed at some level (Purves et al., 2008).Those terms imply complete awareness of these processes of evaluation and reflection. And they seem most comprehensible if we think of situations when people faced with several different options have to decide for themselves what to do. But many decisions are made unconsciously and it has been argued that analogous processes shape behaviour outside the domain of reward-guided decision making (Rushworth et al., 2009).

Interest in decision making has sometimes focussed on rule-guided decision making – the process by which an appropriate action is selected on the basis of a learned association with a particular stimulus. A key element in many of the paradigms studied is that one implicit or explicit "rule" unambiguously predicts the correct decision to make in a given situation.

However in some situations environmental cues do not provide sufficient information about which action to make (Rushworth, 2008). Therefore people are thought to instead rely crucially on (a) their predictions about action outcomes; (b) the expected values/utilities of these outcomes (c) the ability to detect actual decision outcomes; (d) evaluation of the discrepancy between predictions and actual outcomes (prediction error); (e) the ability to update predictions based on the prediction error weighted against the reinforcement history (learning rate); (f) (second order) predictions about the uncertainty of these decision outcome predictions (similar to the idea of "risk"); (g) valuation of the uncertainty, depending on risk susceptibility (people seem to differ in judging risks as being acceptable or unacceptable, enjoyable or unbearable); (h) the ability to detect the actual risks (mean outcome variance); (i) evaluation of discrepancy between risk predictions and actual risks; (j) the ability to update risk predictions. Several areas in the brain have been argued to be central to such processes, especially areas in the medial frontal cortex (MFC), the basal ganglia, the substantia nigra and the ventral tegmental area (Schultz et al., 1997; Rushworth, 2008; Schultz et al., 2008; Rushworth et al., 2009).

A strict distinction between reward-guided and rule-guided decision making, however, cannot capture some of the important aspects of decision making. For example the Wisconsin Card Sort Test actually requires: (A) learning rules for classifying the card deck based on feedback; (B) applying the learned rules to a

certain situation (i.e. a certain card); (C) making "rule predictions"; (D) evaluating those predictions in the light of feedback and (E) switching between task rules.

Moreover it has been known for a long time that we use rules, strategies and heuristics to guide decisions about what course of action is likely to yield the greatest reward. These strategies and heuristics employed when people make reward-oriented decisions could be considered as "rules" that have proven to be adaptive, although they might differ from those rules that unambiguously guide actions (such as traffic regulations).

It therefore seems reasonable to expect similar prediction processes in rule-guided behaviour and even in perception (Summerfield et al., 2006; Bar, 2007; Rushworth et al., 2009; Summerfield and Egner, 2009). It appears implausible to think that the brain "waits" for incoming information to represent the environment independently of its actual affordances. Instead the environment might only be represented in an affordance-dependent way. It might be preferable to view the brain as constantly making predictions about the future. Such predictions might not just be about what rewards are expected to ensue but about environmental states, stimuli, and action affordances that are expected to be encountered next, and even what internal motivational states might be expected next. By attempting to minimize the prediction error such predictions may become more accurate.

A frontal cortex-basal ganglia loop (modulated by monoaminergic projections) could be important for selective updating processes following prediction violations (Hazy et al., 2007). Additionally mechanisms of rule-guided action selection are thought to rely on similar neuronal mechanisms and similar frontal cortex – basal ganglia pathways as those of information updating (Hazy et al., 2007).

Explicit or implicit rules about appropriate and inappropriate responses in certain situations could guide (1) the predictions about the actions that might have to be made; (2) predictions about stimulus identities and how they are going to bias competition between potential responses based on the (1); (3) predictions about the uncertainty of (1) and (2). For example if one approaches the traffic lights the knowledge about traffic light rules will guide the (1) the predictions about which actions might be required in the upcoming situation (i.e. action A = slowing down, action B = driving on), (2) the stimulus identities (i.e. stimulus a = red, stimulus b = green, we would be surprised to find the traffic lights turning blue) and how they bias competition between the actions in (1) (i.e. stimulus a → action A, stimulus b → action B), and predictions about the uncertainty of (1) (is there any chance that one might have to choose a third action?) and (2)(is there any chance, that the traffic light might turn blue or that green means slowing down?). If those predictions turn out to be poor it might be sensible to update the "rule" (e.g. employing another level of control, e.g. if an ambulance is passing the crossroads than red ∩ green → slow down) or even switching to another rule (e.g. red → drive on, green → slow down).

This would suggest that the brain does not simply encode information about the organism's internal states and the environment. It might instead encode the differences between the environment experienced and the environment predicted or the internal state experienced and the internal state predicted. This would imply (a) the whole idea of predictive coding being a ubiquitous property of brain function, for example such predictive coding might even be present within the motor system (Bestmann et al., 2008b)), and (b) predictions, prediction error evaluation and updating of predictions (learning) might be occurring in hierarchically organised systems for complex control.

Rule-Guided Behaviour

To act appropriately in a changing environment we need to associate certain actions and strategies with predictions about their outcomes. Additionally we need to keep track of the actual action outcomes in order to revise both: our predictions about those action outcomes and our strategies to create reward-maximizing action selection rules.

Rules are constructs that guide predictions about *situation – action – outcome contingencies*. It has been suggested that human behaviour largely relies on explicitly or implicitly learned rules for action (Bunge, 2004; Bunge et al., 2005; Bunge and Wallis, 2007). Thinking about cognitive control and decision making as processes mostly guided by rules can be fruitful for investigation of the underlying neural processes although it might have its caveats.

Decision making has been studied in psychological and neuroscience laboratories by devising relatively simple cognitive tasks. Subjects are often asked to learn arbitrary rules that unambiguously guide their action selection. However every-day decision making and action selection surely does not solely rely on *arbitrary* and *unambiguous* rules. Rather humans try to build up theories about their every-day environment trying to explain why some actions could be appropriate in certain situations to *reduce* arbitrariness. Moreover the rules we use to guide our decisions are almost never unambiguous. They rather need constant revision and reconsideration. A person that obsessively sticks to learned rules will not act sensibly in an uncertain and changing environment. Nevertheless answering the core question of the rule-guided decision making field could contribute substantially to our concept of how the brain generates

behaviour: the questions of acquisition, long-term storage, retrieval, maintenance and implementation of rules and task sets and how we flexibly switch between those.

This research has mainly focused on the lateral PFC. Although lateral PFC was often observed to be engaged during the performance of working memory tasks converging evidence suggests the lateral PFC being equally important for learning and updating of rules, retrieving them from long-term memory, and using them to control behaviour (Bunge, 2004; Buckley et al., 2009).

Basal ganglia and its dopaminergic inputs are proposed to be crucially involved in the incremental acquisition of rules. However medial temporal lobes could be important for declarative instead of procedural rule learning (Bunge, 2004). Posterior medial temporal gurus and pre-SMA are thought to represent rules, task and action sets (Bunge, 2004; Nachev et al., 2008). Ventro-lateral PFC as well as the inferior frontal junction area (Brass et al., 2003; Bunge, 2004; Bunge and Wallis, 2007) are supposed to be responsible for rule retrieval. If rules and response set representations have to be maintained "online" pre-SMA, several areas in the ventro-lateral and dorso-lateral PFC as well as the frontal pole are found to be active. Those findings were interpreted as ventro-lateral PFC being important for retrieval and maintenance, whereas dorso-lateral PFC is engaged in rule manipulation or overriding a strong but inappropriate response tendency. The fronto-polar cortex seems to be active when several possible rules and strategies have to be retrieved and different subtasks have to be coordinated (Sakai and Passingham, 2003; Bunge, 2004; Koechlin and Hyafil, 2007; Rowe et al., 2007). Hierarchies in the lateral PFC are proposed to facilitate rule implementation and action selection (Fuster, 2001; Koechlin et al., 2003; Fuster, 2004; Petrides, 2005; Badre and D'Esposito, 2007; Koechlin and Summerfield, 2007; Badre, 2008; Badre et al., 2009; Kouneiher et al., 2009). Areas in the medial frontal cortex are thought to

contribute to rule switching and conflict resolution (Rushworth et al., 2004; Dosenbach et al., 2006; Nachev et al., 2007; Rushworth, 2008).

Based on the notion of PFC being a source of top-down bias on information processing in other brain areas (Miller and Cohen, 2001) rules could be thought of as more or less complex and nested biasing patterns. They might, therefore, have a hierarchical structure.

However although many areas in the brain, especially in the frontal lobes have been discussed in relation to different processes of rule-guided behaviour, the general neural mechanisms remain to be explained. These areas might be involved in learning, updating, retrieving and employing rules, but the underlying neural mechanisms remain to be clarified. If we cannot explain how distributed neural networks can learn rules, robustly maintain and selectively update them, how they chose rules in certain situations and how they use them to guide behaviour, we would have to assume a very smart, rule-guided homunculus though, but still a homunculus.

Therefore several mechanisms that are crucially important for processes of learning, retrieving, selecting and applying rules remain to be explained on a neural level. Among these processes are: (1) Rapid updating – the working memory system has to be able to rapidly encode and robustly maintain information (rules, relevant stimuli, past events) in the face of the interference from ongoing processing or other stimulus input (Hazy et al., 2007). (2) Selective updating of multiple separate working memory representations (e.g. several currently relevant rules, stimuli, contexts) – working memory has to know when and what to update. Updating of certain representations should not interfere with others, although updating of one representation can depend on another one (e.g. if stimuli have to be interpreted in the light of a context, "episode" or rule) (Hazy et al., 2007; Koechlin and Summerfield, 2007). (3) Selective

output-gating for top-down biasing of information processing – working memory representations have to be able to bias processing (updating, control and output) at a certain time point in order to achieve "goals". (4) Learning what and when to gate – both maintenance and output gating are not pre-programmed processes. The system has to learn when and which information should be maintained, updated or used to bias processing in other areas. This is difficult especially in complex situations (episodes) when the benefits of having updated and maintained information are only available later in time. But this is important for learning new rules and learning when to use them.

A PFC-basal ganglia- thalamus- PFC loop has been proposed to carry out those processes (Hazy et al., 2007). Such a loop is thought to work in parallel with one centred on M1 (a motor cortex- basal ganglia- thalamus- motor cortex loop) and controlling motor output (Alexander et al., 1986). This loop conveys go- signals (via the *direct* striatum→ globus pallidus internus/ substantia nigra pars reticulata→ thalamus) and no-go-signals (via an *indirect* striatum→ globus pallidus externus→ globus pallidus internus→ thalamus or even via a *hyperdirect* subthalamic nucleus pathway) therefore guiding motor output (go) and motor inhibition (no-go) in the motor cortex. Updating (go) and maintenance (no-go) of information in the PFC could employ the same circuits. The PFC is argued to contain a huge number of "isolated" groups of interconnected neurons. Hazy, Frank and O'Reilly (Hazy et al., 2007) call these neuron groups "stripes". Human PFC is estimated to comprise about 20,000 stripes. The big number of stripes and their proposed functioning is suggested to be one source for human's extraordinary intelligence (Hazy et al., 2007). These stripes and their corresponding basal ganglia loops are suggested to realise rapid but selective updating or maintenance of information (Hazy et al., 2007). Temporal and contextual

updating activity is then modulated by dopaminergic projections from substantia nigra and ventral tegmentum to the basal ganglia and the PFC (Hazy et al., 2007).

Research in humans and nonhumans as well as theoretical and computational approaches have led to some hypotheses about brain regions and how they use rules, strategies and constructs to guide decisions and actions (Bunge and Wallis, 2007). An important next step will be to characterise the temporal dynamics of interactions of these regions to elucidate the neural bases of these processes.

Task switching

Everyday life requires appropriate configuration of mental resources and processing. These configurations bias perception and action in a goal-directed way. We are able to pursue multiple goals or tasks simultaneously but can also decide to prioritise one in accordance with the current internal motivational state and with environmental demands. If necessary we can switch rapidly from one task set to another which can change the way we interact with our environment dramatically: how we process sensory information, how we allocate executive control resources, how we select and organise responses.

The whole concept of "tasks" is somehow artificial but it could serve as a fruitful model for behaviour in everyday life and therefore help to explain how the brain organises processing sequences appropriately. Task-switching paradigms became popular for investigating the transitions between two environmental, attentional, intentional, motivational, cognitive or motor states. Typically such paradigms are divided into "switch" and "stay" trials when the task either changes from one trial to the next or remains the same. Despite the obvious simplification inherent in such a

paradigm it seems reasonable to think the brain might actually represent the real world in a somewhat similar way as it attempts to estimate the stability or volatility of the world and to represent the dynamic changes that occur (Rushworth, 2008).

In task-switching experiments subjects have to learn (usually before but sometimes during the actual testing phase) several different tasks. In the *alternating-runs paradigm* the task is switched every N trials, where N is held constant (Monsell, 2003). Switch trials are compared to stay trials. An alternative is to give subjects *pre-specified task sequences*. In *task-cueing paradigms* the trial type (switch vs. stay) is unpredictable and a task instruction cue is presented at the same time (e.g.(Hyafil et al., 2009)), before (e.g. (Sakai and Passingham, 2003)), or even after (e.g. (Ruge et al., 2009)) the target stimulus. In *intermittent-instruction paradigms* task cues are presented to instruct a series of trials (an episode).

Generally responses take longer to initiate on a switch trial than on a task-repetition trial (switch cost or repetition benefit) (Monsell, 2003). If advance knowledge is given about the task and subjects have time to prepare a certain task set (as in (Brass and von Cramon, 2002; Sakai and Passingham, 2003)) switch costs are usually reduced (*preparation effect*). However they do not disappear completely. The remaining switch costs are called *residual costs* (Monsell, 2003). Although performance (reaction time, error rate) recovers after the switching, the responses in the successive stay trials are still slower and more inaccurate than when just one task has to be performed throughout the entire block (often referred to as *mixing costs*) (Monsell, 2003).

Research has tried to determine one single cognitive process that slows down response time after switching. To change task a process of task set reconfiguration, of mental gear shift might be necessary (Monsell, 2003; Bunge and Wallis, 2007).

Retrieving action rules, enabling a different response set and adjusting response criteria might be the source of those substantial task-set-reconfiguration costs. If this were true switch costs should dissolve if the subjects were given enough time to reconfigure the task set before stimulus presentation. This is not true (Monsell, 2003; Bunge and Wallis, 2007). Residual costs led to the notion that a new task set cannot be reconfigured in the absence of a concrete stimulus. Rather the new task set is believed to emerge during the course of task implementation and resolution of conflict between the two competing task sets. Additionally it was argued that a transient carry-over of the old task set that hence needs to inhibited contributes to switching costs (Monsell, 2003; Bunge and Wallis, 2007). This would explain longer lasting switching costs that slow down performance in the first stay trials after switching and higher switching costs in trials with bivalent stimuli (Brass et al., 2003; Monsell, 2003; Crone et al., 2006). Even when performing only one task performance slows down if the same stimuli were used in another task block a few minutes ago (Koechlin et al., 2003; Monsell, 2003). Whether processes of "inhibiting associative retrieval" are really similar to those of response inhibition (see above) and whether they might even engage similar similar brain areas (such as right IFC, see 1.1.2) still needs to be clarified. One could imagine that connectivity between areas of "inhibitory control" (e.g. rIFC) and areas of "task set representation" varies with bivalent vs. univalent block-contrasts (similar to (Koechlin et al., 2003; Miller and D'Esposito, 2005; Kouneiher et al., 2009)).

Studying neural processes of task switching could help to tease apart different processes of task switching. Imaging studies supported the view of task switching involving several processes, such as (1) *task retrieval, abstract task representation and task set reconfiguration* in the frontopolar cortex (Sakai and Passingham, 2003),

the inferior frontal junction (Brass and von Cramon, 2002; Derrfuss et al., 2005), PFC and its dopaminergic innervations (Cools et al., 2004; Bunge and Wallis, 2007), the pre-SMA (Braver and Barch, 2006; Dosenbach et al., 2006) or the anterior cingulate cortex (Hyafil et al., 2009); and (2) *resolution of conflict between competing task sets* in the anterior cingulate cortex (Kerns et al., 2004), the pre-SMA (Isoda and Hikosaka, 2007; Rushworth, 2008; Mars et al., 2009) or the lateral prefrontal cortex (Brass et al., 2003; Hyafil et al., 2009). Although very challenging the investigation of task switching has proven fruitful and significant for the understanding of functional organisation of the nervous system and human behaviour.

1.1.2. Brain Areas Involved in Cognitive Control

Theories of rostro-caudal organisation of the frontal lobe

At the beginning of the 19th century many scientists believed that the most anterior parts of the human brain might be critical for higher cognitive functions. Such beliefs were inspired by the fact that the frontal lobes are far more developed in humans and great apes than in other mammals. Although phrenology can only be considered a pseudoscience it encouraged exploration of how different cognitive functions are localised in the brain.

Important work on cortical localisation came from clinical-pathological studies such as those conducted by Broca and by Wernicke. Physiologist started to investigate cortical localisation in animals. In the late 1860s Hitzig and Fritsch showed that more posterior regions in the frontal lobes were associated with motor function (Purves et al., 2008; Fritsch and Hitzig, 2009). Lesions to those motor regions led to lack of control over movements. However damage to more anterior regions of the frontal lobes caused no obvious sensory or motor deficits. Hitzig and Fritsch therefore speculated those anterior areas could be important for higher cognition (Hitzig and Fritsch, 1873). Lesion studies by Ferrier in the 1870s and Bianchi at the beginning of the 20th century led to further insights into frontal lobe function and suggested a few fundamental operations that might be carried out by the frontal cortex.

Since then the frontal lobes have been associated with reasoning, problem solving, decision making and intelligence. Brodmann compared the relative proportion of the whole brain that the frontal lobes constituted in different species. He estimated that

only 7%of a dog's and less than 4 % of a cat's cortex is prefrontal. In contrast he found that the prefrontal cortex constituted about 10 %, 20%, and 30% of the whole brain volume in monkeys, great apes, and humans respectively (Brodmann, 1912).

However recent research has suggested that Brodmann's estimates are probably wrong. Structural MRI studies found the proportional size of the frontal lobes in humans is about the same as in great apes (Semendeferi et al., 1997; Passingham, 2002; Semendeferi et al., 2002). Nor were there any significant differences in *prefrontal* cortex proportion. However the most anterior portion of the prefrontal cortex, the frontopolar cortex could turn out to be proportionally larger in humans (Semendeferi et al., 2001). Additionally the human neocortex is over three times as large as expected for a primate matched for body size. And intelligence might be determined by the absolute size of the prefrontal cortex (amount of tissue, number of cells) (Passingham, 2002). However recent research has questioned the frontal lobes as being the only factor that determines human intelligence (Glascher et al., 2009; Nachev et al., 2009).

Although prefrontal cortex integrity may not be the sole determinant of intelligence it is widely agreed that, together with the basal ganglia, it is the core component of a system for cognitive control. Lesions to the frontal lobes lead to environmental dependency syndrome, perserveration, impairment of working memory and problem solving abilities, disinhibition and socially inappropriate behaviour (Lhermitte, 1986; Lhermitte et al., 1986; Shallice and Burgess, 1991; Hornak et al., 2003). But there has been an ongoing debate as to how exactly the prefrontal cortex exerts and implements cognitive control. Moreover it has turned out to be difficult to explain flexible, coordinated and purposeful behaviour in humans in terms of the capacity of a distributed network of neurons rather by simply invoking a homunculus.

An influential theory proposes that representations in the prefrontal cortex that can be considered as attentional templates, rules or goals provide a top-down bias to information processing in other areas of the brain and therefore facilitate the flow of activity along pathways that lead to the "correct" performance of a task (Miller and Cohen, 2001). Rather than being the processing module for all those higher order cognitive functions, PFC exerts a biasing influence that ensures that a particular set of brain areas exerts the greatest control over behaviour. Just as the perforated paper rolls in the player pianos* - that of course do not actually play the piano, or even move the felt covered hammers that strike the piano strings, but which carry the exact instructions for the correct sequence of key presses, that constitutes the playing of a particular musical piece, so the PFC can be seen as an active memory with the role of controlling activity patterns of biasing signals to other structures.

Properties required to support this role would be: 1. multimodal convergence of behaviourally relevant information (i.e. connections from the higher order sensory areas associated with the different modalities), 2. feedback pathways that can exert biasing influences on other structures throughout the brain, 3. maintenance of relevant information that is robust to interference, 4. rapid updating of information and plasticity (important: *selective* updating of representations) (Miller and Cohen, 2001; Hazy et al., 2007). The prefrontal cortex seems to exhibit these properties and converging evidence supports this view.

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* Player pianos are self-playing pianos, containing a pneumatic or electro-mechanical mechanism that operates the piano action via pre-programmed music stored on perforated paper rolls.

The predictions of this theory about a modular structure and functional organisation of the frontal cortex are not entirely clear though. However based on these hypotheses theories of a rostro-caudal axis of the PFC supporting a cognitive control hierarchy whereby more anterior areas in the lateral frontal cortex mediate more abstract control processes have been developed.

Cognitive control is often defined as the ability to select actions in accordance with external environmental demands and internal goals. But it is not completely clear what this exactly means and which cognitive processes this might involve. It has therefore proven to be fruitful to study certain areas that seem to be important for those cognitive processes and to describe the computations conducted by those areas. This could help elucidating the components of a complex cognitive operation. In this way neuroscience can help to answer cardinal questions in cognitive psychology.

The idea of a hierarchical organisation in the frontal lobe has proved very popular (Fuster, 2001; Koechlin et al., 2003; Fuster, 2004; Petrides, 2005; Koechlin and Jubault, 2006; Badre and D'Esposito, 2007; Hazy et al., 2007; Koechlin and Summerfield, 2007; Badre, 2008; Haggard et al., 2008; Badre et al., 2009). The contributions made by different prefrontal regions may be distinguished from one another in a variety of ways. For example there might be basic processing differences between regions. Alternatively, areas might differ from one another in terms of the nature of the representations they process, in other words they might perform computationally similar operations but on quite distinct inputs

The perception-action cycle (Fuster, 2001, 2004) was one of the first theories linking the hierarchical organisation of behaviour to a hierarchical organisation of the brain. Already in the spinal cord posterior parts are largely devoted to sensory functions (posterior horn, posterior funiculus) and anterior structures to motor functions

(anterior horn, anterior funiculus). This division can also be discerned in the cerebral cortex and we can view the posterior part of the brain as primarily sensory cortices and their sensory representations and the anterior part of the brain (the frontal lobe) as primary motor cortex (M1) and associated executive representations (Fuster, 2001, 2004). Mnemonic representations are formed in the cortex from the bottom up, i.e. from primary areas of sensation and action, respectively to higher areas of association. Executive memory is therefore stored alongside a posterior-to-anterior axis in the frontal lobes. The lowest hierarchical level is M1, representing and integrating executive memories for simple movements. At increasingly higher levels (and therefore more anterior areas in the frontal cortex) more complex memories, schemas and plans of goal-directed behaviour are represented. Those schemas require the mediation and integration of cross-temporal contingencies (Fuster, 2001). Information about the environment and our behaviour in interaction with it flows along a perception-action cycle from sensory and sensory-association areas to execution and execution-association areas. Strong connections exist between sensory and executive areas of equivalent rank and between different levels of sensory and executive representation, respectively. Increasing environmental demands recruit higher sensory and executive association areas and therefore information flows alongside consequently increasing loops in the perception-action cycle (Fuster, 2001, 2004).

Similar representational hierarchies have emphasised working memory as the cardinal functions of many areas in the PFC (Courtney, 2004; Passingham and Sakai, 2004; Petrides, 2005; Badre, 2008). Domain specificity of working memory content has been hypothesised to distinguish rostral from caudal PFC with caudal PFC representing domain specific information and rostral PFC maintaining information from multiple domains.

According to other authorities, the rostro-caudal gradient of abstractness derives from relational complexity. More anterior parts of the PFC subserve more complex manipulations of working memory content (Badre and D'Esposito, 2007; Badre, 2008).

Some approaches tried to combine ideas about working memory domain specificity and relational complexity (Petrides, 2005). Similar concepts compiled notions of relational complexity and representation of control demand (Badre and D'Esposito, 2007; Badre et al., 2009).

Another approach proposes a top-down executive control hierarchy with different levels processing information (in an information theoretic manner) to guide actions in time (Koechlin and Summerfield, 2007; Haggard et al., 2008). The first level of executive control is called the level of "sensory control" and processes information conveyed by stimuli to guide immediate action. Simple stimulus-response mappings will therefore involve "sensory control" (Koechlin et al., 2003; Koechlin and Summerfield, 2007; Kouneiher et al., 2009). Neural correlates of this level can be found in dorsal premotor cortex (Koechlin et al., 2003; Kouneiher et al., 2009). To reduce uncertainty about immediate decision contextual information is processed in the level of "contextual control". Neural correlates of this level can be found in posterior PFC (BA 9/44). To process information guiding actions in a whole subsequent episode, "episodic control" levels will be recruited. For example stimuli that inform us which task set to employ in the following period of time will engage anterior PFC (BA 46) (Koechlin et al., 2003; Kouneiher et al., 2009). The level of "branching control" allows us to select action representation based on a pending temporal context. Processing of "branching control information" takes place in the most anterior part of the lateral PFC, in the frontopolar cortex (FPC, BA 10)

(Koechlin and Hyafil, 2007; Koechlin and Summerfield, 2007). FPC is therefore crucially important for all types of multitasking. Effective connectivity analysis of the fMRI data supporting this theory has suggested: along the hierarchy information is passed from higher to lower levels (from anterior to posterior) accumulating in the premotor and motor areas to guide behaviour (Koechlin et al., 2003; Miller and D'Esposito, 2005; Koechlin and Summerfield, 2007; Haggard et al., 2008; Kouneiher et al., 2009). Another hierarchy of motivational control has been proposed in the medial frontal cortex (MFC) (Kouneiher et al., 2009) with the dorsal MFC "energising" areas of executive control in the lateral PFC in simple highly incentive situations (contextual motivation, equivalent to contextual control) and the anterior MFC recruiting lateral PFC areas in ongoing highly incentive episodes (episodic motivation).

Although these theories of frontal lobe executive control hierarchies seem extremely attractive to many working in cognitive neuroscience they have limitations: The prefrontal cortex might not be the only area implementing cognitive control. It might be sensible to include the basal ganglia in those theories of control hierarchies as well as the parietal cortex (Coulthard et al., Neuron, 2008). Moreover those models often propose only one function (corresponding to one level of executive control within the hierarchy) for each area in the lateral PFC. A within-trial time-series analysis of the activity in PFC regions might help us to understand the different contributions of a single area to both executive control and decision making during the time course of a decision. More time-sensitive measurements of neural processing than fMRI could also help answering the question of frontal lobe architecture and function.

Right Inferior Frontal Cortex (rIFC) and Response Inhibition

The concept of inhibition has been very influential and it might be argued that it has been overextended. It remains to be proven if it can be applied to processes in working memory, emotion, cognitive control or other forms of higher cognition in a sensible way (Aron, 2007).

Inhibitory mechanisms in executive control could serve as a helpful model for the research on inhibition. It is not objectionable to employ deductive strategies (i.e. use general principles to explain or predict individual phenomena) in empirical science. And the theoretical concept of inhibition could proof to be fruitful in cognitive neuroscience. But empirical data needs to show whether the hypothetical notion of inhibition is grounded in actual brain mechanisms and how it is implemented in behaviour.

A sensible heuristic framework could be the idea that certain brain areas influence the processing in other areas in a top-down manner (Miller and Cohen, 2001). An influence exerted by one brain area (the source) over another brain area (target area) that leads to measurable decrease of activity or output of the target area could then just be considered as inhibition (Aron, 2007).

However it is not easy to test this hypothesis in human participants as it is not clear to which extend inhibition can be reliably uncovered in imaging experiments (Waldvogel et al., 2000). As discussed previously some neuroscientific concepts tried to propose integrated theories of the frontal lobe and how neuronal processing could elicit cognitive control (Fuster, 2001; Miller and Cohen, 2001; Sakai and Passingham, 2003; Koechlin and Summerfield, 2007; Badre, 2008). Others try to map distinct processes to discrete regions of the prefrontal cortex.

Converging evidence suggests that the prefrontal cortex exerts its effects on posterior cortical and subcortical areas by inhibitory mechanisms and that the right inferior frontal cortex (see Figure 1) might play a crucial role. Patients with lesions in rIFC have been reported to show impaired stopping performance (Aron et al., 2003a). Moreover the stop signal reaction times (SSRTs) were positively correlated with the volume of brain damage in pars triangularis (BA45) and pars opercularis (BA44).

Imaging studies have shown an increased BOLD response in rIFC in stop as opposed to go conditions (Konishi et al., 1998a; Aron and Poldrack, 2006; Aron et al., 2007a). Subjects who inhibited more quickly (shorter SSRTs) activated rIFC more strongly.

Studies employing action reprogramming (Mars et al., 2007b), cognitive set shifting and task switching (Konishi et al., 1998b; Konishi et al., 1999; Dove et al., 2000) and response inhibition paradigms other than the go/no-go task (Garavan et al., 1999) showed a right hemispheric dominance of inhibitory control and suggested that rIFC areas play an essential role in this process.

However activity in rIFC areas during response inhibition does not necessarily imply an inhibitory influence of rIFC over the motor cortex on a neural level (Aron et al., 2004a). Evidence comes from monkey neurophysiology (Sasaki et al., 1989). A no-go potential i.e. activity specific to no-go trials was recorded 110 to 150 ms after cue onset (consistent with common SSRTs) over the caudal part of the dorsal bank of principal sulcus and also in the rostroventral corner of the prefrontal cortex in five macaca fuscata monkeys. Electrical stimulation of those no-go foci produced reduced electrical activity in the motor cortex and cancelled manual responses in go-trials.

TMS experiments have shown increased short-intracortical-inhibition (SICI, a measurement of internal M1 excitability that is thought to reflect the activity of

inhibitory interneurons) related to stop trials and decreased SICI in go trials in go/no-go tasks (Waldvogel et al., 2000; Coxon et al., 2006). Inhibition was found to be impaired after a repetitive TMS virtual lesion of the rIFC (Chambers et al., 2006).

Pathways have been suggested through which the prefrontal cortex might influence the motor cortex. Motor output is primarily regulated by basal ganglia loops (Nambu et al., 2000; Nambu et al., 2002; Nambu, 2008). The so called "direct pathway" arises from GABA-ergic striatal neurons and projects monosynaptically to the globus pallidus internus. The "indirect pathway" arises from different GABAergic striatal neurons and projects polysynaptically via the globus pallidus externus and the subthalamic nucleus to the globus pallidus internus. Traditionally the direct pathway is thought to release the thalamo-cortical drive therefore facilitating motor output whereas the indirect pathway was suggested to inhibit the motor cortex. The direct pathway though has a much shorter conduction time than the indirect pathway due to a smaller number of synapses. However in go/no-go experiments SSRTs are much shorter than go-RTs (normally around 170 compared to 380 ms, of course depending on the exact experimental paradigm, (Aron et al., 2007a; Aron et al., 2007b; Aron and Verbruggen, 2008)) indicating that already initiated motor commands can easily be overtaken and abolished. This requires a much faster and more efficient inhibitory pathway.

Recent anterograde double-labelling studies have shown that the subthalamic nucleus (STN) in monkeys receives projections from the motor cortex, the supplementary motor cortex, from dorsal and ventral premotor (Nambu et al., 1996; Nambu et al., 2002). Ventral premotor cortex (PMv) is in fact the most posterior part of the rIFC and could together with its projections to the STN (PMv → STN → pallidum) be considered the centre of an inhibitory network that was often attributed to rIFC (see

Table 1). (Chikazoe et al., 2009) suggests that there are at least two subregions in rIFC which are anatomically very close to each other but functionally different. A region referred to as the "posterior inferior frontal gyrus" (pIFG) is specifically activated during response inhibition, but not during processing of infrequent and surprising stimuli. The "inferior frontal junction" however was found activated primarily during processing of infrequent stimuli. This pIFG region could be identical with PMv.

The hyperdirect pathway (cortico-subthalamo-pallidal) conveys powerful excitatory effects from frontal areas to the globus pallidus externus bypassing the striatum with shorter conduction times than the direct and indirect pathway and inhibits thalamo-cortical drive. This hyperdirect pathway is thought to play a role in preparation of movement initiation by inhibiting motor programs widely to clear the way for distinct motor outputs via the direct pathway. It could however also play a role in response inhibition (Nambu et al., 2002; Aron and Poldrack, 2006; Aron et al., 2007a; Aron et al., 2007b; Isoda and Hikosaka, 2008; Nambu, 2008).

Imaging studies have shown the subthalamic nucleus active predominantly during no-go trials in a go/no-go task (Aron and Poldrack, 2006; Aron et al., 2007a). Activity in STN was correlated with right IFC activity. A DTI analysis revealed a "direct" connection between rIFC and STN in 9 out of 10 subjects (Aron et al., 2007a). Moreover both areas were found to be connected to the pre-supplementary motor area (STN in 7 out of 10, rIFC in 8 out of 10 subjects). Those findings were interpreted in terms of pre-SMA monitoring response conflict, control demand or uncertainty recruiting a rIFC-STN inhibitory control system ((Aron et al., 2007a); however note the completely converse findings and hypotheses in (Duann et al., 2009)).

Lesions to the basal ganglia in experimental animals and humans impairs stopping performance (Eagle and Robbins, 2003). Deep brain stimulation improves inhibition

of motor responses in Parkinson's disease (van den Wildenberg et al., 2006). Recordings from neurons in the subthalamic nucleus in monkeys found neurons that were related to action reprogramming in an antisaccade task (Isoda and Hikosaka, 2008). The majority of those neurons appeared to inhibit the undesired response. However others were related to facilitation of desired action. The inhibition related activity emerged 174 ms after cue onset (Isoda and Hikosaka, 2008).

Computational modelling of prefrontal cortex basal ganglia interactions has proposed a more general role of those circuits in executive function and working memory updating (Hazy et al., 2007).

However the cortico-subthalamo-pallidal pathway is unlikely to be the only pathway for response inhibition. The ventral premotor cortex (the most posterior area in rIFC) is most likely to influence motor output via direct cortico-cortical projections (Tomassini et al., 2007) during execution of more complex action sequences (Koechlin and Jubault, 2006; Davare et al., 2008; Davare et al., 2009) or when action sequences have to be inhibited (Buch et al., submitted). Both right IFC and pre-SMA could influence motor output not only via the cortico-subthalamo-pallidal route (Aron et al., 2007a) but also through direct cortical projections, e.g. via dorsal premotor cortex (Johansen-Berg et al., 2004; Mars et al., 2009). If the cue telling the subject to revoke a certain motor response occurs very early in the stage of motor planning, (e.g. if the interval between go and no-go signal is very short) it might be that response inhibition operates on an earlier stage in frontal planning regions. A go/no-go imaging experiment focusing on the activity of the hyperdirect pathway (via STN) found the no-go related activity in STN correlated with the stop signal delay (SSD) (Aron and Poldrack, 2006) indicating that early stage inhibitory processes (short SSD) might employ other, intra-cortical pathways.

Recent research has distinguished a global mechanism of stopping (Coxon et al., 2007; Aron and Verbruggen, 2008) that appears to affect not just the initiated action tendency but multiple tendencies from a much slower selective stopping mechanism. Global stopping could be achieved via the hyperdirect pathway whereas selective stopping could engage the indirect pathway or intra-cortical pathways. However further research needs elucidate the different neuronal bases of those two stopping processes.

Some studies suggest other areas in the frontal and parietal lobes being equally important for inhibition in executive control. The already mentioned DTI/fMRI triangulation study (Aron et al., 2007a) did not only find the STN to be connected to the right inferior frontal cortex but both areas to be directly connected to pre-SMA. The neuronal recordings in the subthalamic nucleus showed (Isoda and Hikosaka, 2008) similar activity patterns to recordings in the pre-SMA (Isoda and Hikosaka, 2007) in the same monkeys performing the same task. Imaging studies have shown areas in the medial frontal cortex (Nachev et al., 2005; Braver and Barch, 2006; Dosenbach et al., 2006; Forstmann et al., 2008b) and dorsolateral prefrontal cortex (Brass et al., 2003; Li et al., 2006) being related to response inhibition and task switching. Inhibition of unconscious response tendencies could rely on the supplementary motor cortex (Sumner et al., 2007) and the indirect striatal-pallidal-thalamic pathway (Aron et al., 2003b).

Research investigating the organisation and representation of complex action sequences such as precision grasping sometimes found a network centred on ventral premotor cortex exerting facilitatory and inhibitory control over motor output (Olivier et al., 2007; Davare et al., 2008; Davare et al., 2009; Buch et al., submitted). However this area of action *programming* and *reprogramming* could be similar to the area in

the right inferior frontal cortex that has been linked to selection and inhibition of motor plans (see table Table 1:) (Konishi et al., 1998b; Garavan et al., 1999; Dove et al., 2000; Huettel et al., 2005; Aron and Poldrack, 2006; Koechlin and Jubault, 2006; Aron, 2007; Aron et al., 2007a; Mars et al., 2007b; Braver et al., 2009). Regrettably those studies rarely refer to each other.

x	y	z	behavioural context/ paradigm	study
44	**12**	**18**	SSRT related activity in a go/no-go task, referred to as **right IFC**	(Aron and Poldrack, 2006)
50	**16**	**20**	Activity related to inhibition and conflict in a go/no-go task, referred to as **right IFC**	(Aron et al., 2007a)
39	**15**	**22**	Activity related to cognitive set shifting in a WCST paradigm, referred to as **right inferior frontal areas**	(Konishi et al., 1998b)
36	**23**	**33**	Activity related to inhibition in a response-inhibition task, referred to as **right inferior frontal gyrus**	(Garavan et al., 1999)
60	**18**	**2**	Activity related to action reprogramming, referred to as **right inferior frontal gyrus**	(Mars et al., 2007b)
40	**8**	**36**	Activity related to task switching, referred to as **right inferior frontal sulcus**	(Dove et al., 2000)
44	**12**	**32**	Activity related to organisation of action chunks, referred to as **BA 44**	(Koechlin and Jubault, 2006)
55	**10**	**35**	Activity related to decision uncertainty, referred to as **right inferior frontal gyrus**	(Huettel et al., 2005)
36	**16**	**4**	Conjointly analysed data from mixed design experiments using ten different tasks and 183 subjects. Aim of the study was to extract a core system for the implementation of task sets, the authors found (a) start cue related activity (b) sustained block activity and (c) error related activity in posterior MFC and the **frontal operculum**	(Braver and Barch, 2006; Dosenbach et al., 2006)
56	**16**	**16**	Subjects performed a go/no-go task, activity related to no-go trials compared to *infrequent* go-trials, referred to as **posterior IFG**	(Chikazoe et al., 2009)
58	**13**	**19**	Interaction with primary motor cortex at rest and during grasping, referred to as **ventral premotor** (**PMv**)	(Davare et al., 2008)
60	**16**	**23**	Involved in precision grasping, especially in positioning of fingers on the object, referred to as **PMv**	(Davare et al., 2006)
55	**15**	**30**	Inhibitory influences onto the motor cortex when grasping movements had to be inhibited and reprogrammed, referred to as **PMv**	(Buch et al., submitted)

Table 1: Studies on action programming, reprogramming and inhibition with activations in posterior rIFC and PMv

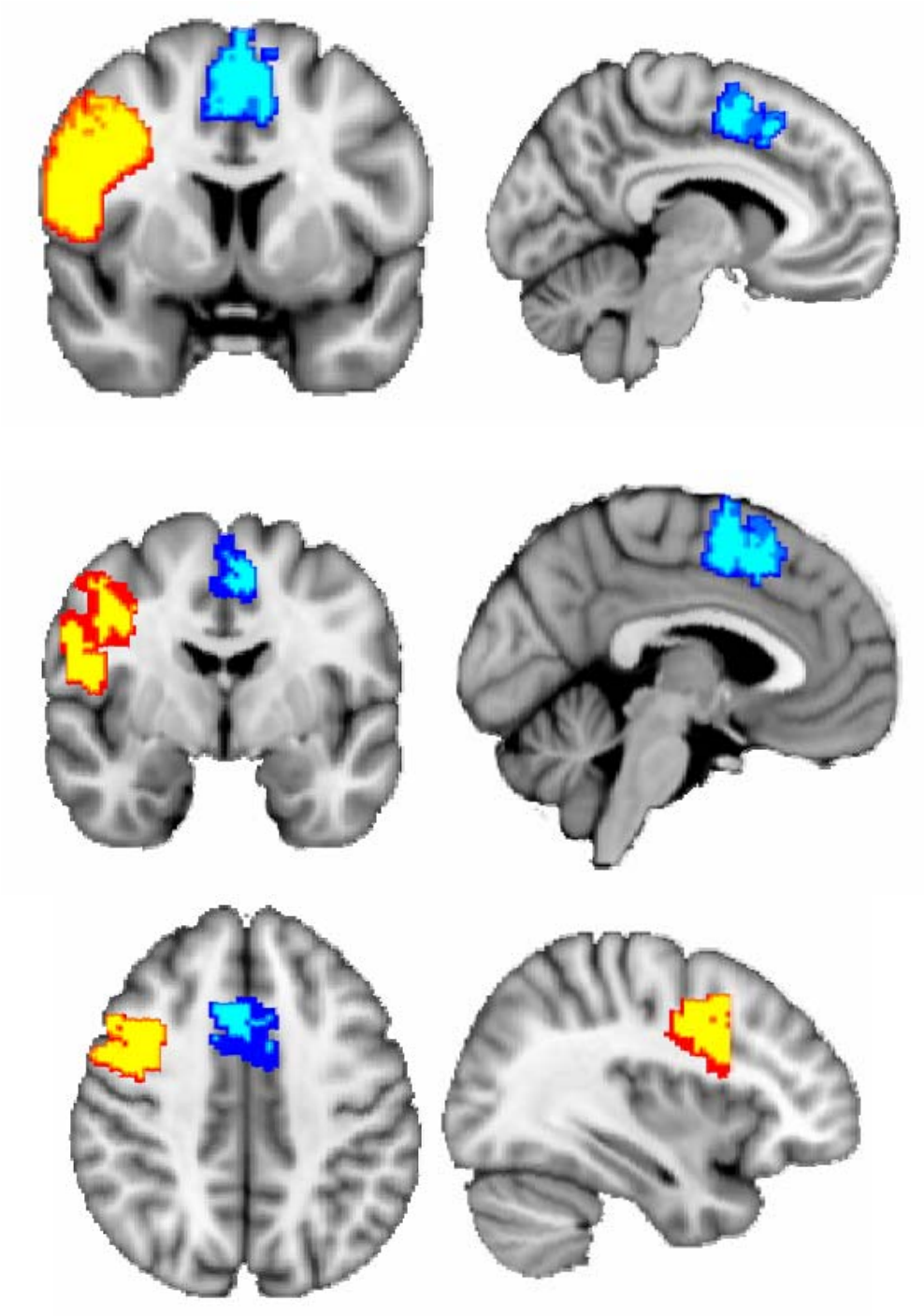


Figure 1: Mask of left and right pre-SMA (blue) derived from a study investigating connectivity profiles of dorsal medial PFC regions by (Johansen-Berg et al., 2004) and kindly provided by Johansen-Berg and colleagues. Mask of right PMv (orange) derived from a study by (Tomassini et al., 2007) and kindly provided by Johansen-Berg and colleagues. Masks are projected onto the T1-weighted MNI-152 brain template. These masks were used as seed masks in the Probabilistic Diffusion Tractography, described in Chapter 2.2.

Functional Role of the Pre-Supplementary Motor Areas

The pre-supplementary motor area is located on the medial aspect of the brain (see Figure 1) that is often called the "dorso-medial PFC". Lesions to this area can lead to a variety of different neuropsychological symptoms, such as alien limb syndrome, utilisation behaviour, or motor neglect (Nachev et al., 2008).

It is under debate whether the supplementary motor area (SMA), the supplementary eye field (SEF) and the pre-supplementary motor area (pre-SMA) can be subsumed as the "supplementary motor complex (SMC) (Nachev et al., 2008). It has even been argued that instead of discrete subregions, there might be a rostro-caudal continuum of graded change in structure and function. This rostro-caudal gradient might then even be organised as a hierarchy responsible for the resolution of conflict of increasing complexity (Nachev, 2006; Nachev et al., 2008). However there is strong evidence for big differences in connectivity patterns in pre-SMA (anterior of the plane of the anterior commissure, i.e. $y > 0$) and SMA ($y < 0$) (Johansen-Berg et al., 2004).

Whereas the SMA makes substantial contribution to the corticospinal tracts (~ 10%) and shows reciprocal connections with the primary motor cortex, pre-SMA seems more directly connected to the PFC and premotor areas. Pre-SMA, as well as SEF and SMA are connected to the basal ganglia via direct, indirect and hyperdirect pathways (Nambu et al., 1996; Nambu et al., 2002; Nachev et al., 2008). Moreover, electrical stimulations to the SMA elicited (mostly complex) movements and suggested a somatotopic organisation of SMA, whereas stimulations to the pre-SMA inhibited actions or improved stopping and "switching behaviour" (Isoda and Hikosaka, 2007; Nachev et al., 2008).

The lack of clear somatotopy and the stronger connections to the PFC inspired suggestions of the pre-SMA being more akin to prefrontal cortex and playing a role in more complex cognitive processes such as internally guided actions (Kennerley et al., 2004; Lau et al., 2004; Nachev et al., 2005; Nachev et al., 2008; Soon et al., 2008), learning and executing complex actions and action sequences (Shima and Tanji, 1998; Rushworth et al., 2002b; Isoda and Tanji, 2004; Kennerley et al., 2004; Koechlin and Jubault, 2006), imagination of movements and temporal judgements (Nachev et al., 2007; Forstmann et al., 2008b; Soon et al., 2008) and of course in decision making processes for detecting and/or solving response conflict, task switching and response inhibition (Ridderinkhof et al., 2004; Rushworth et al., 2004; Nachev et al., 2005; Braver and Barch, 2006; Dosenbach et al., 2006; Nachev et al., 2007; Sumner et al., 2007; Taylor et al., 2007; Forstmann et al., 2008b; Forstmann et al., 2008a; Nachev et al., 2008; Rushworth, 2008; Duann et al., 2009; Kouneiher et al., 2009; Mars et al., 2009). Several attempts to interpret the multiplicity of functions indicated by these and other data as aspects of one fundamental function have been made (Ridderinkhof et al., 2004; Rushworth et al., 2004; Nachev et al., 2007; Nachev et al., 2008; Rushworth, 2008).

Pre-SMA was found to be active in self-initiated actions as opposed to externally cued actions (Nachev et al., 2008) especially when participants were completely free to choose their actions and when they were asked to pay attention to the intention to move (Lau et al., 2004; Soon et al., 2008). Therefore it has been argued that pre-SMA represents motivational and volitional states (Lau et al., 2004; Kouneiher et al., 2009) and conscious awareness of those states might just enhance the pre-SMA activity. Pre-SMA was even found to predict the “free” choice of subjects about 5 seconds before they entered awareness (Sohn et al., 2003). It especially seemed to predict the

timing of the movement. Others ascribed the self initiation of movements to more anterior structures in the MFC, especially to the anterior cingulated cortex (Rushworth et al., 2004).

Pre-SMA activity has also been associated with performance of movement sequences (Shima and Tanji, 1998; Isoda and Tanji, 2002, 2003, 2004). Pre-SMA was found to be active at the beginning of sequences, at the transition between single sequence components or with respect to the number of movements that remain to be made in order to finish the sequence. Especially the latter finding has led to the notion that pre-SMA might represent the general cognitive aspects of sequential behaviour in an effector-independent manner (Isoda and Tanji, 2004; Nachev et al., 2008). Moreover dependent modulations of those motor-related signals could suggest an organisation of sequential behaviour with respect to motivational states or the expectation of reward occurrence (Isoda and Tanji, 2004; Kouneiher et al., 2009). Whereas the dorsal premotor cortex (PMd) is associated with the selection of single actions in response to stimuli, pre-SMA seems to represent, select and retrieve action and even task sets (Rushworth et al., 2002b; Kennerley et al., 2004; Rushworth et al., 2004; Koechlin and Jubault, 2006). An imaging experiment investigating the hierarchical organisation of human behaviour and the representational hierarchy of simple and complex action chunks reported both, an anterior-to-posterior hierarchy of action sequencing in the lateral, inferior frontal cortex and sustained activity in the pre-SMA (Koechlin and Jubault, 2006).

Notions about the role of pre-SMA in representing the architecture of behavioural sets and sequences could be related to its potential role in cognitive control and task set switching: pre-SMA was proposed to be part of a core system for task set implementation (Braver and Barch, 2006; Dosenbach et al., 2006). In the analysis of

mixed design experiments using 10 different tasks and 183 subjects only pre-SMA and IFC were found to be consistently active in every task type and showed start-cue activity, sustained activity and error related activity. Although a popular view is that the anterior cingulate cortex (ACC) monitors conflict and subsequently recruits lateral PFC areas to adjust cognitive control (Miller and Cohen, 2001; Kerns et al., 2004) many authors now rather point to pre-SMA (Ridderinkhof et al., 2004; Rushworth et al., 2004; Nachev et al., 2008). At the end they could both play a role in recruiting lateral prefrontal executive control areas in cognitively demanding but incentive situations (Kouneiher et al., 2009). Pre-SMA was also implicated to be of special importance if voluntary action plans need to be changed (Nachev et al., 2005; Isoda and Hikosaka, 2007; Mars et al., 2009) or undesired actions ought to be inhibited (Passingham et al., 2002; Isoda and Hikosaka, 2007; Sumner et al., 2007; Forstmann et al., 2008a).

It is tempting to subsume all those functions that pre-SMA was thought to carry out under one single general function. And it was suggested that this function might be the resolution of competition between motor plans in order to produce unitary actions (Nachev et al., 2007; Nachev et al., 2008) or the flexible change of task and action sets (Rushworth et al., 2004; Rushworth, 2008). But we might be well advised to resist this temptation as it seems unlikely that the multiplicity of these processes can be easily accounted for by one single function without running the risk of simplification.

1.2. Methodology

1.2.1. TMS

In the Middle Ages magnets were attributed great and bizarre powers to heal all kinds of diseases, such as arthritis, gout, baldness or epilepsy (Walsh and Pascual-Leone, 2003). Paracelsus (1493-1541) claimed every person had magnetic powers and based on these notions Franz Mesmer (1734-1815) later developed his ideas of "magnétisme animal" or "mesmerism". Every animate being was supposed to comprise a magnetic fluid or ethereal medium. This "animal" magnetic force (opposed to mineral magnetism in e.g. ferric oxide containing stones) could get disorganised and hence caused diseases like hysteria, ophtalmoplegia, delirium, aches and pains (Porter, 2003; Walsh and Pascual-Leone, 2003). But trained and gifted individuals were supposed to be able to use their own magnetic forces to reorganise the "nervous system" to harmony. In developing his techniques of animal magnetism Mesmer actually created a very early form of hypnosis instead of exploring a proper medical treatment based on magnetism. But it appears ironical that TMS as a technique actually based on the principle of electromagnetic induction might eventually return to psychiatry and psychotherapy in order to treat depression or schizophrenia (Hallett, 2000, 2007; Rossini and Rossi, 2007).

D'Arsonval 1896 in Paris, Beer 1902 in Vienna, Thompson 1910 in London and Dunlap 1911 in Baltimore had already reported that rapidly changing magnetic fields applied to the retina, to the optic nerve or the occipital cortex could induce phosphenes (Walsh and Pascual-Leone, 2003). In 1965 Bickford and Fremming applied magnetic stimulations to peripheral nerves and muscles obtaining twitches in healthy human subjects. In 1980 Merton and Morton excited the human primary

motor cortex with transcranial electrical stimulation (TES). On 12th February Anthony Barker and his colleagues from the University of Sheffield attempted for the first time to stimulate the human brain with magnetic pulses in Merton's laboratory at the National Hospital in London (Barker et al., 1985; Walsh and Pascual-Leone, 2003). The experiment was immediately successful with clearly visible muscle twitches without discomfort for the subject (opposed to TES, which is a relatively painful procedure). A very short report about the experiment was published a few months later in The Lancet (Barker et al., 1985)(see Figure 2).

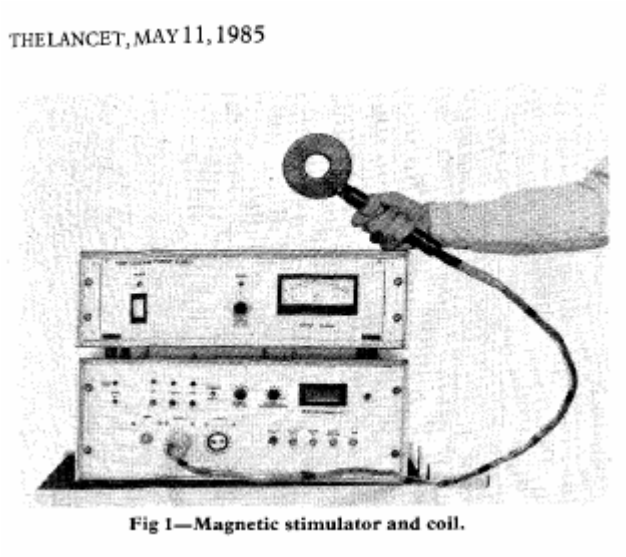

THE LANCET, MAY 11, 1985

Fig 1—Magnetic stimulator and coil.

Figure 2: Picture from the original publication by (Barker et al., 1985) reporting the first application of TMS to a human brain.

Since then, TMS became increasingly famous in physics, mathematics, computer science, neurophysiology, cognitive neuroscience (e.g. in vision, visual cognition, action, language, memory, reasoning etc.) and medicine (e.g. diagnostic, therapeutic applications etc.). The number of TMS papers published in peer-reviewed journals has increased progressively and steadily in the last 20 years (Rossini and Rossi, 2007). Many attempts have been made to combine TMS with other techniques in

neuroscience such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), electroencephalography (EEG), diffusion tensor imaging (DTI) and single unit recordings in animals (Hallett, 2000; Walsh and Cowey, 2000; Hallett, 2007; O'Shea et al., 2008; Driver et al., 2009).

The sequence of delivery of a single magnetic pulse begins with an electric current of up to 8 kA, generated by a capacitor and discharged into a circular or figure-of-eight shaped coil (Walsh and Cowey, 2000; Stewart et al., 2001a; Wasserman et al., 2008). The electric current hence produces a magnetic field of up to 3 Tesla. The pulse has a rise time of about 100 μs and lasts for less than 1 ms. The magnetic field induces an electric field in a nearby coil (Faraday's principle of electromagnetic induction) which is, in the case of transcranial stimulation, the brain tissue (see Figure 3).

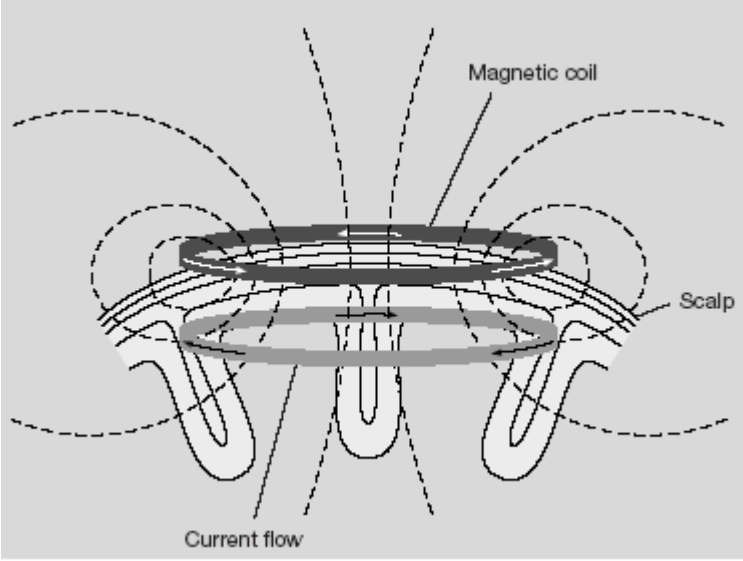


Figure 3: A high-current pulse is produced in a coil of wire (dark grey circle) which is placed above the scalp. A magnetic field is induced perpendicular to the plane of the coil (dotted lines). An electric field is induced perpendicular to the magnetic field and proportional to the change of the magnetic field (light grey circle). Figure taken from (Hallett, 2000).

The induced electric field is proportional to the change of the magnetic field (Wasserman et al., 2008). Neuronal elements are activated by the electric field by two mechanisms. If the field is parallel to the neuronal element, then the field will be most effective where the intensity changes as a function of distance. If the field is not completely parallel to the neuronal element, activation will occur at the bends of the neuron or its axon (Hallett, 2007). The effects of the magnetic pulse on neural populations has been shown to depend crucially on (1) the waveform of the coil-current (mostly biphasic and monophasic waves); (2) the direction of the current (electric current in the brain is always directed opposite to the current in the coil); (3) intensity of the stimulation (often expressed as a "percentage of maximum stimulator output" or percentage of the resting motor threshold- see below); (4) form and type of the TMS coil (e.g. round coil, figure-of-eight shaped coil, iron-core coils, H-coils). It is worth paying attention to the exact design of a TMS experiment as the effects and findings crucially depend on those parameters and can change dramatically when only one single parameter is modified (Wasserman et al., 2008).

Among the many techniques used in cognitive neuroscience TMS is the only one that allows us to interfere actively with brain function (Pascual-Leone et al., 2000). Although the experimenter's input to the brain during a TMS experiment is always very similar – a magnetic pulse – the output he is actually interested in can be rather diverse and there are many measures and quantifications of TMS effects on the brain (see Table 2).

The first application of a transcranial magnetic pulse was over the primary motor cortex (Barker et al., 1985). In fact TMS has especially been used to measure various

parameter	definition	application	physiological effect	use
Resting Motor Threshold (RMT)	intensity required to elicit a 50-µV MEP with 50% probability in a completely relaxed muscle	TMS over the motor cortex	membrane excitability of excitatory interneurons that directly project onto corticospinal neurons	clinical, research, pharma-cology
Active Motor Threshold (AMT)	intensity required to elicit a 100-200 µV MEP in a slightly contracted muscle	TMS over the motor cortex	membrane excitability of corticospinal neurons and excitatory interneurons that directly project onto corticospinal neurons	clinical, research, pharma-cology
Phosphene Threshold	minimum magnetic stimulation intensity capable of eliciting phosphenes	TMS over the visual cortex	excitability of (primary) visual cortex	research
Central motor conduction time (CMCT)	conduction time from the motor cortex to the spinal cord and brainstem motoneurons	TMS over M1 and MEP latency measurement	conduction velocity of the pyramidal tracts	clinical: diagnosis surgery
recruitment curve	relationships between TMS pulse intensity and muscle twitch (MEP size)	TMS at different intensities over M1	progressive recruitment of less excitable interneurons and pyramidal neurons	clinical, research, pharma-cology
Motor Evoked Potential (MEP)	EMG-activity induced by the stimulation of M1 (peak to peak amplitude or area under the curve)	TMS over the motor cortex, EMG recording	cortico-spinal excitability, integrity of the cortico-spinal tracts	clinical, research, pharma-cology
Silent Period (SP)	suppression of voluntary EMG activity following the magnetic pulse applied over M1.	TMS over M1, EMG recording	spinal cord refractoriness (only first 50 ms of SP) and intra-cortical inhibitory mechanisms (possibly GABA-B mediated; similar to LICI? → see Table 3)	clinical, research, pharma-cology

paired-pulse TMS measure-ments	a conditioning pulse (CP) preceding the actual test pulse changes the amplitude of the test pulse MEP depending on (1) CP intensity; (2) inter-pulse interval; (3) area of CP stimulation; (4) cognitive state	two TMS pulses in quick succession over the same or different brain area(s).	excitatory or inhibitory influences of “distinct” interneuron-populations or distant brain areas onto M1 cortico-spinal excitability (→ Table 3)	clinical, research, pharma-cology
cortical mapping	area in the motor cortex where TMS elicits MEPs from a certain muscle	TMS over the motor cortex	Cortical representation of muscles in the primary motor cortex	clinical, research
plasticity	depression or increase of excitability of the stimulated region after application of different repetitive TMS protocols	low-frequency rTMS, high-frequency rTMS, theta-burst stimulation	Long-term potentiation (LTP), long-term depression (LTD)	clinical, research
behavioural effects after virtual lesion	behavioural impairment following TMS over a certain brain area	TMS over a certain brain area, RTs, error rates	Magnetic stimulation leads to artificial and inefficient activity in the stimulated area, introduces noise and disrupts processing	research

Table 2: Measures and quantifications of TMS effects on the brain (Hallett, 2000; Pascual-Leone et al., 2000; Walsh and Cowey, 2000; Walsh and Pascual-Leone, 2003; Di Lazzaro et al., 2004; Hallett, 2007; O'Shea and Walsh, 2007; Rossini and Rossi, 2007; O'Shea et al., 2008; Wasserman et al., 2008).

parameters in the motor system. The most common definition of *the resting motor threshold* (RMT, see Table 2) is the intensity of the magnetic stimulation (often measured in percentage stimulator output, not in kA or Tesla) necessary to elicit a very small twitch (a 50-μV potential in the EMG recording) with 50% probability in a completely relaxed muscle (Hallett, 2000, 2007; Rossini and Rossi, 2007; Wasserman et al., 2008). This measurement allows us to evaluate M1 corticospinal excitability and changes in excitability due to brain plasticity and disease. RMT can be influenced by drugs that affect Na^{+} and Ca^{++} channels and is therefore likely to indicate membrane excitability (Hallett, 2007; Wasserman et al., 2008). The TMS induced posterior-to-anterior brain current flow across the central sulcus (anterior-to-posterior flow in the TMS coil) is supposed to stimulate excitatory interneurons that directly project onto pyramidal neurons (I_1 waves in epidural spinal cord recordings) (Di Lazzaro et al., 2004; Wasserman et al., 2008). Therefore RMT does not represent the excitability of pyramidal neurons (as for example TES) but the excitability of excitatory interneurons that directly (I_1 waves) or indirectly ($I_{2\text{-}4}$ waves) project onto the pyramidal neurons. Pyramidal neurons are recruited at much higher intensity (possibly more than 150% RMT). Additionally RMT is often used as an individual gauge to determine the intensity of the magnetic pulses in different TMS paradigms (see for example Chapter 2.1). The *active motor threshold* (AMT, see Table 2) is a very similar parameter. It is defined as the pulse intensity required to elicit a 100-200-μV EMG-twitch in a slightly contracted muscle. It is normally a bit lower than the RMT because active muscle contraction increases cortico-spinal excitability. Whether it recruits pyramidal cells in addition to "upstream" excitatory interneurons is under debate (Rossini and Rossi, 2007; Wasserman et al., 2008). The *phosphene threshold* (Table 2) is thought to be a similar measurement in the visual system and is defined as

the intensity of a magnetic pulse necessary to evoke the perception of light-flashes (in the absence of visual stimuli). It is thought to reflect the excitability of the visual cortex (Boroojerdi et al., 2002; Kammer et al., 2003; Walsh and Pascual-Leone, 2003). The phosphene threshold is normally higher than the RMT. RMT, AMT as well as the phosphene threshold are very stable within subjects across different TMS sessions, but there does not seem to be an intra-individual correlation between RMT and the phosphene threshold (Stewart et al., 2001b; Boroojerdi et al., 2002).

TMS can also be used to test the speed of conduction in the cortico-spinal pathway. To obtain the *central motor conduction time* (CMCT, see Table 2) which is the time from the motor cortex to the motor neuron pool in the anterior horn of the spinal cord (for arm, body and leg muscles) or to the motor neurons in brainstem nuclei (for face and neck muscles), one has to subtract the peripheral conduction time from the motor evoked potential (MEP) latency (Hallett, 2000, 2007; Wasserman et al., 2008). Slowing in CMCT is seen in patients with multiple sclerosis and other degenerative diseases and it can indicate a spinal stenosis even before it becomes clinically apparent. *Recruitment curves* (Table 2) reflect the growth of the muscle twitches (measured as MEPs) as a function of increase of pulse intensity. This measurement is less well understood but the increase in the MEP with increasing TMS pulse intensity could reflect the recruitment of surrounding interneurons in addition to the "core" interneurons activated at threshold (RMT or AMT) and at very high intensities (>150% RMT) the recruitment of pyramidal cells (Hallett, 2007; Rossini and Rossi, 2007). The *MEP* (Table 2) can be recorded from the muscle of interest and can be quantified as the peak to peak amplitude or the area under the rectified MEP (often referred to as "area under curve"). The MEP has been used to investigate the excitability of the M1 cortico-spinal system and how it is influenced by drugs,

additional pulses over the same or other areas of the brain, cognitive states, diseases etc. The *silent period* (SP, see Table 2) is a pause in ongoing voluntary EMG activity following the application of a magnetic pulse over M1. The duration of the SP is longer in small hand muscles (up to 300 ms) than in leg muscles (less than 100 ms). The first part of the silent period (the first 50 ms) is thought to be due to spinal motoneuron refractoriness. The latter part is entirely due to cortical mechanisms. These mechanisms seem to be mediated by GABA-B receptors (just as long-interval intracortical inhibition, see Table 2 and Table 3). The SP duration has been shown to be altered in several pathological conditions. Some studies indicated a greater sensitivity of SP abnormalities than MEP abnormalities. Therefore exploring the SP duration in pathological conditions could contribute to a better understanding of the underlying pathophysiological mechanisms.

It has been discovered that conditioning pulses preceding the actual M1 pulse (so called “test pulse”) can change the excitability of the motor cortex and hence the MEP amplitude. This approach is often referred to as *paired-pulse TMS* (Civardi et al., 2001; Oliveri et al., 2003; Mochizuki et al., 2004; O'Shea et al., 2007b; Davare et al., 2008; O'Shea et al., 2008; Reis et al., 2008; Davare et al., 2009; Mars et al., 2009; Rothwell et al., 2009). These paired-pulse TMS paradigms vary (1) the intensity of the conditioning pulse; (2) the inter-pulse-interval (IPI) between the conditioning-pulse and the test pulse; (3) the area that is stimulated with the conditioning pulse; (4) the cognitive state: for example paired pulse experiments at rest or while subjects perform a simple task. By varying these parameters the paired-pulse TMS experiments can quantify inhibitory and excitatory influences of distinct interneuron-circuits and other brain areas onto the M1 cortico-spinal output (see Table 3 and for review (Reis et al., 2008)).

TMS can also be used to localise brain function in *space* and *time*. For example magnetic stimulations can be used to determine the representation of muscles in the motor cortex ("*space*") and evaluate plastic changes of these representations (Pascual-Leone et al., 2005; Hallett, 2007). The spatial resolution of TMS is restricted as a TMS pulse will normally change activity in (1) the targeted region; (2) immediately surrounding areas in the cortex and (3) cortical areas anatomically and functionally connected with (1) and (2) (Walsh and Cowey, 2000; Hallett, 2007; O'Shea and Walsh, 2007; Sack et al., 2007). However the effective resolution may be in the order of a few millimetres to 1cm and very good inferences can be made about the role of a certain area if the TMS experiment is designed appropriately and the control experiments are chosen well.

The temporal resolution of TMS is relatively high compared to other brain imaging techniques (such as fMRI or PET) and the functional contribution of a certain brain area to a cognitive operation can be clarified by applying single TMS pulses or short trains of pulses during a cognitive task. TMS introduces noise into the neural system stimulated for a very short period of time and can therefore be used as a lesion technique interrupting information processing in a certain area of the brain on a certain time point during task performance (elucidating brain function in "*time*") (Walsh and Cowey, 2000; Walsh and Pascual-Leone, 2003; Hallett, 2007; O'Shea and Walsh, 2007; Miniussi et al., 2009). This technique is known as the *"virtual lesion"* approach and has several advantages over lesion studies in neuropsychological patients and non-human animals. Whereas brain lesions are often less focal and the damaged brain might have undergone months of plastic reorganisation and compensation of deficits the short and very focal TMS pulse reversibly interrupts

paired-pulse effect	conditioning pulse applied over…	inter-stimulus interval	conditioning pulse intensity	neuronal populations
short-interval intracortical inhibition (SICI)	ipsilateral M1	1-5 ms	~70-80% RMT	inhibititory interneurons (GABA-A receptor type)
short-interval intracortical facilitation (SICF)	ipsilateral M1	1-1.5 ms, 2.5-3.0 ms, ~4.5 ms	= />RMT	cortical interneurons
intracortical facilitation (ICF)	ipsilateral M1	10-15 ms	~80% RMT (slightly higher than SICI)	net facilitation consisting of strong facilitation and weaker inhibition (NMDA-rec. type interneurons?)
long-interval intracortical inhibition (LICI)	ipsilateral M1	50-200 ms	> RMT; 110% RMT	earlier effects result from spinal inhibitory mechanisms, later from cortical, GABA-B rec. mediated
interhemispheric inhibition (IHI)	contralateral M1	7 ms or more	~RMT	transcallosal route, I2 and I3 waves are inhibited
interhemispheric facilitation (IHF)	contralateral M1	4-5 ms	~RMT	transcallosal route, I3 waves are facilitated
ipsilateral silent period (iSP)	contralateral M1			transcallosal route (posterior half of the trunk?), I2 and I3 waves are inhibited
interhemispheric PMd-M1	contralateral PMd	8-10 ms	90-110 % RMT	Commissural fibres between PMd and contral. M1, inhibition
cerebellar inhibition CBI	inion	5-6 ms		Cerebello-thalamo-cortical pathway
long-latency afferent inhibition (LAI)	median nerve	20-600 ms		at longer latencies (more than 200 ms) this inhibition has been shown to be mostly cortical in origin

Table 3: Paired-pulse TMS effects, their respective protocol and the presumed underlying neurophysiological mechanisms (Civardi et al., 2001; Mochizuki et al., 2004; Hallett, 2007; Rossini and Rossi, 2007; O'Shea et al., 2008; Reis et al., 2008; Wasserman et al., 2008; Rothwell et al., 2009).

processing of an area in healthy subject's brains without allowing long-term compensatory mechanisms. Measurements of behavioural impairments can be drawn from reaction times, error rates etc. (just as in lesion normal studies). The virtual lesion (TMS) condition is contrasted with a no-TMS condition, a sham-TMS condition or a virtual lesion condition of another brain area.

TMS can also be used to induce plastic changes in the brain. These paradigms can be a model to asses and understand brain plasticity and its underlying mechanism. Additionally these paradigms could possibly be used therapeutically. In animal experiments it has been possible to change the efficacy of synaptic transmission by repetitive electrical stimulation of central nervous pathways through mechanisms of long-term potentiation (LTP) and depression (LTD). Repetitive TMS (rTMS) has tried to transfer these effects to human subjects (Huang et al., 2005). RTMS at slow rates of 0.2 to 1 Hz has been shown to decrease brain excitability (see Chapter 2.3), whereas rTMS at faster rates (more than 5 Hz) is supposed to increase excitability. Repetitive stimulation protocols delivering very short, very high frequency trains of pulses (e.g. three pulses of 50 Hz) at theta frequency of about 5 Hz have been shown to yield even stronger excitatory or inhibitory effects (Huang et al., 2005). This protocol is referred to as theta burst stimulation (TBS). A typical TBS paradigm would be three pulses at 50 Hz repeated at 5 Hz. If given intermittently as 2 seconds of stimulation every 10 seconds this leads to increased excitability. If given continuously it leads to decreased excitability. Reasonably short TBS applications (40 seconds) have been shown to yield relatively long lasting effects (up to 60 minutes) (Huang et al., 2005; Hallett, 2007). These longer lasting effects of rTMS applications were hoped to be useful in clinical contexts.

As mentioned before, TMS can help to diagnose several pathological conditions, such as demyelisation in multiple sclerosis, compressive myelopathy following spinal disc herniation, or the severity of stroke in the acute stage (Hallett, 2000, 2007; Wasserman et al., 2008). It has as well been used to investigate processes of pathological plasticity and pathogenesis. The representation of muscles in the motor cortex has been shown to change after the amputation of a limb. Cortical representations of the muscles proximal to the amputation seem to expand into the territory of the amputated part (Hallett, 2007). Some of these changes were argued to occur rapidly. Hand muscle representations increased over a 5 day period in normal subjects as they learned a skilled task with their hands (Pascual-Leone et al., 2005). TMS has been used to understand the pathogenesis of psychiatric and neurological diseases such as epilepsy, focal dystonia, Parkinson's disease, Huntington's disease, Tourette's syndrome, cerebellar ataxia and migraine (Wasserman et al., 2008). In epileptic patients a loss of intracortical inhibition (see Table 3), both short-interval (SICI) and long-interval (LICI) and an increase of intracortical facilitation (ICF) has been reported, although the motor threshold seems to be normal (Hallett, 2007; Wasserman et al., 2008). In patients with focal hand dystonia (such as musician's and writer's cramp) the slope of the recruitment curve (see Table 2) was found to be steeper and SICI was diminished. Parkinson's disease patients and patients with Tourette's syndrome showed shortened SPs (see Table 2), whereas Huntington's disease patients show prolonged silent periods. Cerebellar inhibition (CBI) (see Table 3) is reduced in patients with cerebellar ataxia. Classic migraine patients were reported to have lower phosphene thresholds (Hallett, 2007; Wasserman et al., 2008). It is obvious that a thorough understanding of the physiological basis of TMS and

phenomena such as SICI, SP or CBI could contribute critically to the understanding of these and other diseases.

Protocols using rapid rTMS over M1 were applied to patients with Parkinson's disease and yielded improvements of their motor performance (Wassermann and Lisanby, 2001; Hallett, 2007; Wasserman et al., 2008). Regrettably these changes often lasted only a few minutes. Repeated applications over a period of days could produce long-lasting effects (Khedr et al., 2003). 1 Hz rTMS applied to the M1 or the premotor cortex has been shown to ameliorate performance and normalise intracortical inhibition in patients with focal hand dystonia (Hallett, 2007). Some reports have suggested that ipsilesional or contralesional rTMS applications in the acute stages of stroke could improve clinical outcome (Hallett, 2007; Rossini and Rossi, 2007; Wasserman et al., 2008). Attempts to use TMS as a therapeutic tool to treat epilepsy, chronic pain, aphasia and tinnitus have been made (Rossini and Rossi, 2007). TMS has as well been used to treat psychiatric conditions. Given the well known efficacy of electroconvulsive therapy (ECT) for drug-resistant major depression, the idea arose that rTMS might be an equally effective, more focal treatment and might have less side effects (Hallett, 2007; Rossini and Rossi, 2007). However although meta-analyses reported an overall efficacy of rTMS in depression (Herrmann and Ebmeier, 2006), the high variability among studies and the absence of significant outcome predictors could suggest a general, non-specific, effect of TMS in depression. Few studies have been conducted in schizophrenia, posttraumatic stress disorders, bipolar disorders and anxiety disorders (Hallett, 2007; Rossini and Rossi, 2007; Wasserman et al., 2008).

Another interesting field of research has combined TMS with imaging techniques such as fMRI and PET as well as with EEG (Hallett, 2000; Walsh and Cowey, 2000;

Hallett, 2007; O'Shea et al., 2008; Driver et al., 2009). This allows combining the advantage of TMS being able to causally interfere with the human brain non-invasively with the ability of fMRI, PET or EEG to disclose whole-brain activity and to discover functional networks (Bestmann et al., 2008a; Ruff et al., 2009).

Many clinicians and researchers were concerned about the safety of TMS when it was first introduced. There was a fear of producing short-term and long-term damages to the brain and inducing seizures (Rossini and Rossi, 2007). Now after 20 years the technique appears to be safe. Single-pulse and paired-pulse TMS protocols are extremely unlikely to cause seizures. Rapid-rate rTMS is more likely to induce seizures, especially in patients with a history of epilepsy (Pascual-Leone et al., 1992; Rossini and Rossi, 2007). The risk depends on the intensity and the frequency of the stimulus, and careful planning of the study will help to prevent complications.

1.2.2. DTI

The idea that complex systems, such as language, literature, politics or society can be best analysed and understood as complex structures of interrelated parts originally came from "structuralism", an influential linguistic, philosophical and sociological approach in the 20th century inspired by de Saussure's work about general linguistics. Structuralism became one of the most important approaches in human sciences in the second half of the 20th century and was soon applied to other fields, such as anthropology, psychoanalysis, literary theory and architecture. The French anthropologist and ethnographer Lévi-Strauss analysed cultural phenomena including mythology, mental structures of the human mind and food preparation. The French philosopher and historian Foucault examined the history of science to study how structures of epistemology shaped the way in which people imagined knowledge and knowing. The American historian Kuhn tried to understand the structural formations of science and scientific paradigms. The approach to analyse complex and ramified systems in terms of their underlying structure, i.e. the relation and connectivity of its single elements, instead of just describing and defining the essence of these elements (as traditional philosophy would have done) was also applied to biological systems.

For a very long time neuroscience tried to elucidate the nature of certain brain areas and their role in cognition. Beginning with phrenology as a pseudoscience and the seminal work of 19th century localisationists such as Broca and Wernicke neuroscience always attempted to ascribe cognitive functions to defined areas in the brain and to clarify these area's particular characteristics. Even today modern brain

imaging techniques obtain exceptional attention. However the function of a cortical area might not only be determined by intrinsic properties but as well by extrinsic connectivity. Hence the whole brain as a complex system and the way how it accomplishes cognitive processes might be easier to understand if we know how its single elements are connected (Passingham et al., 2002; Johansen-Berg and Behrens, 2009).

In order to understand the brain as a complex system it might be necessary not just to know its activation patterns but also how its components are interconnected, interrelated and how they cooperate. Connections of a brain area constrain information flow (input and output). The functionality of an individual network node in a network is at least in part designated by the node's interconnections. Connectional properties of an area might therefore critically determine its function. To analyse these connectivity patterns the notion of connectivity fingerprints was introduced (Passingham et al., 2002). It has been suggested that these connectional area-specific fingerprints underlie the area-specific functional activations in different tasks, referred to as "functional fingerprints". Cytoarchitectonic fingerprints led Brodmann's classification of cortical maps (Brodmann, 1909). Connectivity fingerprints, i.e. the unique set of input and output connections of each brain area, could therefore lead to new connectivity based cortical maps and a description of the full connectivity structure of an organism's brain, a "connectome" (Johansen-Berg and Behrens, 2009).

Information about these fingerprints could be derived from tract tracing studies (Johansen-Berg and Behrens, 2009). Tracers such as Horseradish Peroxidase (HRP), Wheat Germ Agglutinin (WGA), Cholera Toxin B Fragment (CTB) or several fluorescent dyes can be injected into predetermined locations of the central nervous

system of living non-human primates or rodents. The tracers are then assimilated by receptor-guided endocytosis and transported along the cytoskeleton, either from the neuron soma to the terminal (anterograde transport) or from the terminal "backwards" to the soma (retrograde transport). A new generation of tracers are neurotropic and neuroinvasive viruses such as the Herpes Simplex Virus Type 1 (HSV 1, commonly known from cold sores or fever blisters) and the Rabies Virus (known from canine madness). These viruses are replicated by recipient's neurons and transported trans-synaptically. Therefore they allow intense transneuronal labelling and depending on the post-injection survival period the study of whole neuronal circuits.

However these techniques are invasive and do not allow in-vivo whole brain analyses or longitudinal studies of human white matter configuration and tracts. Therefore the development of diffusion MRI was a revolution for the investigation of human brain structure and function. The concept of diffusion MRI is to produce quantitative maps of the microscopic, natural displacement of water molecules in brain tissue (Le Bihan, 2003; Jellison et al., 2004; Assaf and Pasternak, 2008). These microscopic displacement processes are based on the physical processes of diffusion, or Brownian motion. When Brown first saw the random motions of pollen grains under the microscope he first believed there was some life force causing these motions. But he found the same random motions when studying dust and other dead matter (Johansen-Berg and Behrens, 2009). Einstein when seeking evidence that would undoubtedly imply the existence of atoms again brought up the random movement of microscopically visible bodies suspended in a liquid and used a probabilistic framework to describe these motions. He introduced the concept of displacement distribution for this purpose, which quantifies the fraction of particles that will have traversed a certain distance in a given period of time. For example in free diffusion

the displacement distribution will be a Gaussian function and can be visualised as a sphere. However if the mobility of these particles is asymmetrically hindered by certain properties of the surrounding medium (e.g. brain tissue) diffusion in some directions might be "faster" as diffusion in others (e.g. in the direction of white matter tracts) and the displacement distribution might no longer be spherical. Such interactions of water molecules with (brain) tissue micro-architecture can be measured with diffusion MRI.

In the early years of diffusion MRI it was recognised that (unidirectional) diffusivity of water molecules could change depending on brain microstructure and cellular alterations and could therefore indicate brain ischemia even when conventional scans (T1-weighted, T2-weighted) could not. Thus the introduction of diffusion-weighted imaging was met with enthusiasm as another MRI-based technique gaining new contrast in the brain and complementing T1-weighted and T2-weighted imaging. About the same time as the finding that diffusivity was reduced in brain ischemia, it was recognised that in certain parts of the brain, especially in the white matter, diffusivity depended strongly on the direction that was measured. For example in the corpus callosum water diffusivity was found to be high parallel to the x-axis (MNI) but low parallel to the y-axis. Whereas in some brain structures diffusivity was found to be similar along all three major axes (e.g. in ventricles, but also in grey matter) and therefore more or less isotropic, diffusivity in other areas (white matter) was found to be highly directional and anisotropic. To characterise diffusion displacement profiles under anisotropic condition diffusivity is measured in several different directions and the results are modelled with a diffusion tensor model ((Basser and Jones, 2002; Johansen-Berg and Behrens, 2009), see Figure 4). Three so-called eigenvectors (έ1, έ2 and έ3) and their eigenvalues (λ1, λ2 and λ3, see Figure 4) are used to describe

each voxel's diffusion displacement profile. The tensor can be visualised as a diffusion tensor ellipsoid (see Figure 4). The main diffusion direction (ἐ1) is the direction of the eigenvector with the largest eigenvalue. Another clinically important measure is the trace, which reflects the orientation-independent mean-diffusivity and was found to be changed in several pathological conditions (Johansen-Berg and Behrens, 2009). The fractional anisotropy (FA) is as well orientation-independent and allows us to draw conclusion on the degree of directionality (anisotropy) in the tissue.

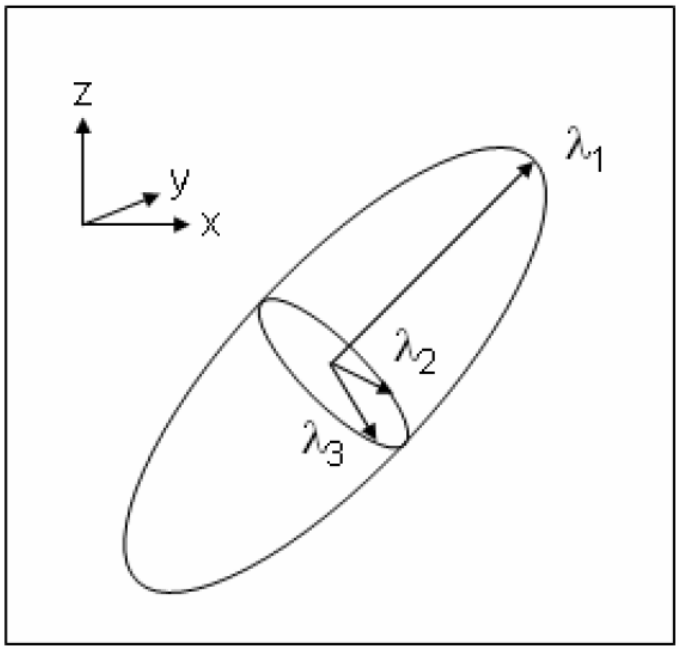


Figure 4: Three so-called eigenvectors (ἐ1, ἐ2 and ἐ3, arrows) and their eigenvalues (λ1, λ2 and λ3) describe each voxel's diffusion displacement profile and can be visualised as a diffusion tensor ellipsoid. Picture taken from "Introduction to MRI" at the hompage of the NMR research group at the Department of Cardiovascular Physiology, Heinrich Heine university Duesseldorf (www.nmr.uni-duesseldorf.de/sets/theory.html).

The interesting discovery that diffusion is highly anisotropic in certain areas of the brain raised hope that information about the microscopic properties of the tissue that actually hindered diffusion in some directions could be inferred just from non-

invasively, voxel-wise measuring and modelling diffusivity profiles. But which microscopic features of (white matter) brain tissue do actually influence those diffusivity profiles? The white matter of the central nervous system (CNS) constitutes the tracts in which axons extend from one area to another. White matter accounts for 40% of the CNS tissue in humans and consists of (1) neuronal axons, (2) oligodendrocytes and their myelin containing extensions, (3) astrocytes that play numerous support roles including maintenance of the blood-brain-barrier, (4) microglia as the immunocompetent cells of the CNS and (5) endothelial cells that form the walls of blood capillaries. Although the myelin sheaths that surround the majority of the CNS axons were initially thought to be the primary determinant of diffusion anisotropy and main diffusion direction of the water molecules it was shown that axonal membranes play the most critical role and myelin only modulates anisotropy in a given tract (Johansen-Berg and Behrens, 2009). Hence the axon density, the amount of myelination and the number of fibre directions present in each voxel can influence anisotropy and the FA.

Differences in white matter architecture are likely to be of importance for brain structure and function and certain anisotropy patterns could therefore be associated with specific physiological and behavioural characteristics (Bengtsson et al., 2005; Boorman et al., 2007; Johansen-Berg and Behrens, 2009; Mars et al., 2009). And in fact certain white matter micro-structural patterns were reported to be associated with handedness, gender (Westerhausen et al., 2003; Westerhausen et al., 2004), experience and expertise in populations of professional musicians (Gaser and Schlaug, 2003; Bengtsson et al., 2005), with performance in bimanual motor tasks (Johansen-Berg et al., 2007), performance in simple cognitive paradigms (Wolbers et al., 2006) and even with measures of functional connectivity (Boorman et al., 2007;

Mars et al., 2009; Buch et al., submitted). One of the most interesting questions now is whether behaviourally relevant inter-individual variations in white matter micro-structure are due to nature or nurture. If they were mainly formed by experience and learning then we should expect these inter-individual variations not just in single white-matter pathways but in distributed functionally related networks and therefore might find intra-individual correlations between functionally related pathways. But if these white matter pathways are mainly determined by nature, i.e. by genes, we might find variations in single white matter tracts independent of functionally related pathways. Conversely these "single tract variations" might then give rise to individual behavioural patterns.

However studies addressing these and other exciting questions crucially rely on accurate whole-brain voxelwise analyses of diffusion MRI data. Therefore issues of cross-subject registration (image alignment), white matter delineation, statistical modelling and multiple comparison correction as well as how to interpret positive and negative results need to be solved. The recently proposed "Tract-Based Spatial Statistics" (TBSS) approach tries to address these problems. It attempts to combine ideas from (1) tractography-based and (2) voxel-based-morphometry-style (VBM) cross-subject comparison approaches (Smith et al., 2006; Smith et al., 2007; Johansen-Berg and Behrens, 2009). As a TBSS analysis will be used later on to elucidate correlations between measures of structural and functional connectivity, the ideas of TBSS will be discussed in more detail in Chapter 2.2.

As every other technique in neuroscience diffusion MRI has its limitations and does therefore not constitute an alternative to tract-tracing techniques but rather a complement (Johansen-Berg and Behrens, 2009). Diffusion MRI does not inform about the direction of a connection and the number of its synapses. Additionally the

spatial resolution is limited and reliably modelling crossing fibres within a voxel turns out to be challenging. However diffusion MRI is the only technique that allows non-invasive in-vivo imaging of white matter architecture in humans. It could help to understand the physiological and behavioural consequences of microscopic white matter structure and therefore allow important insights in structure, function, development and disease of the human CNS.

2. Functional and Anatomical Networks of Executive Control

2.1. Functional Networks of Inhibition and Action Reprogramming

2.1.1. Inhibition and Action Reprogramming in the Motor Cortex

Introduction

Converging lines of evidence show that action selection and the withholding of prepared actions involve a network of cortical and subcortical structures. These include regions in the lateral prefrontal cortex (PFC), medial PFC and basal ganglia (Aron et al., 2004a; Isoda and Hikosaka, 2007; Mars et al., 2007b; Isoda and Hikosaka, 2008; Chambers et al., 2009; Mars et al., 2009; Stinear et al., 2009; Buch et al., submitted). As the target of influence from these regions, M1 is likely to be a site of convergence for processes underlying response selection, inhibition and action reprogramming. Therefore the motor system is likely to be affected by preparatory processes and expectations (Bestmann et al., 2008b; Sinclair and Hammond, 2008; Stinear et al., 2009). General corticospinal excitability of the motor system depends on the balance of M1 intrinsic excitatory and inhibitory interneuron circuits (Sohn et al., 2002; Wasserman et al., 2008). Theories of response inhibition and action reprogramming suggest that the fronto-basal-ganglia network influences corticospinal excitability and intracortical inhibitory interneurons within M1.

Intracortical interneurons, found in the superficial layers of the motor cortex (i.e. layer II and III), have excitatory and inhibitory synaptic connections with corticospinal neurons in layer V (pyramidal neurons). These pyramidal neurons project directly to the motoneurons in the anterior horn of the spinal cord or in the brainstem nuclei. Inhibitory and excitatory interneurons integrate inputs from other cortical and subcortical regions and then modulate the excitability and output firing of corticospinal pyramidal neurons.

Corticospinal excitability of the motor-cortex can be studied with single-pulse TMS and excitatory and inhibitory intracortical networks can be investigated with paired-pulse TMS. Single and paired-pulse TMS are safe, painless and non-invasive methods for stimulating corticospinal neurons and intracortical interneurons. A single, supra-threshold TMS pulse applied over M1 causes direct and trans-synaptic excitation of corticospinal neurons. Corticospinal volleys reach the corresponding spinal motoneuron pool and activity in the target muscles can be observed and quantified, using standard electromyography (EMG) recordings, measuring size and latency of a motor evoked potential (MEP). When this suprathreshold TMS pulse ("test pulse") is preceded by a subthreshold TMS pulse ("conditioning pulse"), the resulting MEP is either facilitated or inhibited, depending on inter-pulse-interval and conditioning pulse intensity (Hallett, 2000, 2007; Reis et al., 2008). These effects can be quantified calculating the ratio between paired-pulse TMS MEP size and single-pulse TMS MEP size with values smaller than 1 (or 100%) indicating inhibition and values bigger than 1 indicating facilitation. Thus TMS can be used to measure changes in motor-cortico-spinal excitability and M1 internal excitatory and inhibitory circuits.

Two principal types of local intracortical inhibition can be studied using a paired-pulse TMS paradigm. Short interval intracortical inhibition (SICI, see Table 3) can be

elicited by a subthreshold (i.e. ~70-90% RMT) conditioning pulse preceding the test pulse by 1-6 ms. These effects are thought to reflect activity of GABA-A receptor sensitive inhibitory interneurons (Reis et al., 2008; Rothwell et al., 2009). Long-interval intracortical inhibition (LICI) is elicited by a supra-threshold conditioning pulse preceding the test pulse by 50-200 ms and thought to be mediated by the activity of GABA-B receptor sensitive inhibitory interneurons (Reis et al., 2008; Wasserman et al., 2008).

M1 excitability increases progressively in the 80-120 ms before movement onset (Leocani et al., 2000). This increase in corticospinal excitability is preceded by a release of SICI which persists during action execution (Reynolds and Ashby, 1999; Stinear et al., 2009). Hence these processes of diminished intracortical inhibition measured as "SICI" could be a relevant component of action selection and initiation. Additionally corticospinal excitability is modulated in correspondence with response signal expectancy (van Elswijk et al., 2007). Corticospinal excitability and intracortical inhibition are modified in a muscle-specific way during movement selection and initiation. MEPs were found to be suppressed in muscles not required for the task and inhibition was found to be increased (Stinear et al., 2009).

In go/no-go paradigms MEPs were facilitated in go trials 50 ms prior to movement onset and suppressed in no-go trials 250 ms after the no-go cue (Hoshiyama et al., 1997; Leocani et al., 2000). This inhibition of prepared actions seemed to be muscle-unspecific and could be observed even in nearby hand muscles not involved in the pre-prepared action. Decrease of M1 excitability could be achieved by these "SICI interneurons" (GABA-A receptor sensitive inhibitory interneurons). Paired-pulse TMS can test the activity of these SICI circuits in go/no-go or stop-signal paradigms. SICI was found to be enhanced in no-go trials and released in go trials compared to

resting-state SICI (Waldvogel et al., 2000; Sohn et al., 2002). SICI was found enhanced during volitional inhibition of a prepared response in a stop-signal task approximately 140 ms after stop cue presentation (Coxon et al., 2006).

The aim of this study was to investigate the time course of M1 corticospinal excitability and M1 internal short-interval inhibitory mechanisms (i.e. SICI) in a much more complex task, requiring inhibition of prepared responses and action reprogramming. Understanding M1 internal mechanisms during action reprogramming and the time-course of these mechanisms could then serve as an informative foundation for the subsequent research on interactions between premotor and prefrontal areas and M1 during action reprogramming and response inhibition.

Methods

Participants. Sixteen healthy volunteers (age range 18 – 30 years, mean age = 22.4 +/- 3.41, 8 females) with no personal or familial history of neurological or psychiatric disease participated in one of the two experiments investigating inhibition and action reprogramming in the primary motor cortex (M1). The experiment was approved by the Oxfordshire Research Ethics Committee and conducted in accordance with the declaration of Helsinki. The same behavioural task and experimental setup was used in two separate experiments to investigate neuronal processes internal to M1 during (1) action reprogramming and inhibition of prepared responses (subsequently called the "switch experiment") and (2) execution of prepared responses (subsequently referred to as "stay experiment"), respectively. 8 Participants (3 females, mean age = 22.3 +/- 3.45) participated in the "switch experiment". 8 participants (5 females, mean age = 22.6 +/- 3.58) participated in the "stay experiment". All participants were right-handed and gave written informed consent. They were all screened for adverse reactions to TMS and risk factors by means of a safety questionnaire.

Experimental setup. Participants were seated in a darkened room and wore a tight-fitting EEG cap, on which TMS sites were marked and earplugs to protect against TMS noise. A chin rest was used to minimise head movements.

Behavioural Task. The task (see Figure 5 and 6) was modelled on the paradigm developed by (Isoda and Hikosaka, 2007) and required participants to respond with the left or right index finger in response to visual stimuli presented on a 17 inch computer screen ~85 cm in front of them. Each trial began with the presentation of a central white fixation square (4.7º width) followed 1000 ms later by the presentation

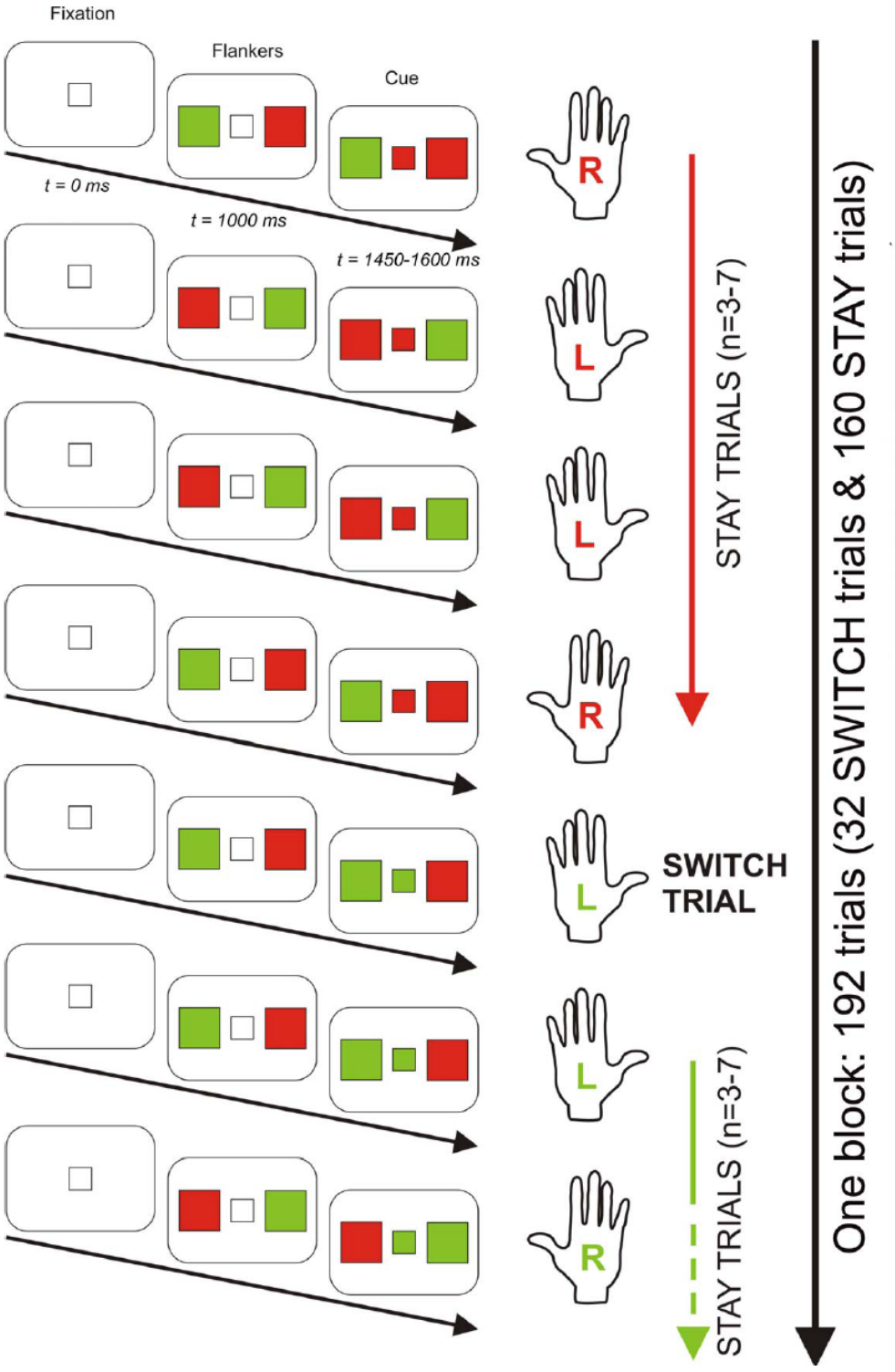


Figure 5: This behavioural task was modelled on the paradigm developed by (Isoda and Hikosaka, 2007). Each "row" in the figure represents a single trial (i.e. 7 trials represented in the figure, one experimental block contained 192 trials). On each trial the participants were presented with a centrally displayed white fixation square. Subsequently two differently coloured flankers appeared on either side of the fixation. After a variable delay (450-600 ms) the central fixation turned red or green instructing the participants to respond with the left or right index finger (represented as left and right hand shapes).

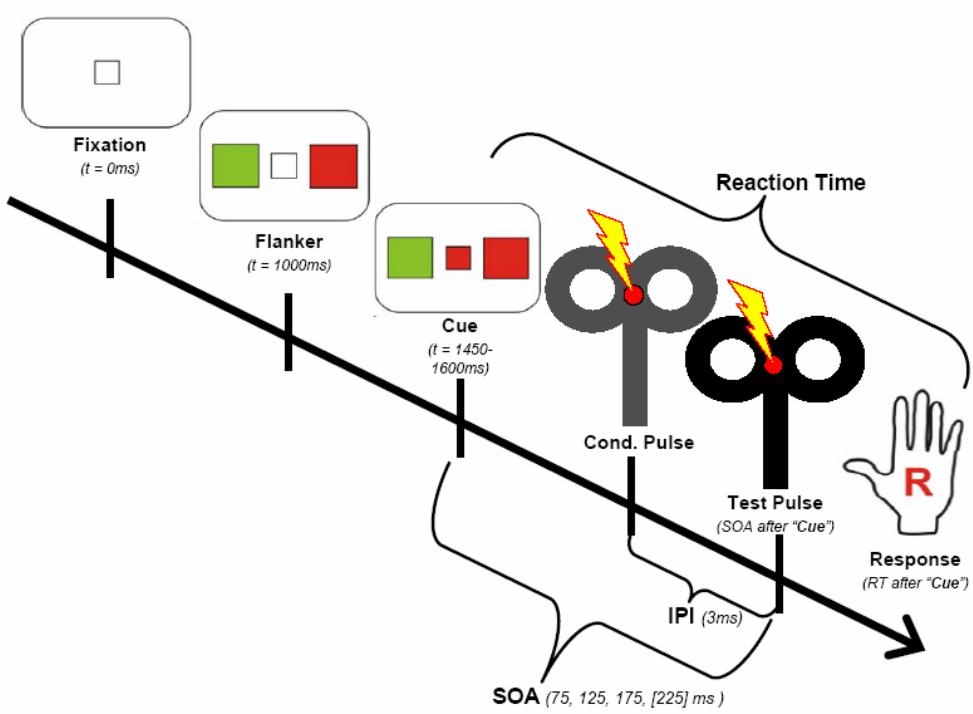


Figure 6: Single TMS trial (each block contained 36 TMS trials). The TMS pulse(s) were delivered after centre colour cue onset.

of two flanker stimuli, one on each side of the white square (6º width). On each trial one of the flanker squares was green, the other one was red, with trial-wise random assignment of colour to side of display. After a variable delay of 450-600 ms (uniform distribution), the white central square turned either red or green, instructing the subjects to respond with the index finger on the side corresponding to the flanker of the same colour. The critical manipulation imbedded in the task was that the central cue repeatedly turned the same colour for trains of between three and seven consecutive trials (uniform distribution, for example, it turned red for the first four trials in Figure 5). This afforded participants the opportunity to prepare a movement, in the period between the onset of the two flanker squares and the onset of the centre colour cue (450-600 ms). However after taking the same colour for a series of between three and seven trials the centre cue changed (for example, after turning red for four consecutive trials, the centre cue turns green on the fifth trial in Figure 5).

This manipulation meant that there were two types of trials: stay trials, in which the fixation square turned into the same colour as in the previous trial, thus allowing the participants to perform the already prepared response, and switch trials, in which the fixation square turned into a different colour as in the previous trial, thus requiring participants to inhibit an already prepared response and to reprogramme their actions. It is noteworthy that trials requiring inhibition and action reprogramming were not announced by a distinct instructional cue. Moreover the participants were not even told that some trials would require inhibition or reprogramming behaviour. Rather switch trials violated an implicit expectation that was built up during the previous train of stay trials. Stimuli were pseudo-randomly generated and a different stimulus order was used for each block. Custom software written in Presentation (version 0.53) controlled the experiment. Before the actual experiment participants were familiarised with the task in one behavioural training block (without TMS pulses, 30 trials) and with the whole experimental setup including TMS pulses in a second training block (with TMS pulses, 30 trials). The "switch experiment" consisted of seven experimental blocks. The stay experiment consisted of five experimental blocks. Each block contained 32 switch and 160 stay trials. Reaction times were recorded, defined as the duration between the onset of centre square colour and the index finger response button press.

TMS. TMS pulses were delivered through a single 70 mm diameter figure-of-eight coil connected via a BiStim module to two high-power Magstim 200 MonoPulse machines (The Magstim Company®). The magnetic pulse was monophasic, with a rise time of ~ 100 μs, decaying back to zero over ~ 800 μs. TMS was delivered over the left M1, with the coil placed tangentially to the scalp, inducing posterior-to-

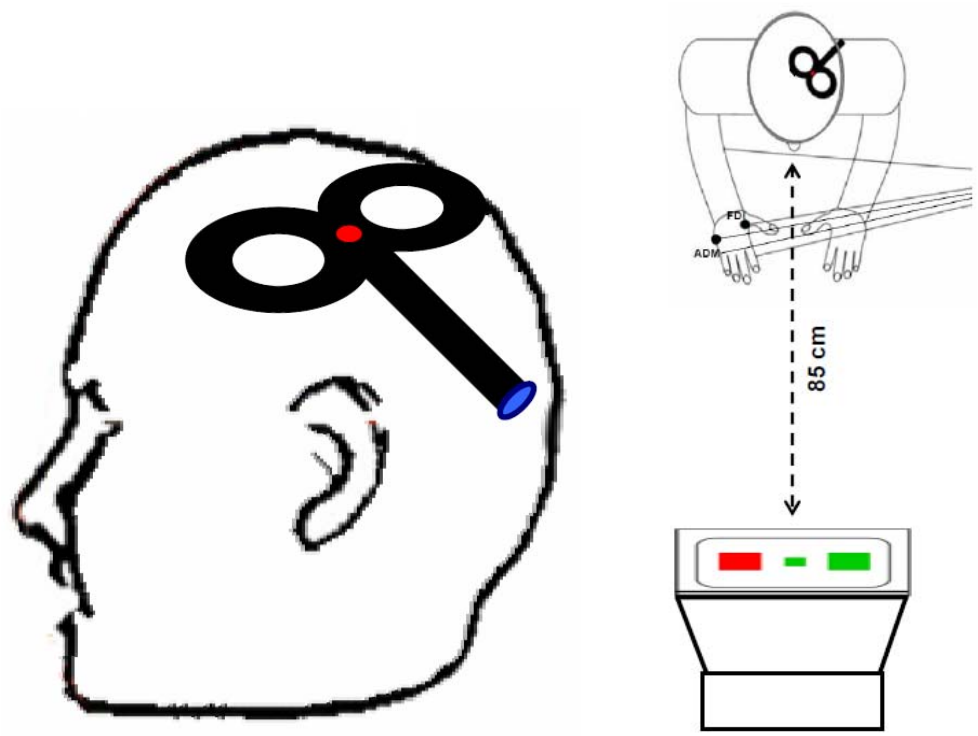


Figure 7: Experimental setup. Single and paired-pulses were delivered through a single 70 mm diameter figure-of-eight coil placed tangentially to the scalp.

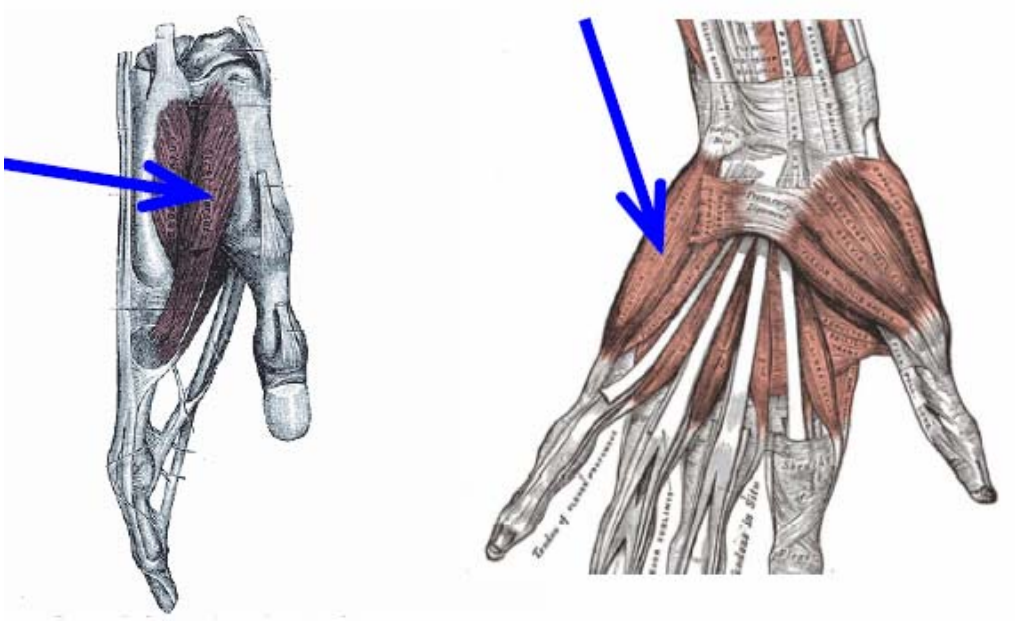


Figure 8: The first dorsal interosseus (FDI, left, radial side) is a flexor and abductor in the second metacarpophalangeal joint and thus inflects and abducts the index finger. The abductor digiti minimi (ADM, right, palmar surface) is an abductor in the fifth metacarpophalangeal joint and thus abducts the little finger. Figure taken from (Gray and Howden, 1918).

anterior current flow perpendicular to the central sulcus (see Figure 7). TMS pulses were delivered on some trials to investigate M1-excitability and internal M1 inhibitory processes during action execution and action reprogramming. There were two types of TMS trials. On half of the trials, so-called "single-pulse" trials, a single TMS test pulse was delivered over the M1-representation of the right hand first dorsal interosseus muscle (FDI, see Figure 8). The M1-representation of the FDI is defined as the scalp site at which the largest mean MEP amplitude from a train of five single-pulses was elicited. The intensity of this TMS test pulse was such that an MEP of 1 – 1.5 mV was evoked in the relaxed, contralateral FDI. This intensity was 48.37% (SEM+/- 2.397) of the maximum stimulator output in the switch experiment and 44.87% (SEM +/- 2.239) for the stay experiment. On the other half of the trials, so-called "paired-pulse" trials, the test pulse was preceded by a "conditioning pulse" by 3 ms. Intensity of the test pulse was exactly the same as in "single-pulse trials". The intensity of the preceding "conditioning pulse" was set at 80% of the resting motor threshold (RMT) of the same FDI muscle. RMT is defined as the TMS pulse intensity necessary to evoke an MEP of 50μV in the completely relaxed muscle with 50% probability (5 out of 10 trials). Average RMT was 42.62% (+/- 2.789) of maximum stimulator output in the switch experiment and 42.25% (+/-2.11) in the stay experiment. Stimulator intensity (80% of FDI RMT) and interval between conditioning and test pulse (inter-pulse interval, IPI, 3 ms) was chosen based on the extensive literature on short-interval intracortical inhibition (SICI, (Sohn et al., 2002; Coxon et al., 2006; Reis et al., 2008; Wasserman et al., 2008; Rothwell et al., 2009; Vucic et al., 2009). During "switch experiments" pulses were almost exclusively delivered during switch trials. In the "stay experiments" pulses were exclusively delivered during stay trials. TMS was delivered 75 ms, 125 ms, 175 ms or 225 ms

after onset of the centre colour cue (stimulus onset asynchrony, SOA, see Figure 6) during switch experiments and 75 ms, 125 ms and 175 ms in stay experiments. TMS pulses were not delivered at the longest interval, 225 ms after colour cue onset, in the stay experiments, as this time-point was too close to the actual responses in stay trials. The presence or absence of TMS could not serve as a pre-cue indicating trial identity, as the pulses were only applied after the centre colour cue had already indicated a switch or stay trial. However, in the switch experiments we delivered a small number of pulses on stay trials, preventing the subjects from detecting any relationship between trial type and TMS delivery. Hence in every block TMS was delivered on 36 out of 192 trials (switch experiment: 32 TMS trials on switch trials and 4 TMS trials on stay trials, only switch trials were analysed; stay experiment: 36 TMS trials on stay trials). For the switch experiments a total of 14 TMS trials per hand (left vs. right hand), SOA (75, 125, 175, 225 ms) and pulse type (single vs. paired) were delivered and used for the analysis. For the stay experiment a total number of 15 TMS trials per hand, SOA (75, 125, 175 ms) and pulse type was delivered and analysed. TMS trials were presented at least 7 seconds apart, to ensure that pulses on adjacent trials did not influence each other. In each block, TMS trials were distributed evenly over response hands, respective SOAs and single- or paired-pulse TMS.

Electrophysiological recordings. MEPs were recorded from two muscles in the right hand using two surface Ag-AgCl electrodes for each muscle in tendon-belly montage. The two electrodes recording EMG activity in right FDI (see Figure 8) were placed on the muscle belly and the second metacarpo-phalangeal joint. The two electrodes recoding right abductor digiti minimi (ADM) were placed on the muscle belly and the fifth metacarpo-phalangeal joint. An earth electrode was placed on the right elbow (over the posterior border of the ulna ~5cm distal of the olecranon). EMG responses

were band-pass filtered between 10-1000 Hz, with an additional 50 Hz notch filter, sampled at 5000 Hz, and recorded using a CED 1902 amplifier, a CED micro 1401 Mk.II A/D converter, and a PC running Spike2 (Cambridge Electronic Design ®, Cambridge, UK).

Analysis. For the analysis of the difference between action reprogramming (switch experiment) and action execution (stay experiment) in M1 corticospinal excitability and intracortical inhibition we concentrated on a between-session and hence between-subjects design: We analysed MEP data from the switch trials in the switch experiment (32 out of 36) and the stay trials in the stay experiment (all 36 trials). This was necessary because obtaining an adequate number for both, switch and stay trials at each SOA with both hands and both single and paired pulses would have resulted in the participants receiving a very large number of TMS pulses and an exceedingly long experiment. Analysis of electrophysiological data concentrated on peak-to-peak amplitudes of the MEPs (see Figure 9) measured on TMS trials (switch trials in the switch experiment, stay trials in the stay experiment). Peak-to-peak MEP amplitude was defined as the voltage difference between the minimum and maximum EMG signal in a window from 15 to 40 ms after TMS delivery. MEP amplitudes determined automatically were verified using a manual cursor for every trial. The experimenter was blinded to the type of trial. Trials with incorrect responses, trials with premature (RT<150) responses, trials in which the response occurred before TMS delivery, trials in which the test pulse failed to elicit a reliable MEP (amplitude<0.1 mV), and trials in which participants pre-contracted the FDI muscle prior to application of the TMS pulse (EMG amplitude>0.1 mV in the 80 ms before the pulse) were discarded from the analysis. Following this pre-processing, on average 2.945 (SEM± 0.195) trials (of a total of 14 trials per condition) in the switch experiment and 3.332 (± 0.243) trials

(of a total of 15 trials) in the stay experiment were excluded. Two ANOVA tests on the number of deleted trials in every condition in the switch experiment and the stay experiment, respectively with the within-subjects contrasts of *"hand"* (left vs. right), *"SOA"* (75, 125, 175, 225 ms in the switch experiment and 75, 125, 175 ms in the stay experiment) and *"pulse"* (single vs. paired-pulse TMS) did not show any significant effects and interactions (all $p>0.2$) indicating that there was no difference in the number of excluded trials for any condition. Two paired-samples t-tests contrasting the number of excluded trials in single-pulse and paired-pulse TMS trials in each experiment (switch experiment and stay experiment) could not find any difference (both $p>0.7$). This is important as a difference in number of excluded MEPs between single and paired pulse TMS trials could possibly obscure very strong inhibition in one trial type, because MEPs smaller than 0.1mV were excluded beforehand.

To account for differences in coil placement between experimental blocks, MEP sizes were median- normalised within each block. Analyses of MEPs were carried out on the mean of the normalized MEP amplitudes in each condition. Analyses of both behavioural and electrophysiological data were conducted using ANOVA tests, using repeated measures where possible. Significant effects were identified based on Huynh-Feldt corrected ANOVA values, using SPSS 16.0. Post-hoc two-sided t-tests were used to further investigate significant effects in the ANOVAs.

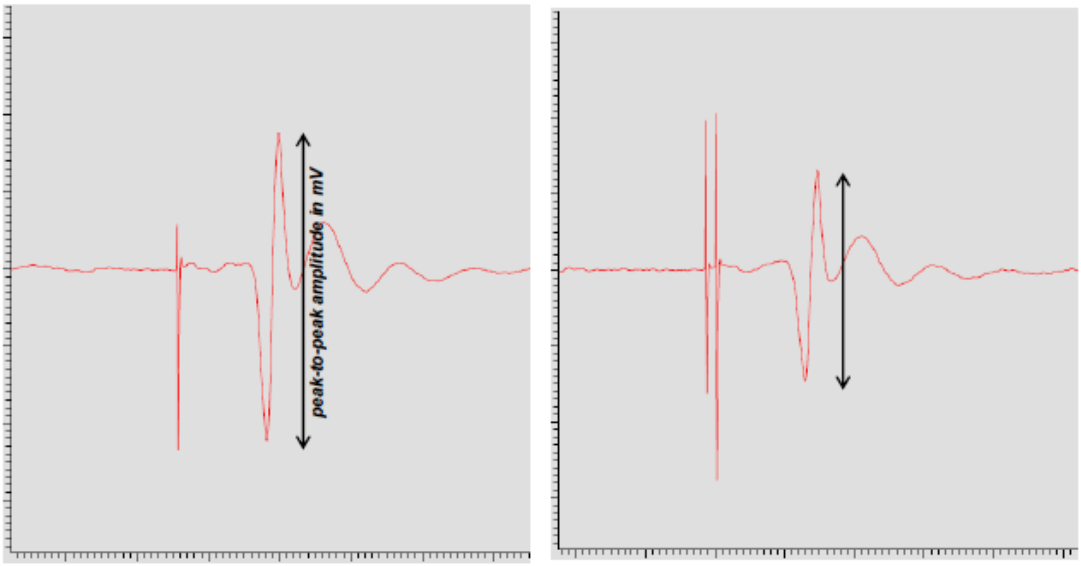


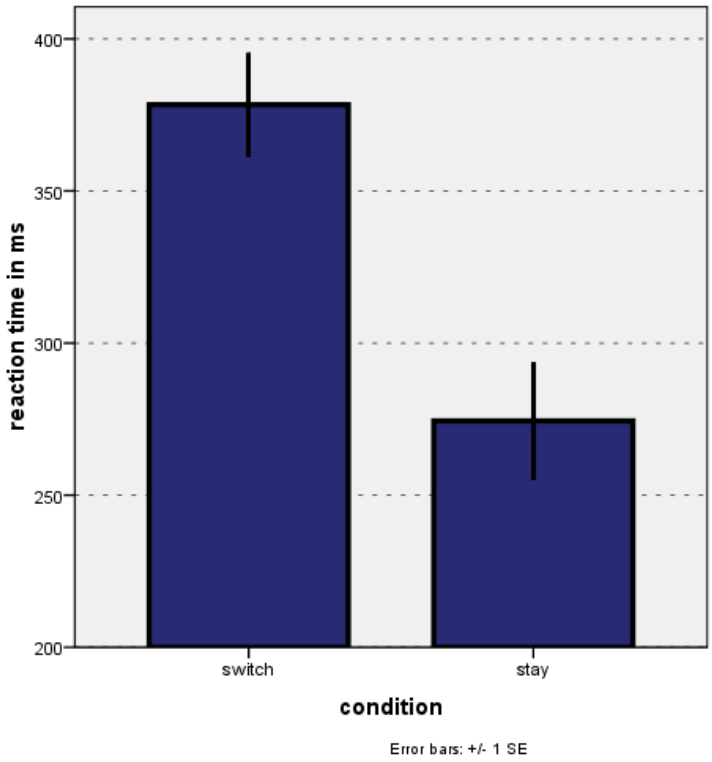
Figure 9: Corticospinal excitability can be quantified as the peak-to-peak amplitude of an MEP elicited by a single TMS pulse over M1. A conditioning pulse preceding the test pulse can change M1 excitability. This can be quantified as a [single-pulse TMS MEP / paired-pulse TMS MEP] ratio.

Results

Behavioural results. ANOVAs of median RTs on correct trials and of error rates (incorrect responses/total number of trials) with *"trial type"* (switch vs. stay) as a within-subject factor and *"experiment"* (switch and stay experiment) as a between-subject factor showed a main effect of *"trial type"* ($F_{1,14}$= 57.804, $p<0.001$ for RTs; $F_{1,14}$=26.401, $p<0.001$ for error rates), but no main effect of *"experiment"* and no interaction between *"experiment"* and *"trial type"* ($p>0.2$). A post-hoc paired-samples t-test confirmed subjects were significantly slower on switch trials than on stay trials (RT 372.1 ms on switch vs. 278.3 ms on stay trials, t_{15}=7.070, $p<0.001$) and made significantly more mistakes (error rate 29.265% on switch trials vs. 2.23% on stay trials, t_{15}=5.312, $p<0.001$) (see Figure 10). This confirmed the effectiveness of the task manipulation. These effects of slower and more inaccurate performance in switch trials can be measured as switching-costs. RT switching costs are defined as the ratio [median RT in switch trials] / [median RT in stay trials]. Error switching costs are defined as [error rate in switch trials] / [error rate in stay trials]. RT switching costs were 1.3614 (i.e. performance slowed down by 36.14% in switch trials) and error switching costs were 17.044 (i.e. subjects made 17 times as many errors in switch trials as in stay trials, or error rate on switch trials was 1704.4% of the error rate on stay trials). Behavioural effects did not differ between the two experiments, as indicated by the absence of any *"experiment"* effect or interaction between "*experiment"* and *"trial type"* Independent-samples t-test comparing behavioural measures (RTs, RT in switch trials, RTs in stay trials, RT switch-costs, error rate, error rate in switch trials, error rate in stay trials and error switch costs) did

not yield any significant differences ($p>0.5$) between the switch and the stay experiment.

TMS results will be presented in the following order: First results from the switch experiment will be presented to show the change of M1-excitability during action reprogramming and intracortical inhibition during action reprogramming. Then results from the stay experiment shall be reported to show M1-excitability during action execution and intracortical inhibition during action execution. At the end processes of excitability and inhibition during action reprogramming and action execution will be compared in a between sessions and hence between-subjects-contrast.



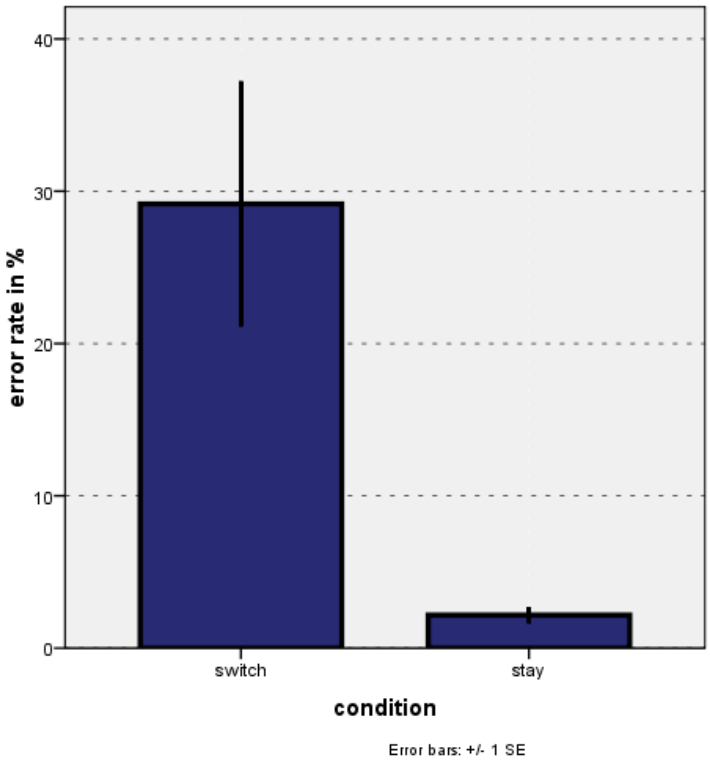


Figure 10: Behavioural data across the two experiments. Subjects were significantly slower on switch trials than on stay trials (top) and made significantly more mistakes (bottom).

M1-corticospinal-excitability during action reprogramming and response inhibition (i.e. switch trials). The main question addressed in this experiment was whether the excitability of the motor-corticospinal system and intracortical inhibitory mechanisms would change depending on the hand (left vs. right) that was chosen for a response and on whether it had been pre-prepared (stay trials) or had to be reprogrammed (switch trials, for the comparison between stay and switch trials see p. 99). Note that we applied TMS pulses only to the left M1 and recorded MEPs only from the right hand FDI and ADM. Therefore we could only observe differences in the left M1-excitability depending on whether the right hand was to be selected or inhibited. In "left hand trials" we would therefore expect to find less M1 corticospinal excitability as in right hand trials, as in left hand trials the recorded hand (i.e. the right hand) was to be inhibited from responding. MEP amplitudes in the single-pulse trials reflected the excitability of M1 and the interconnected corticospinal tract. Single-pulse FDI MEPs from switch trials were analysed (see Figure 11 top) with an ANOVA with within-subjects factors of *"hand"* (left vs. right hand) and "*SOA"* (75, 125, 175 and 225 ms) revealed a significant "*hand"* by *"SOA"* interaction ($F_{3,21}$=10.764, p<0.001). Post-hoc t-tests revealed a significant difference for the MEPs 75 ms (t_7=3.081, p=0.018, left>right) and 225 ms (t_7=-3.170, p=0.016. right>left!) after cue onset between left and right hand responses. Note that the difference between excitability of the motor cortex in "choose" (i.e. right hand) and "inhibit" (i.e. left hand) trials reversed over the time course of action reprogramming. The motor cortex was more excited 75 ms after cue onset if the action was to be inhibited than if it was to be chosen, probably due to the expectation that the opposite hand was to be moved. One hundred and fifty ms later the difference between "inhibit" and "chose" trials was completely inverted, indicating that action reprogramming was successfully achieved.

We also found significant differences between the two time-points, 75 ms and 225 ms after cue onset, in left hand response trials (t_7=2.491, p=0.042) as well as in right hand responses (t_7=-3.816, p=0.07), indicating that changes in M1 excitability during the time-course of action reprogramming where due to both, inhibition and facilitation, and that both, inhibitory and facilitatory mechanisms occurred at about the same time during action reprogramming. This is interesting, as it could suggest that both processes, inhibition of the prepared but inappropriate response and facilitation of the unprepared but required response occur at about the same time, just before the average response time of the stay trials (288.3 ms) and could therefore indicate that both processes are relevant for successfully reprogramming action plans.

Intracortical inhibition during action reprogramming and response inhibition. To ascertain whether some processes affecting the excitability of M1 during action reprogramming were mediated by the interneuron circuits indexed by SICI (a population of GABA-A receptor sensitive, inhibitory interneurons in the motor cortex, see Chapter 1.2.1), we analysed FDI MEP data from paired-pulse TMS trials in switch trials and calculated the ratio [paired-pulse TMS trial MEP] / [single-pulse TMS trial MEP] for every condition separately (hand, SOA; see Figure 11 bottom). This ratio (multiplied with 100) can be interpreted as percentage MEP change, due to the sub-threshold conditioning pulse, that precedes the test pulse by 3 ms and selectively activates these interneuron populations. An ANOVA test on the single and paired-pulse trial MEPs for every condition with within-subjects contrasts of *"pulse"*, *"SOA"*, and *"hand"* revealed significant effects of *"pulse"* ($F_{1,7}$= 6.240, p=0.041), *"SOA"* ($F_{3,21}$= 5.749, p=0.005), *"hand"* ($F_{1,7}$= 12.748, p=0.009) and significant "*hand"* × *"SOA"* ($F_{3,21}$=13.329, p=0.002), "*hand"* × *"pulse"* ($F_{1,7}$= 8.026, p=0.025) and *"hand"* × *"SOA"* × *"pulse"* ($F_{3,21}$= 3.276, p=0.041) interactions. An ANOVA

test on the paired-pulse / single-pulse TMS ratios with within-subjects contrasts of *"SOA"*, and *"hand"* revealed significant effects of *"hand"* ($F_{1,7}$= 13.799, p=0.008) and a significant "*hand" × "SOA" interaction* ($F_{3,21}$= 6.347, p=0.003). Post-hoc paired samples t-tests of the MEP ratios revealed significant differences between left and right-hand responses 125 ms (t_7=-3.952, p=0.006), 175 ms (t_7=-3.197, p=0.015) and 225 ms (t_7=-3.860, p=0.006) after centre cue colour onset, indicating that action-selection and reprogramming processes might employ SICI-interneuron circuits. Paired samples t-tests between the MEP ratios on different time-points during action reprogramming revealed significant differences in right hand response trials between 75 ms and 125 ms (t_7=-3.096, p=0.017), 75 ms and 175 ms (t_7=-3.026, p=0.019), and 75 ms and 225 ms (t_7=-5.019, p=0.002), indicating that the difference between the hands was mostly due to a release of the unprepared but correct hand and to a lesser extend to the increase in inhibition of the prepared but incorrect hand. This is interesting as it could suggest that (1) inhibitory processes indexed by SICI plays a crucial role in action reprogramming; (2) action reprogramming on the level of "SICI-type" interneurons occurs relatively early in the time-course of response selection and earlier than the change in general M1 excitability; (3) hence inhibitory processes indexed by SICI could play a causal role in M1 excitability during action reprogramming; (4) inhibitory changes, as indexed by SICI, during action reprogramming involve a release of the representation of the previously unprepared but correct response. An increase in inhibition of the prepared but incorrect response is less prominent or else occurs later in time.

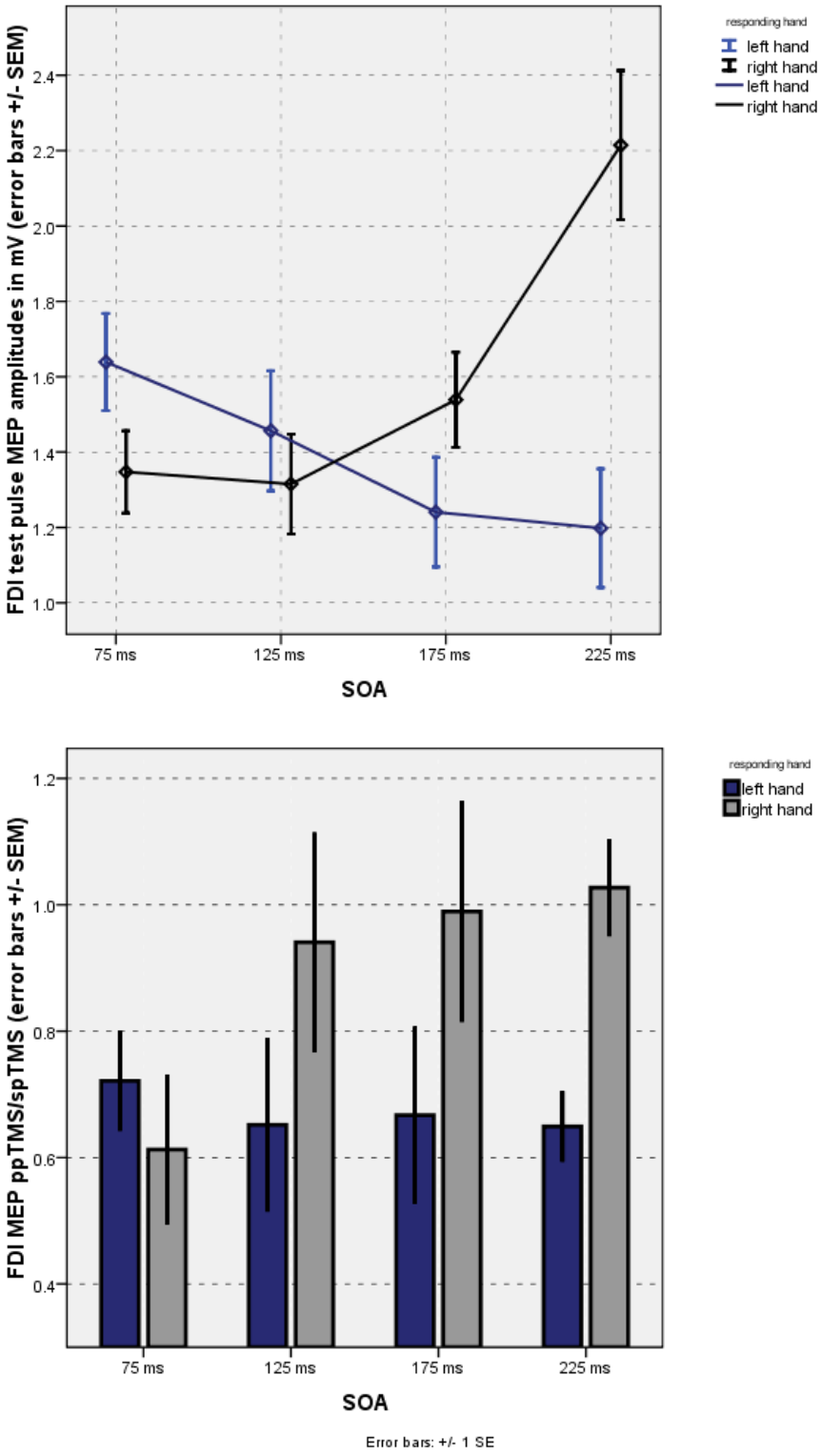


Figure 11: Time course of general M1 excitability (top) during action reprogramming (i.e. switch trials). Single-pulse TMS MEP sizes are plotted for every condition (SOA and hand). Time course of intracortical inhibition during action reprogramming. Single-pulse / paired-pulse TMS MEP ratios are plotted for every condition (SOA and hand).

Functional specificity of the observed effects. To investigate whether the changes of M1 corticospinal excitability and M1-internal inhibitory processes were functionally specific and restricted to the muscles involved in the task (e.g. FDI) or whether these processes could be observed in other nearby muscles that do not functionally contribute to the task (e.g. ADM) we analysed MEPs recorded from the ADM muscle (see Figure 12). However note that the experimental setup was not optimal for ADM muscle recording as TMS pulses were delivered at the M1-representation of the FDI muscle and stimulus intensities were determined based on FDI MEPs. Therefore ADM MEP results should be interpreted with caution. An ANOVA test on ADM single pulse MEPs with the within-subjects factors *"hand", "SOA"* (75, 125, 175 and 225 ms) revealed a significant *"hand"* ($F_{1,7}$= 9.325, p=0.018) and "*hand"* × *"SOA"* interaction ($F_{3,21}$= 3.362, p=0.045). Post-hoc t-tests revealed a significant difference for the MEPs 225 ms (t_7= -7.192, p>0.000 left>right) after cue onset between left and right hand responses, and a significant difference for right hand response trials between 75 ms and 225 ms MEPs (t_7= -4.316, p=0.003). An ANOVA test on the single and paired-pulse trial ADM MEPs for every condition with within-subjects contrasts of *"pulse"*, *"SOA"*, and *"hand"* and an ANOVA test on the ADM MEP ratios with within-subjects contrasts of *"SOA"*, and *"hand"* revealed no significant effects. This could suggest that changes in M1 excitability during action reprogramming are not restricted to the muscles functionally involved in the task and occur in nearby hand muscles as well, whereas changes in SICI are restricted to the hand muscles that are used in the task. However the experimental setup does not allow strong claims about the effects in ADM, as the whole experiment was designed to investigate effects in the muscles controlling the button presses (i.e. FDI). Nevertheless the data could be interpreted as the effects of general M1 excitability

being less muscle-specific than effects of SICI, which tend to be most prominent in the muscles directly involved in the task.

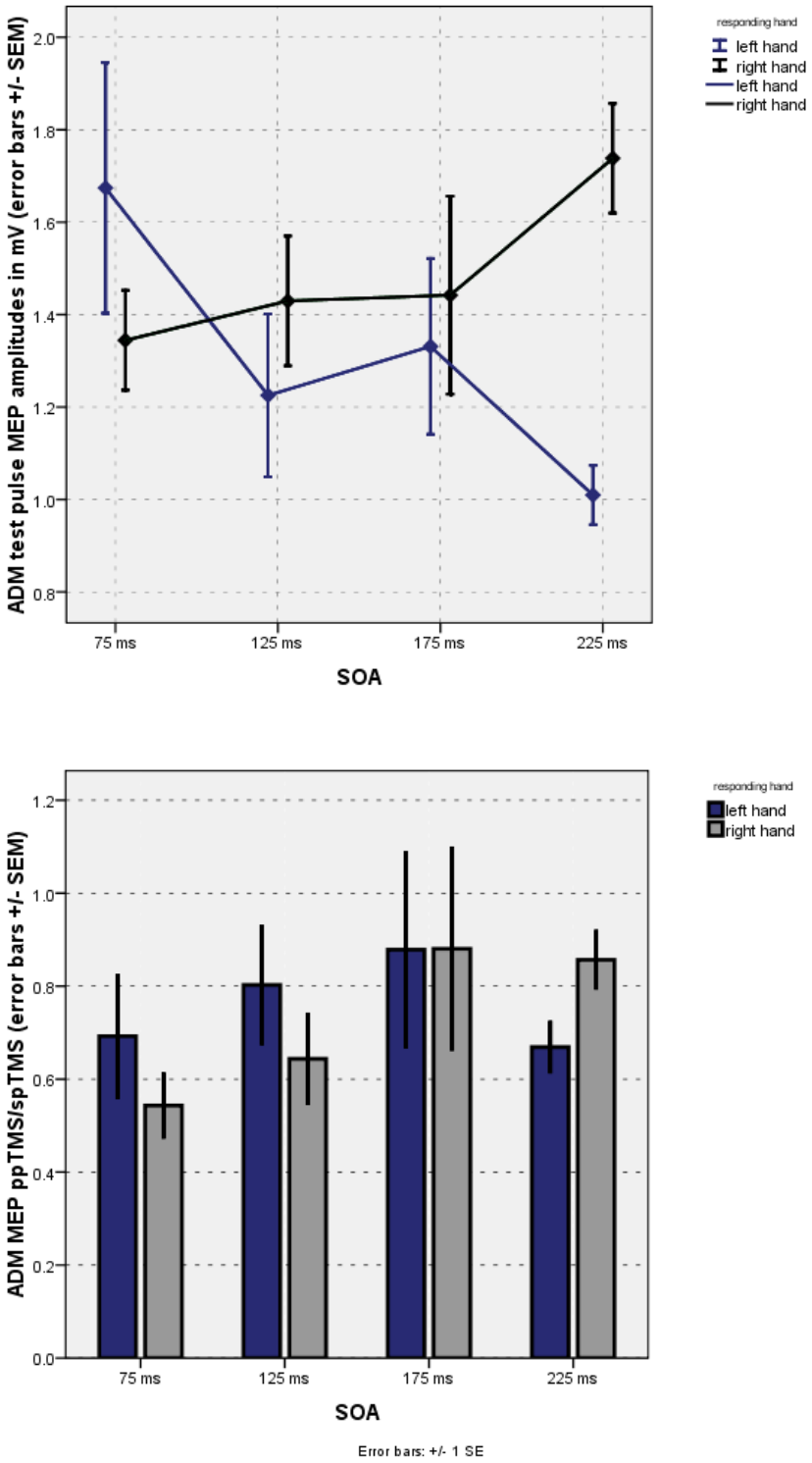
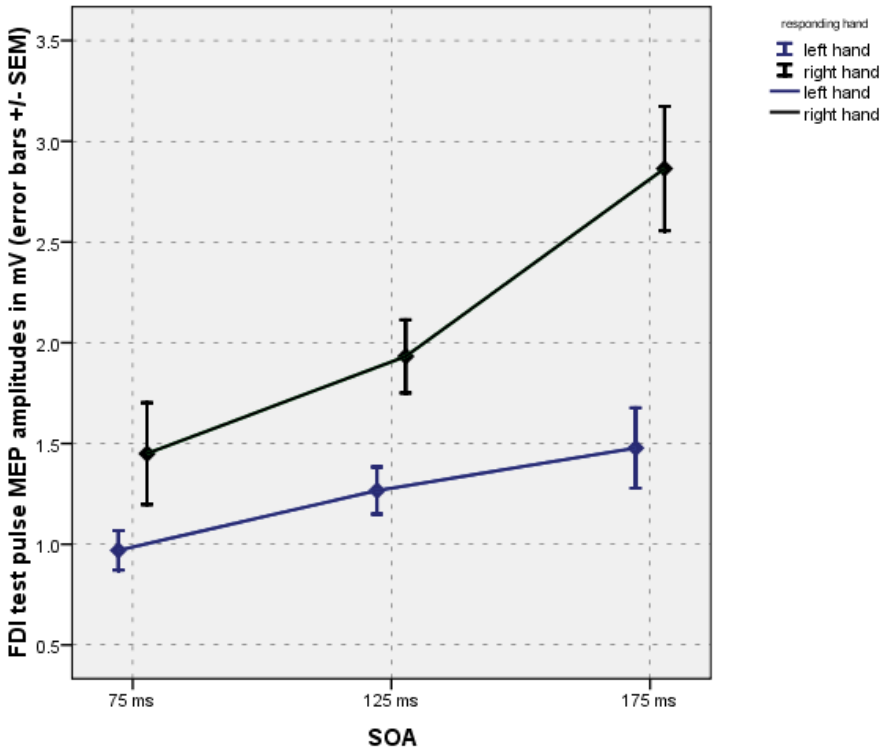


Figure 12: MEP data from the ADM muscle. Time course of general M1 excitability (top) and intracortical inhibition (bottom) during action reprogramming.

M1-corticospinal-excitability during action execution (i.e. stay trials). In a separate experiment we investigated M1 corticospinal excitability on 3 different time points (SOAs: 75, 125, 175 ms) during action execution trials, i.e. when the action, that was prepared before the centre cue colour onset was to be executed (see Figure 13). We did not stimulate 225 ms after cue onset because this would have been to close to the responses (on average 288.3 ms after cue onset). Additionally we wanted to compare the time-course of M1 excitability in action reprogramming and action execution trials (in a between-subjects contrast). Single-pulse FDI MEPs in the stay experiment (only stay trials, see Figure 13 top) were analysed and an ANOVA on the MEPs with the within-subjects factors *"hand"* and "*SOA"* (75, 125 and 175 ms) revealed a significant *"hand"* effect ($F_{1,7}$= 16.842 , p=0.005) and a significant "*hand"* × *"SOA"* interaction ($F_{2,14}$= 3.979, p=0.043). Post-hoc t-tests revealed a significant difference for the MEPs 125 ms (t_7= -2.733, p=0.029, right>left) and 175 ms (t_7=-4.391, p=0.016. right>left) after cue onset between left and right hand responses, significant differences in FDI MEPs in right hand response trials between two time-points, 75 ms and 125 ms SOA (t_7=-2.983, p=0.02) and between 75 ms and 175 ms SOA (t_7= -3.517, p=0.01), indicating that if an action was prepared and had to be executed subsequently, excitability in the corresponding M1 increases as the response approaches. It seems as if excitability increases progressively in the interval between cue onset and response, although this increase is most prominent ~110 ms before the response (i.e. 175 ms SOA).

Intracortical inhibition during action execution. To investigate changes in SICI associated with the execution of an already prepared response, we analysed MEPs in paired-pulse TMS trials and calculated MEP ratios (see Figure 13 bottom). An ANOVA test on the single and paired-pulse trial MEPs for every condition with

within-subjects contrasts of *"pulse"*, *"SOA"*, and *"hand"* revealed significant effects of *"pulse"* ($F=71.034$, $p<0.001$), *"SOA"* ($F_{2,14}= 11.823$, $p=0.003$), *"hand"* ($F_{1,7}=19.246$, $p=0.003$) and a significant "*hand"* × *"SOA"* ($F_{2,14}= 5.085$, $p=0.034$) interaction. An ANOVA test on the paired-pulse / single-pulse TMS ratios with within-subjects contrasts of *"SOA"*, and *"hand"* revealed a significant effect of *"hand"* ($F_{1,7}= 12.247$, $p=0.01$). Post-hoc paired sampled t-tests of the MEP ratios revealed significant differences between left and right-hand responses 75 ms ($t_7= -3.230$, $p=0.014$) and 175 ms ($t_7= -2.752$, $p=0.028$). This could be interpreted as the appropriate response being present already 75 ms after centre cue colour onset, as it was prepared even before cue onset. Additionally we found a difference in right hand response trial MEPs between 75 and 125 ms ($t_7= 3.005$, $p=0.02$). This was somehow surprising, as we would have expected progressive release of SICI in the time course of movement execution, just as we observed it during action reprogramming. However these effects might reflect a process of withholding a prepared response until information from "reprogramming areas" would arrive and subsequently as this information did not arrive (and the trial turned out not to be a switch trial), the prepared response can be released.



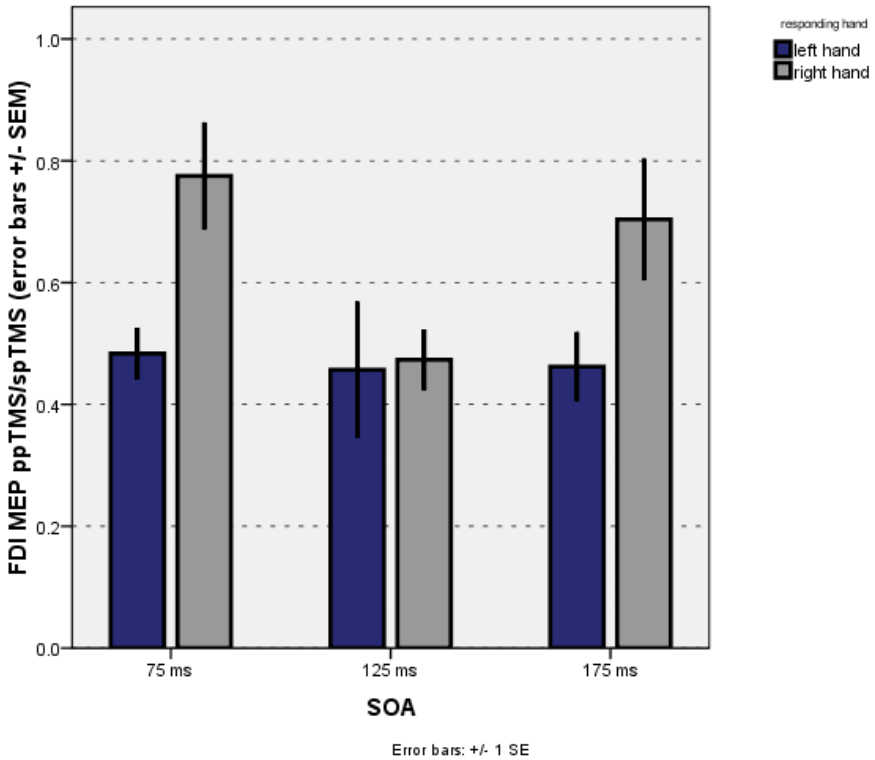


Figure 13: Time course of general M1 excitability (top) during action execution (i.e. stay trials). Single-pulse TMS MEP sizes are plotted for every condition (SOA and hand). Time course of intracortical inhibition during action execution. Single-pulse / paired-pulse TMS MEP ratios are plotted for every condition (SOA and hand).

Functional specificity of the observed effects. To investigate functional specificity of these effects ADM single-pulse MEPs and paired-pulse MEPs were analysed and ADM MEP ratios were calculated for every condition (see Figure 14). An ANOVA test on ADM single-pulse MEPs with the within-subjects factors *"hand"*, *"SOA"* (75, 125 and 175) revealed a "*hand"* by *"SOA"* interaction ($F_{2,14}$= 4.329, p=0.035). Post-hoc t-tests revealed a significant difference between left and right hand response MEPs at 175 ms (t_7= -3.058, p>0.018 left<right) after cue onset, and a significant difference for left hand response trials between 75 ms and 175 ms MEPs (t_7= 2.635, p=0.034). This could indicate that general M1 excitability increases in the time course of action execution again non-specifically, i.e. in the M1 representation of the responding finger and of nearby hand muscles. An ANOVA test on the single and paired-pulse trial ADM MEPs for every condition with within-subjects contrasts of *"pulse"*, *"SOA"*, and *"hand"* only revealed a significant effect of pulses ($F_{1,7}$= 9.981, p=0.02) and an ANOVA test on the ADM MEP with within-subjects contrasts of *"SOA"*, and *"hand"* ratios revealed no significant effects. This could again indicate a greater FDI specificity of the SICI effects, suggesting that SICI selectively inhibits and releases finger muscles which are involved in the experimental task.

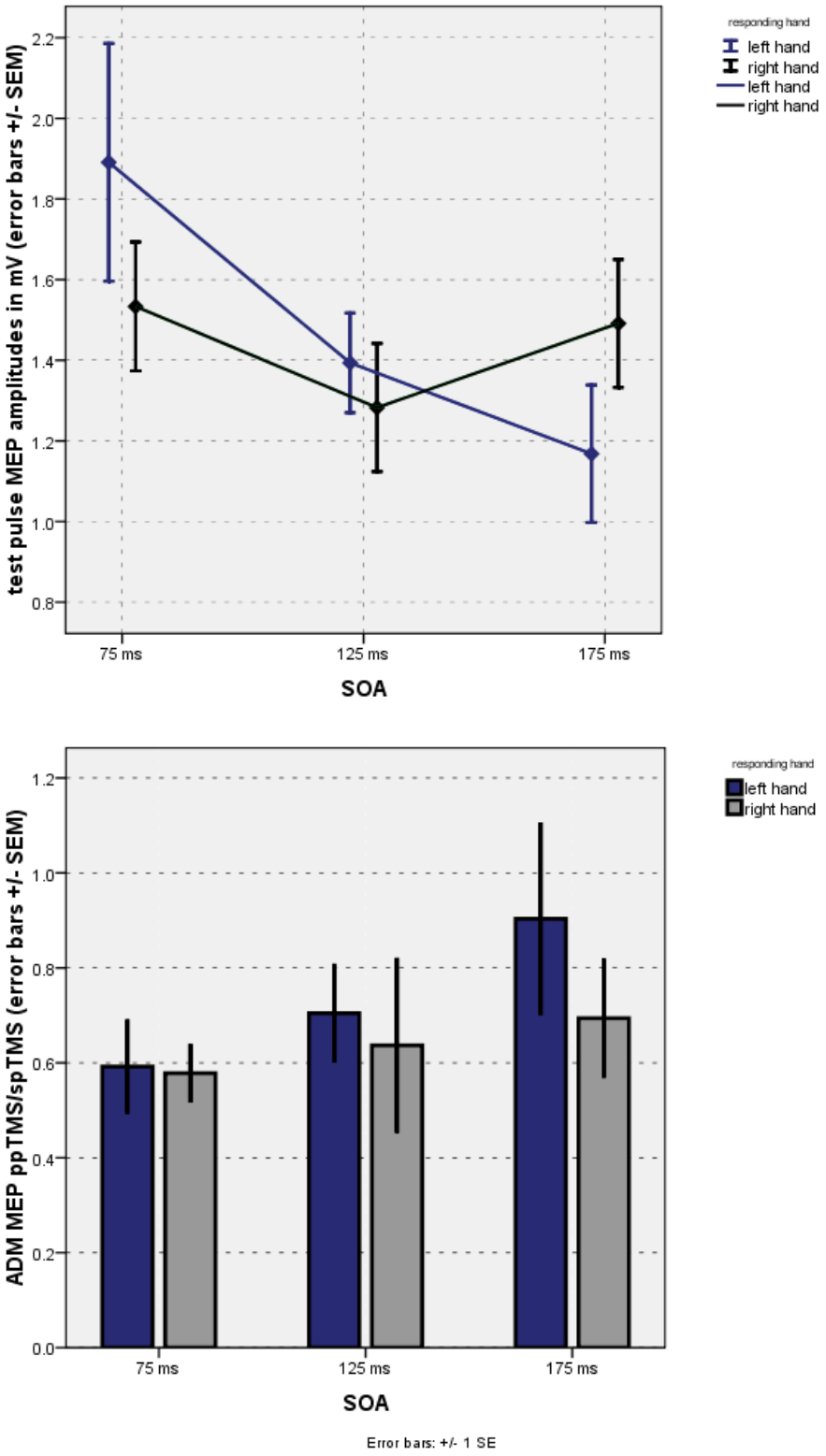
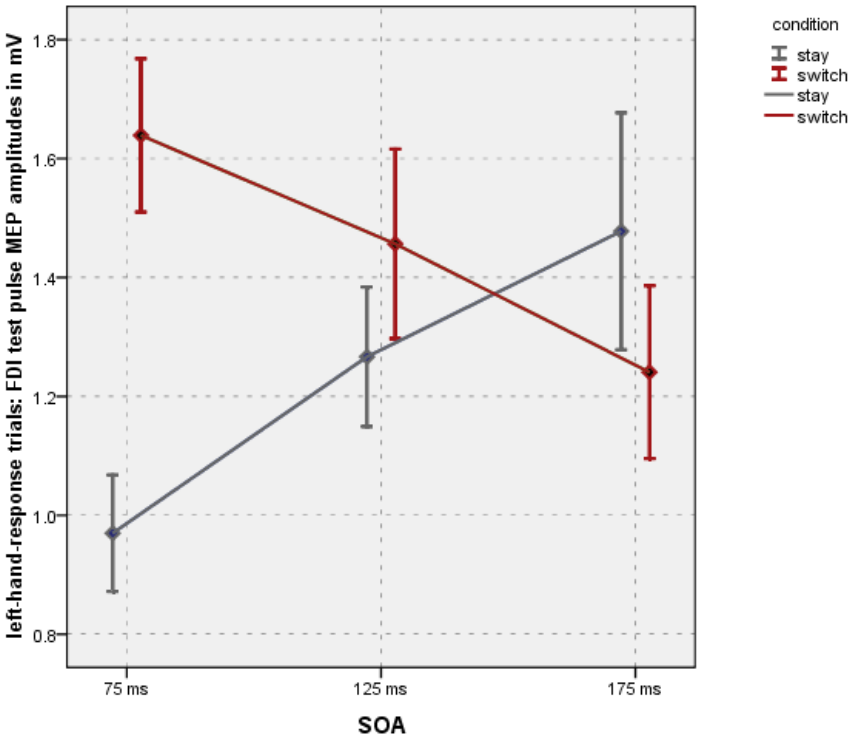


Figure 14: MEP data from the ADM muscle. Time course of M1 cortex excitability (top) and intracortical inhibition (bottom) during action execution.

Action reprogramming vs. action execution (i.e. switch trials vs. stay trials). One important question of this experiment was whether processes of action reprogramming as opposed to action execution would take place or converge on the level of M1 and the motor system. If one wants to study action reprogramming and response inhibition in the brain it appears sensible to examine mechanisms internal to the target area (i.e. M1) of these reprogramming and inhibition processes first. Therefore we aimed to compare M1 corticospinal excitability and short-latency M1 internal inhibitory processes (i.e. SICI) during reprogramming of prepared actions and execution of prepared actions. We thus had to concentrate on a between-subjects design contrasting M1 single-pulse effects and SICI effects in the "switch experiment" with the "stay experiment". Note that the 2 experiments were exactly the same and behavioural data showed no difference in performance between the two groups. We termed the two experiments "switch experiment" and "stay experiment", because the two TMS protocols focused on different behavioural trial types (i.e. switch and stay) in the exact same task and setup. To infer whether M1 excitability differed depending on whether the subjects were just executing the action they had prepared or whether they had to update their prediction, re-programme their action plan and inhibit the prepared but incorrect response, an ANOVA test on FDI single-pulse MEPs with the within-subjects factors *"hand"*, "*SOA"* (75, 125 and 175) and the between subject factor *"condition"* (switch vs. stay) was conducted (see Figure 15). We found an effect of *"hand"* ($F_{1,14}$= 12.973, p=0.003), of *"SOA"* ($F_{2,28}$= 5.906, p=0.014), a significant "*condition" × "hand"* interaction ($F_{1,14}$= 16.053, p=0.001), a significant "*condition" × "SOA"* interaction ($F_{2,28}$= 8.768, p=0.003), and a significant "*hand" × "SOA"* interaction ($F_{2,28}$= 8.672 p=0.001), indicating that the time-course of M1 excitability differed between switch and stay trials. Post-hoc independent samples

t-tests, not assuming equal variances, revealed a significant difference between switch and stay trials for left-hand-response trials at 75 ms SOA (t_{14}= -4.129, p=0.001, switch>stay), right-hand-response trials at 125 ms SOA (t_{14}= 2.746, p=0.017, stay>switch) and 175 ms SOA (t_{14}= 3.980, p=0.003, stay>switch), indicating that inhibition of the prepared but incorrect response in switch trials is not completed 75 ms after cue onset and facilitation of the correct response is much stronger and faster in stay trials compared to switch trials, possibly resulting in shorter RTs in stay trials. To examine whether SICI processes differed depending on the contrast between action execution and action reprogramming an ANOVA test on paired-pulse / single pulse MEP ratios with the within-subjects factors *"hand"*, *"SOA"* (75, 125 and 175) and the between subject factor *"condition"* (switch vs. stay) was conducted (see Figure 16). We found an effect of *"hand"* ($F_{1,14}$= 19.707, p=0.001), a significant "*condition"* × *"SOA"* interaction ($F_{2,28}$= 3.535, p=0.043), and a significant "*condition"* × *"hand"* × *"SOA"* interaction ($F_{2,28}$= 7.813 p=0.002), indicating that the time-course of SICI change differed between switch and stay trials in different ways depending on the hand that responded . Post-hoc independent samples t-tests, not assuming equal variances, revealed a significant difference between switch and stay trials for left-hand-response trials at 75 ms SOA (t_{14}= -2.652, p=0.023, switch>stay), and for right-hand-response trials at 125 ms SOA (t_{14}= -3.001, p=0.016, switch>stay), indicating that SICI release of the prepared but wrong response is still present early (i.e. 75 ms after cue onset) in the time-course of action reprogramming but not action execution and that later on (i.e. 125 ms after cue onset) SICI release of the correct response is stronger during action reprogramming than during action execution. This is interesting as it could suggest that relatively early occurring (125 ms after cue onset) very strong releases of SICI-indexed processes are necessary for switching

from a prepared and automatic response to an alternative one. Hence one way action reprogramming could be achieved via SICI is by overcoming the SICI-indexed release of the prepared but wrong response with an even stronger release of SICI of the unexpected alternative response, which is then followed later on by an increase in inhibition of the prepared but wrong response.



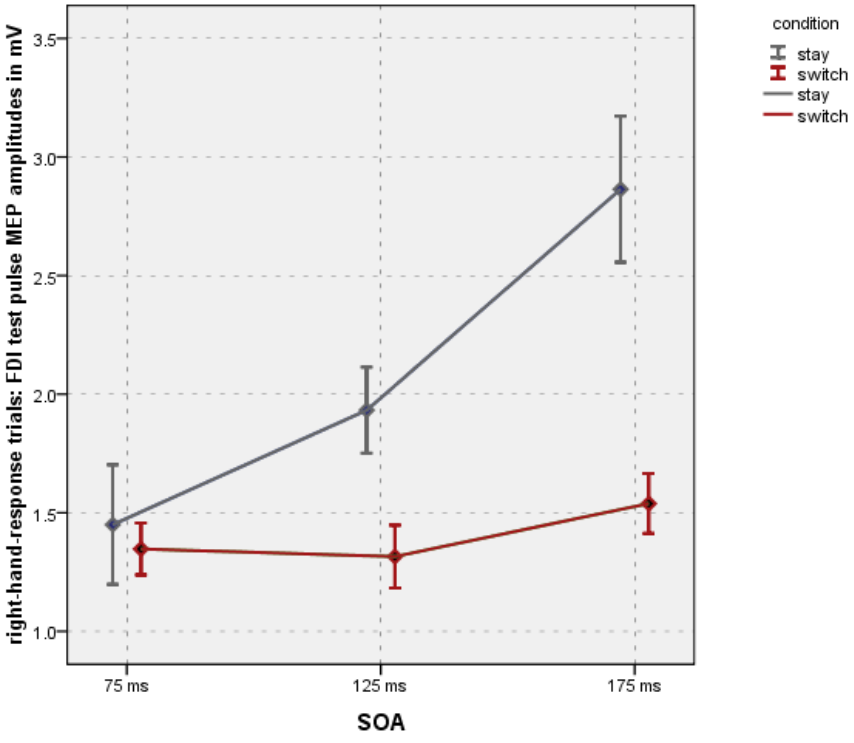


Figure 15: Time course of general M1 excitability during action reprogramming (red) and action execution (grey). Single-pulse TMS MEP sizes are plotted for every SOA, left hand response trials (top) and right hand response trials (bottom). The difference between the two curves is only whether the left (top) and right (bottom) response was chosen as prepared (grey) or after reprogramming (red).

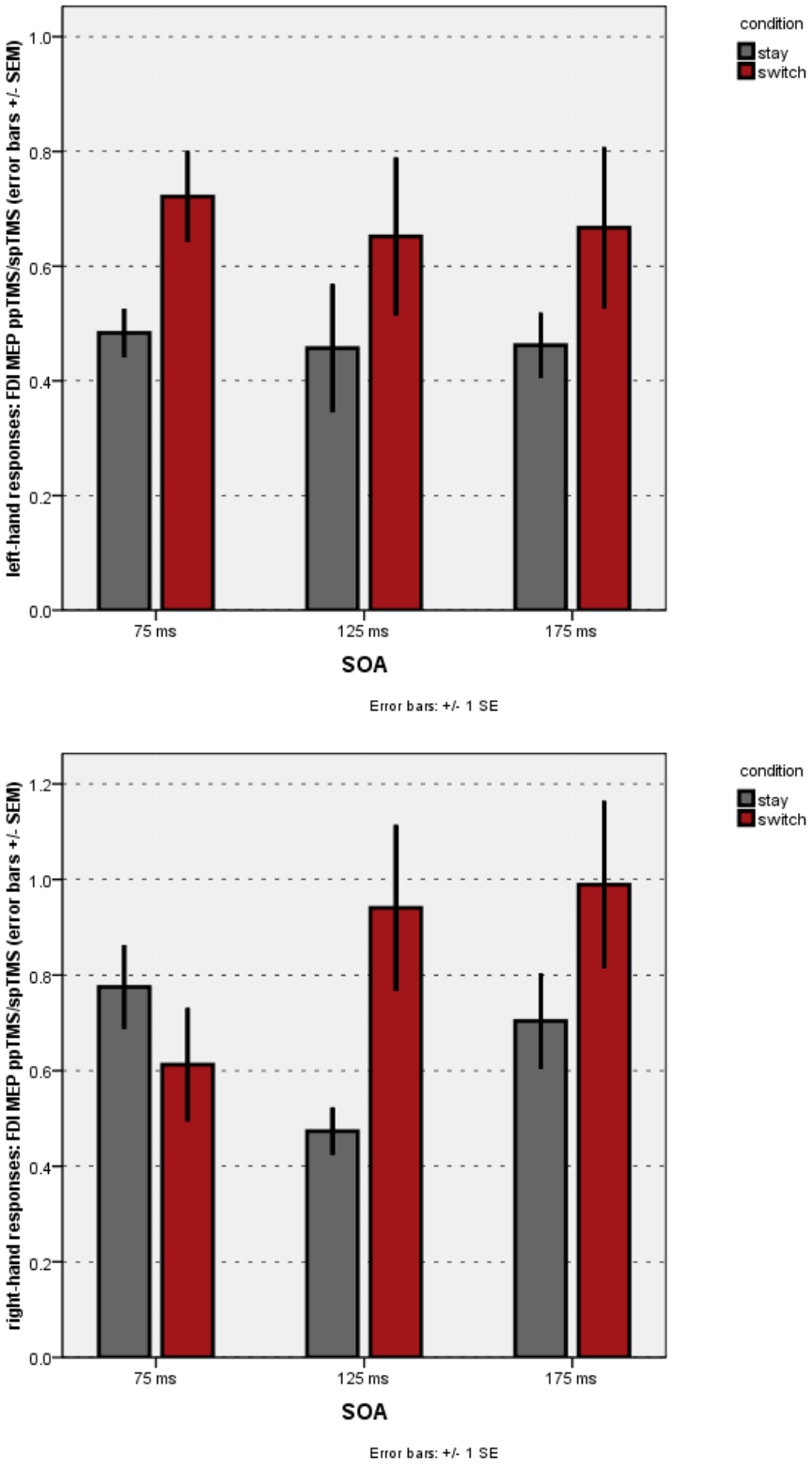


Figure 16: Time course of intracortical inhibition during action reprogramming (red) and action execution (grey). Single-pulse / paired-pulse TMS MEP ratios are plotted for every SOA, left hand response trials (top) and right hand response trials (bottom).

Discussion

If alternative actions have to be selected in the context of ongoing movement plans due to an unexpected change in the environment and environmental demands, prepared but inappropriate responses need to be inhibited and more appropriate response strategies have to developed, selected and initiated. Action reprogramming and response inhibition is likely to involve brain areas different from those known to be active during action execution. A network of several brain areas in the lateral and medial PFC as well as regions in the parietal lobes has been proposed (Garavan et al., 1999; Dove et al., 2000; Aron et al., 2004b; Nachev et al., 2005; Crone et al., 2006; Aron, 2007; Isoda and Hikosaka, 2007; Mars et al., 2007b; Chambers et al., 2009; Mars et al., 2009). As the final stage of action reprogramming and the direct or indirect target area of these networks M1 is likely to reflect these processes. We hypothesised that excitability and internal inhibitory processes in M1 would change during the time course of action reprogramming and would differ from situations when only action execution was required. We used single and paired-pulse TMS and a very simple action reprogramming task to test this hypothesis.

Single-pulse and paired-pulse MEP data recorded from a muscle critically involved in the task and from a nearby hand muscle suggests that processes of action reprogramming change the pattern of M1 excitability as well as intracortical inhibition (i.e. SICI) compared to processes of normal action selection.

In switch trials 75 ms after cue onset excitability of the motor system of the prepared but incorrect response is still bigger than the excitability of the motor system of the unprepared but appropriate response. This effect is reversed 225 ms after cue onset suggesting that action reprogramming on the level of the motor system takes place

between 75 ms and 225 ms after the onset of the instructive cue. This is consistent with other paired-pulse TMS studies investigating action selection and response inhibition in motor and premotor areas and pre-SMA (Sohn et al., 2002; Coxon et al., 2006; Koch et al., 2006; O'Shea et al., 2007b; Mars et al., 2009). Most importantly this effect occurs much earlier than the average response time in action execution trials suggesting that it might be crucial for successful switching. Reprogramming of the ongoing action plan is achieved by facilitating the unprepared but correct and inhibiting the incorrect but prepared response. The major role of SICI-indexed inhibition processes during action reprogramming seems to be the release of the unprepared but correct response. This release occurs 125 ms after cue onset and increases later on in time. SICI release of the correct response appears earlier than the effects in general excitability indicating that SICI-indexed inhibition modulates excitability of M1 and hence plays an important role in selecting an unprepared but correct response. These effects were not observed in normal action execution trials suggesting that the correct response plan was already present even on the level of the motor system 75 ms after cue onset as it could be predicted on the basis of the flanker stimuli. Interestingly the release of SICI for the correct response was greater for the response switching trials than for the action execution trials at 125 ms SOA. This could suggest that one mechanism achieving successful action reprogramming is an even stronger release of SICI to overcome the prepared but incorrect action plan.

It is unlikely that action reprogramming takes place solely at the level of the M1. Imaging and neurophysiological studies have already shown that action reprogramming recruits a network of cortical and subcortical structures including rIFC, basal ganglia and pre-SMA (Garavan et al., 1999; Aron and Poldrack, 2006; Li et al., 2006; Aron et al., 2007a; Isoda and Hikosaka, 2007; Mars et al., 2007b; Isoda

and Hikosaka, 2008; Duann et al., 2009; Mars et al., 2009). It is likely that these areas exert their facilitatory and inhibitory influences on the motor system between 75 ms and 225 ms after cue onset as this is the interval when we observed changes in M1 excitability and SICI associated with response inhibition and reprogramming. The following experiments will try to answer these questions.

2.1.2. Functional Connectivity between rIFC and M1 and Pre-SMA and M1 during Inhibition and Action Reprogramming

Introduction

Due to constant and rapid changes in our environment we have to be able to adjust our behaviour flexibly. This ability to adapt performance to volatile environmental demands (often referred to as "executive control") is argued to crucially depend on (1) detecting and solving response conflict in support of correct and appropriate responses and (2) inhibition of premature and inappropriate responses. Areas in the medial frontal cortex (such as pre-SMA) and the lateral PFC (such as rIFC) have been suggested to be critically important in situations involving (a) the direct competition, (b) inhibition, (c) updating or (d) reprogramming of actions and action plans (Aron et al., 2004a; Nachev et al., 2008). Inhibition is often investigated and measured employing relatively simple tasks, such as the already mentioned go/no-go task. But it has been suggested that inhibition and reprogramming might be general mechanisms by which PFC exerts top-down influence on other brain areas to implement executive control.

The ability to inhibit premature responses was impaired after damage to the rIFG (especially to the pars triangularis [Brodmann area 45] and the pars opercularis [BA 44]) and after damage to the pre-SMA (Aron et al., 2003a; Nachev et al., 2007). The degree of damage to the rIFC was negatively correlated with the performance in the go/no-go task (and SSRTs). Imaging studies reported activity in pre-SMA and rIFC associated with these stopping processes (Aron and Poldrack, 2006; Li et al., 2006; Aron et al., 2007a; Chikazoe et al., 2009; Duann et al., 2009) and suggested they

might be mediated via the STN and a “hyperdirect pathway” (Nambu et al., 2000; Nambu et al., 2002; Aron et al., 2007a; Aron et al., 2007b; Isoda and Hikosaka, 2007, 2008; Duann et al., 2009). Repetitive TMS over pre-SMA during response conflict results in a greater activation in the M1 controlling the competing response, as indexed by the lateralized readiness potential (LRP) (Taylor et al., 2007). RTMS to the rIFC has been shown to transiently impair performance in go/no-go tasks (Chambers et al., 2006). Deep-brain stimulation (DBS) of the STN improved inhibitory control in a go/no-go and a stop-signal task in patients with Parkinson's disease (van den Wildenberg et al., 2006).

These results could be interpreted as pre-SMA, rIFC and STN being a crucial part of a network of inhibitory control. However some important questions remain to be answered: (A) The concept and the term “inhibition” is extremely popular and widely used in the field of cognitive neuroscience (Aron, 2007). However many objections have been raised to the concept of active inhibitory mechanisms in cognitive neuroscience and psychology. The question whether processes of executive control, action reprogramming and task switching rely at least to some extent on inhibitory processes might be difficult to answer with current imaging techniques (Waldvogel et al., 2000; Aron, 2007; Hallett, 2007). However a paired-pulse TMS paradigm could be used to investigate whether these areas that have most often been associated with inhibition and task switching really have an inhibitory physiological influence on the motor-cortico-spinal excitability. (B) Brain areas in the frontal lobe and the basal ganglia have been suggested to play a role in response inhibition and task switching. How they really cooperate and whether all of them exert the same inhibitory influence on inappropriate motor outputs remains to be clarified. (C) Some authors even claim a specialized “network” of response inhibition and action reprogramming (Garavan et

al., 1999; Dove et al., 2000; Li et al., 2006; Duann et al., 2009). The anatomical and functional basis of such a specific inhibitory network could be revealed with a combined paired-pulse TMS-DTI approach (Boorman et al., 2007; Mars et al., 2007a; O'Shea et al., 2007b; Buch et al., submitted) correlating individual differences or even individual patterns of (a) performance (b) functional connectivity and (c) anatomical connectivity.

Functional interactions between dorsal premotor cortex and M1 (Koch et al., 2006; O'Shea et al., 2007b), ventral premotor cortex and M1 (Davare et al., 2008; Davare et al., 2009) or pre-SMA and M1 (Mars et al., 2009) can be demonstrated on a sub-second time-scale with paired-pulse TMS. It has been shown in combined TMS-fMRI experiments that TMS does not only activate the brain area underneath the TMS coil but also areas connected to this area (Hallett, 2000; Walsh and Cowey, 2000; Bestmann et al., 2004, 2005; Hallett, 2007; O'Shea et al., 2008; Driver et al., 2009). Hence a pulse over one area could have a big impact on the activity and excitability of a distant area depending on the connectivity of the stimulated area. This impact can be shown in combined TMS-fMRI paradigms and measured as the BOLD response in the indirectly activated area. However connectivity between areas can change over time in a cognitive task. Therefore the indirect impact of the TMS pulse on a distant but connected area can vary in the time course of a cognitive task requiring interaction of these two areas. The temporal resolution of fMRI does not allow detecting these sub-second time scale changes in functional connectivity. However using a paired-pulse TMS paradigm we can evaluate excitability of the motor system with high temporal resolution and hence can investigate fluctuations in the impact of a pulse over an area functionally connected to M1 on the excitability of the motor system. In such experiments the M1 “test pulse” is preceded by a conditioning pulse over

another brain area milliseconds earlier and its impact on the size of the MEP is measured as a ratio between paired-pulse TMS MEPs and single-pulse TMS MEPs (just as SICI, see Chapter 2.1.1). Conditioning pulses over the pre-SMA have already been shown to facilitate M1 test pulse MEPs in trials that required the subjects to inhibit a prepared response and reprogramme their action plan (Mars et al., 2009). We used the same task as in the previous experiment investigating M1-excitability and SICI, to test whether a conditioning TMS pulse over rIFC would influence M1 excitability in trials requiring the reprogramming of a prepared but no longer appropriate action and the normal execution of a prepared action, respectively.

Methods

Participants. 22 healthy volunteers (age range 19 – 40 years, mean age = 26.36 +/- 4.61250 SD, 10 females) with no personal or familial history of neurological or psychiatric disease participated in one or more of the three experiments investigating functional connectivity between two brain areas during inhibition and action reprogramming. The experiment was approved by the Oxfordshire Research Ethics Committee and conducted in accordance with the declaration of Helsinki. The same behavioural task and experimental setup was used in three separate experiments to investigate time course of functional connectivity between rIFC and left M1 during (1) action reprogramming and inhibition of prepared responses (experiment 1, subsequently called the "rIFC-M1 switch experiment") and (2) execution of prepared responses (experiment 2, subsequently referred to as "rIFC-M1 stay experiment"), respectively and (3) to control whether observed effects were due to processes internal to M1 or to external input from rIFC (experiment 3, subsequently called "M1 control experiment"). 10 Participants (5 females, mean age = 27.6 +/- 5.70 SD) participated in the "rIFC-M1 switch experiment". 10 participants (4 females, mean age = 26.6 +/- 3.57 SD) participated in the "rIFC-M1 stay experiment". 8 participants (4 females, mean age = 24.5 +/- 4.21 SD) participated in the "M1 control experiment". All participants were right-handed and gave written informed consent. They were all screened for adverse reactions to TMS and risk factors by means of a safety questionnaire.

Experimental setup. Participants were seated in a darkened room and wore a tight-fitting EEG cap, on which TMS sites were marked and earplugs to protect against TMS noise. A chin rest was used to minimise head movements.

Behavioural Task. We used the same behavioural paradigm as in the previous experiment (see Chapter 2.1.1) modelled on the task developed by (Isoda and Hikosaka, 2007). Again the task was presented on a PC running Windows XP and the experiment was controlled by custom software written in Presentation (version 0.53) (see Figure 17). Before the actual experiment participants were familiarised with the task in one behavioural training block (without TMS pulses, 30 trials) and with the whole experimental setup including TMS pulses over rIFC and M1 in a second training block (with TMS pulses, 30 trials). Both, the "rIFC-M1 switch experiment" and the "rIFC-M1 stay experiment" consisted of 7 experimental blocks. The "M1 control experiment" consisted of 4 experimental blocks. Each block contained 30 switch and 150 stay trials. Reaction times were recorded.

TMS. The aim of this experiment was to investigate the influence exerted by rIFC on M1 during response inhibition and response execution. This can be achieved using a paired-pulse TMS paradigm with one TMS coil over the M1 (test coil) and another TMS coil (conditioning coil) over rIFC (see Figure 17). TMS pulses were delivered on 30 out of 180 trials per block. There were two types of TMS trials. On half of the trials, so-called *"single-pulse" trials*, a single TMS "test pulse" was delivered over the left M1-representation of the right FDI. As in the previous experiment intensity of this TMS test pulse was such that an MEP of 1 – 1.5 mV was evoked in the relaxed, contralateral FDI. This intensity was 45.7% (SEM+/- 2.399) of the maximum stimulator output in the rIFC-M1 switch experiment, 43.6% (SEM +/- 1.99) for the rIFC- M1 stay experiment, and 48.12% (SEM +/- 2.62160) for the M1 control

experiment. On the other half of the trials, so-called *"paired-pulse" trials*, the M1 test pulse was preceded by a "conditioning pulse" over the rIFC in the "rIFC-M1 switch experiment" and the "rIFC-M1 stay experiment" and by a pulse through the same coil in the "M1 control experiment". The inter-pulse-interval was 8 ms. Intensity of the test pulse was exactly the same as in "single-pulse trials". The intensity of the preceding conditioning pulse was set at 110% of the resting motor threshold (RMT) of the left FDI muscle (right M1). These parameters were obtained from studies investigating functional PMv-M1 connectivity in grasping (Davare et al., 2008; Davare et al., 2009; Buch et al., submitted). This might appear somewhat surprising as action reprogramming and response inhibition has previously been closely associated with rIFC. It is, however, noteworthy that the most posterior part of the IFC actually corresponds to the region recognized as PMv by most researchers working on the motor system (Mayka et al. 2006, Tomassini et al., 2007) and has been proposed to be important for the initiation and cancellation of action sequences (Koechlin and Jubault 2006; Davare, Montague et al. 2009; Buch, Mars et al. submitted) (see Table 1). Moreover given its strong connections with ventral and opercular frontal regions and its projections to the STN that were argued to be crucial for "breaking" ongoing movements and action plans, PMv is well placed to influence M1-corticospinal output during response switching (Nambu et al., 1996; Nambu et al., 2000; Nambu et al., 2002; Aron et al., 2007a; Tomassini et al., 2007; Isoda and Hikosaka, 2008).

Average RMT was 38.6% (+/-1.44 SEM) of maximum stimulator output in the rIFC-M1 switch experiment, 36.6% (+/-1.14 SEM) in the rIFC-M1 stay experiment, and 40.8% (+/-2.52 SEM) in the M1 stay experiment. We hypothesised that the preceding conditioning pulse would change the MEP amplitude elicited by the test pulse, depending on the type of the trial (switch trial vs. stay trial) and the time after centre

cue colour onset (75 ms, 125 ms, 175 ms). In the M1 experiment the two pulses (conditioning pulse and test pulse) were delivered through the same coil over the same area in the motor cortex, testing whether the observed changes of test pulse MEP size in the paired-pulse trials were due to M1 internal mechanisms. In the rIFC-M1 switch and stay experiment TMS pulses were delivered through two 55 mm diameter figure-of-eight coils directly connected to two high-power Magstim 200 MonoPulse machines (The Magstim Company®) (see Figure 17). In the M1 control experiment pulses were delivered through a single 70 mm diameter figure-of-eight coil connected via a BiStim module to the same high-power Magstim 200 MonoPulse machines. The magnetic stimulus had monophasic pulse configuration, with a rise time of ~ 100 μs, decaying back to zero over ~ 800 μs. TMS coil applying test pulses to the left M1 was placed tangentially to the scalp, inducing posterior-to-anterior current flow perpendicular to the central sulcus. TMS coil applying conditioning pulses to the rIFC was placed tangential to the scalp with the handle of the coil pointing forwards and upwards, perpendicular to the sylvian fissure (see Figure 18). MRI scans were acquired for every subject. The location for the conditioning coil was determined using an MRI-aligned frameless stereotaxic neuronavigation system (Brainsight, Rogue Research Inc®). Each subject's head was first coregistered with their anatomical MRI in native space, and a trajectory was plotted from each scalp location at which TMS was applied onto the cortical surface using Brainsight software. Individual subjects' structural MRI scans were then normalized to the MNI 152-mean brain T1 template to evaluate the areas of TMS (left M1 and rIFC) with respect to the Montreal Neurological Institute (MNI) standard space using the linear registration tool "FLIRT" of Oxford Centre for Functional Magnetic Resonance Imaging of the Brain's (FMRIB) Software Library "FSL" (Smith et al., 2004). The

mean MNI location across subjects for the conditioning coil location was x=60.5 (+/- 1.09 SEM), y=14.25 (+/- 1.48) and z=30.11 (+/- 2.15) in the rIFC-M1 switch experiment and x=60.1 (+/- 1.41), y=15.04 (+/- 0.87) and z=29.66 (+/- 1.66) in the rIFC-M1 stay experiment (see Figure 18). The mean M1 test coil MNI location was x=-43.84 (+/- 2.91), y=-6.12 (+/-2.13) and z=63.32 (+/- 2.7) in the rIFC-M1 switch experiment, and x=-46.37 (+/- 2.15), y=-9.36 (+/-1.34) and z=57.12 (+/- 5.17) in the rIFC-M1 stay experiment (see Figure 18). "RIFC-M1 switch experiment", "rIFC-M1 stay experiment" and "M1 control experiment" were separate experiments involving different participants. During "rIFC-M1 switch experiments" pulses were almost exclusively delivered on switch trials (24 TMS trials per block delivered on switch trials, 6 per block on stay trials), only switch-trial MEPs were analysed. In the "rIFC-M1 stay experiments" pulses were almost exclusively delivered on stay trials (24 TMS trials per block delivered on switch trials, 6 per block on stay trials), only stay trial MEPs were analysed. In the "M1 control experiment" pulses were delivered on switch and stay trials (15 TMS trials per block on switch trials, 15 TMS trials per block on stay trials), switch and stay trial MEPs were analysed. TMS was delivered 75 ms, 125 ms and 175 ms after onset of the centre colour cue (stimulus onset asynchrony, SOA) during rIFC-M1 switch and stay experiments and 175 ms SOA in the M1 control experiment (as we found the biggest effect at 175 ms SOA). For the rIFC-M1 switch and stay experiments a total of 14 TMS trials for each condition of hand (left vs. right hand), SOA (75, 125, 175) and pulse type (single vs. paired) were obtained and used for the analysis. In the M1 control experiment a total of 15 TMS trials per hand (left vs. right), SOA (175), condition (switch vs. stay) and pulse type (single vs. paired) were obtained and analysed. TMS trials were presented at least 7 seconds apart, to ensure that pulses on adjacent trials did not influence each other. In

each block, TMS trials were distributed evenly over response hands, respective SOAs and single- or paired-pulse TMS.

Electrophysiological recordings. As in the previous experiment MEPs were recorded from two muscles, the FDI and the ADM (see Figure 8), in the right hand using two surface Ag-AgCl electrodes in tendon-belly montage. An earth electrode was placed on the right elbow. EMG responses were band-pass filtered between 10-1000 Hz, with an additional 50 Hz notch filter, sampled at 5000 Hz, and recorded using a CED 1902 amplifier, a CED micro 1401 Mk.II A/D converter, and a PC running Spike2 (Cambridge Electronic Design, Cambridge, UK).

Analysis. For the analysis of the difference between rIFC-M1 connectivity during action reprogramming (rIFC-M1 switch experiment) and action execution (stay experiment) we concentrated on a between-session and hence between-subjects design: We analysed MEP data from the switch trials in the rIFC-M1 switch experiment (24 out of 30 per block) and the stay trials in the rIFC-M1 stay experiment (24 out of 30 trials per block). We analysed switch and stay trials in the M1 control experiment (30 out of 30 trials per block). As in the previous experiment analysis of electrophysiological data concentrated on peak-to-peak amplitudes of the MEPs measured on TMS trials (switch trials in the rIFC-M1 switch experiment, stay trials in the stay experiment, all trials in the M1 control experiment). Peak-to-peak MEP amplitude was defined as the voltage difference between the minimum and maximum EMG signal in a window from 15 to 40 ms after TMS delivery. Trials with incorrect responses, trials with premature (RT<150) responses, trials in which the test pulse failed to elicit a reliable MEP (amplitude<0.1 mV), and trials in which participants pre-contracted the FDI muscle prior to application of the TMS pulse (EMG amplitude>0.1 mV in the 80 ms before the pulse) were discarded from the analysis.

Following this pre-processing, on average 2.31 (SEM± 0.177) trials per condition (of a total of 14 trials per condition) in the rIFC-M1 switch experiment, 3.38 (± 0.187) trials per condition (of a total of 14 trials) in the stay experiment, and 2.56 (+/- 0.262) trials per condition (of a total of 15 trials) in the M1 control experiment were excluded. Three paired-samples t-tests contrasting the number of excluded trials in single-pulse and paired-pulse TMS trials in each experiment (rIFC-M1 switch, rIFC-M1 stay and M1 control experiment) could not find any difference ($p>0.6$) in the number of MEPs excluded from the analysis between single-pulse and paired-pulse TMS trials in any of the three experiments. To account for differences in coil placement between experimental blocks, MEP sizes were median- normalised within each block. Analyses of MEPs were carried out on the mean of the normalised MEP amplitudes in each condition. Analyses of both behavioural and electrophysiological data were conducted using ANOVA tests, using repeated measures where possible. Significant effects were identified based on Huynh-Feldt corrected ANOVA values, using SPSS 16.0. Post-hoc two-sided t-tests were used to further investigate significant effects in the ANOVAs.

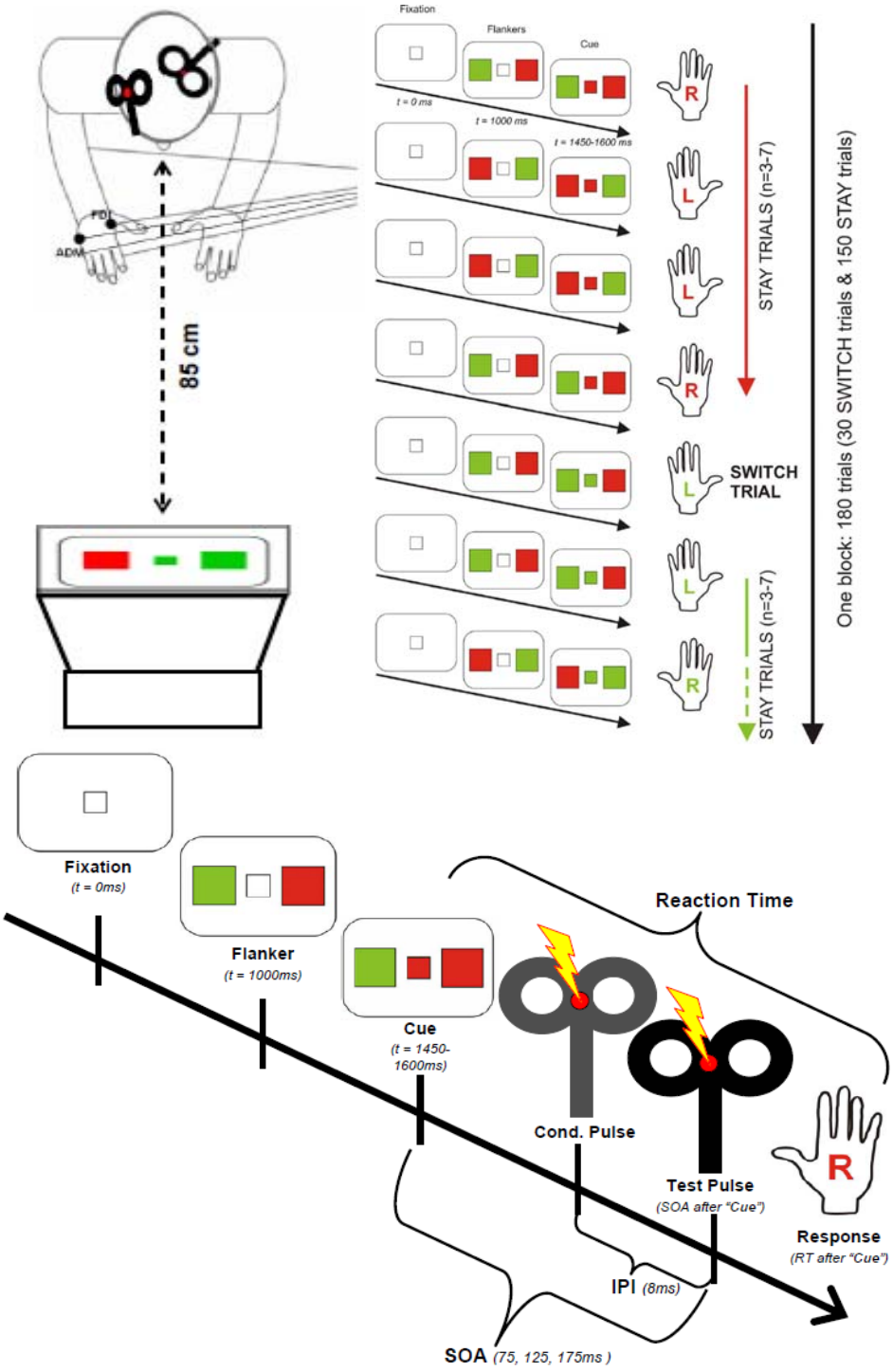


Figure 17: Experimental setup. Pulses were delivered through two 55mm diameter figure-of-eight coils (top). The conditioning coil was placed over rIFC, the test coil was placed over left M1. One experimental block contained 180 trials, 30 switch trials and 150 stay trials (top). On each trial the participants were presented with a centrally displayed white fixation square. Subsequently two differently coloured flankers appeared on either side of the fixation. After a variable delay (450-600 ms) the central fixation turned red or green instructing the participants to respond with the left or right index finger (represented as left and right hand shapes). Scheme of a single TMS trial (bottom). One experimental block contained 30 TMS trials, 15 single pulse TMS trials and 15 paired-pulse TMS trials.

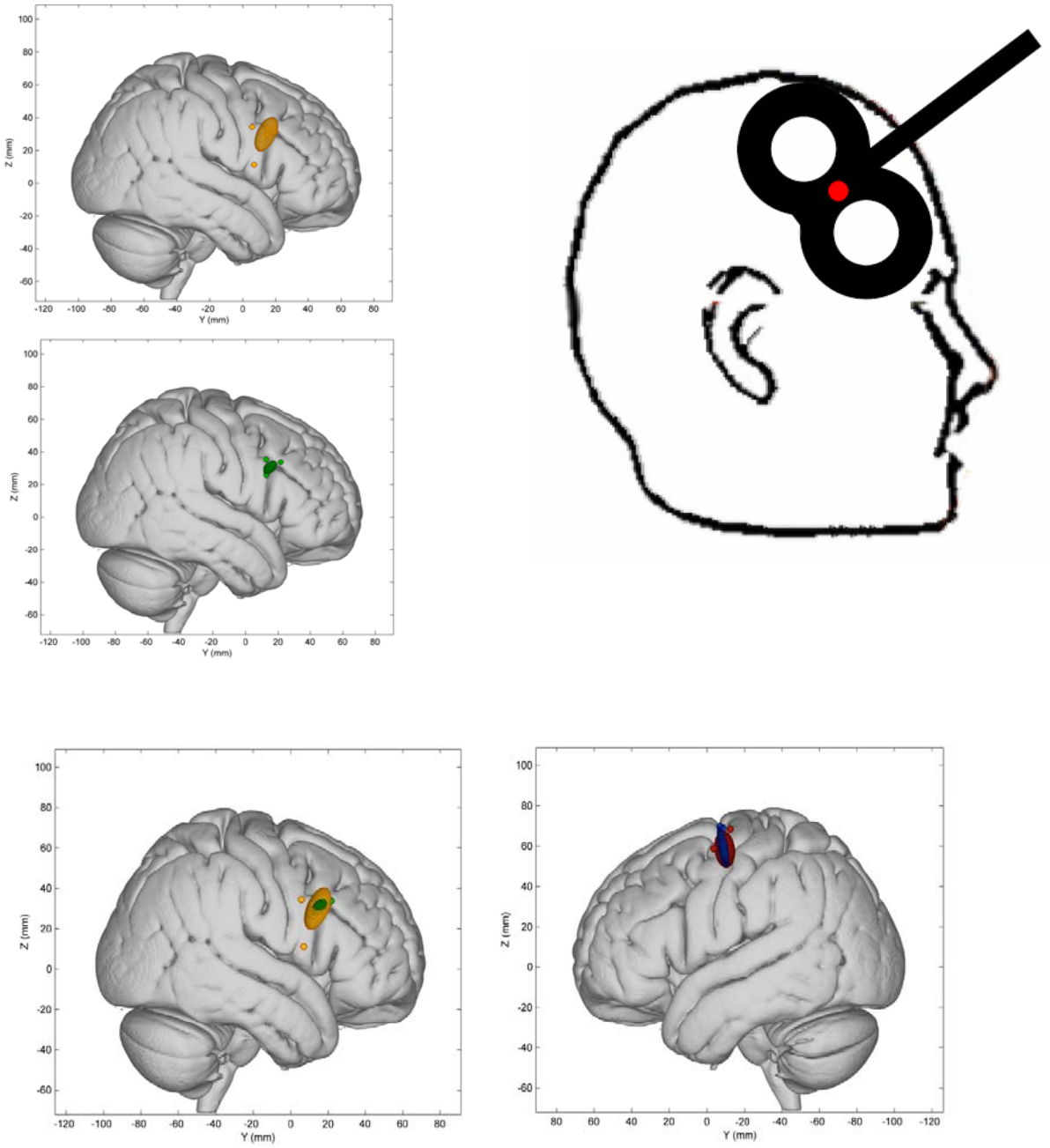


Figure 18: MNI coordinates for TMS targets. Circular symbols indicate individual subjects' stimulation locations in MNI152 space. Ellipsoids represent 95% confidence limits of the mean group stimulation location for each area. Left M1: x=-43, y=-6 and z=63 in the rIFC-M1 switch experiment (red), and x=-46, y=-9 and z=57 in the rIFC-M1 stay experiment (blue). RIFC x=60, y=14 and z=30 in the rIFC-M1 switch experiment (yellow) and x=60, y=15 and z=29 in the rIFC-M1 stay experiment (green). TMS coil applying conditioning pulses to the rIFC was placed tangential to the scalp with the handle of the coil pointing forwards and upwards.

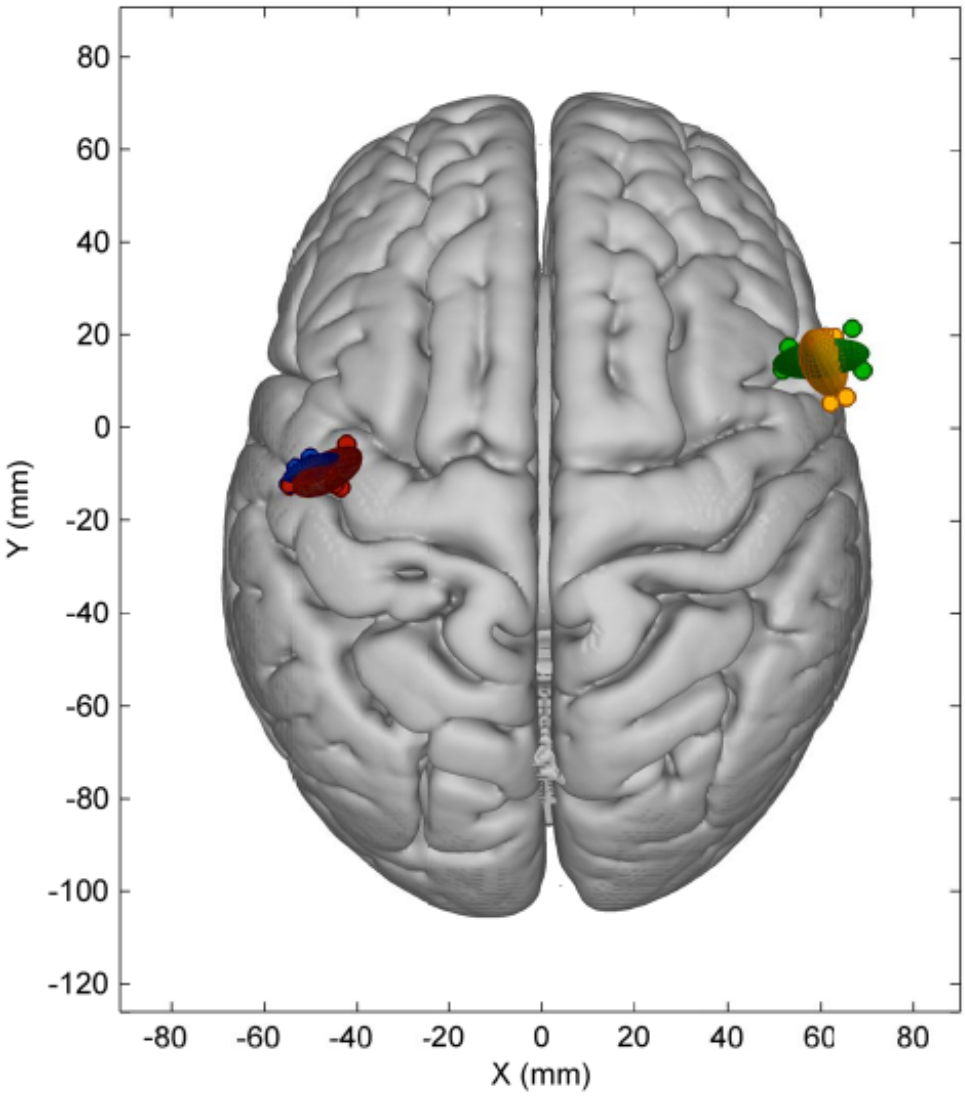


Figure 19: MNI coordinates for TMS targets. Circular symbols indicate individual subjects' stimulation locations in MNI152 space. Ellipsoids represent 95% confidence limits of the mean group stimulation location for each area.

Results

Behavioural results. ANOVAs of median RTs on correct trials and of error rates (incorrect responses/total number of trials) with *"trial type"* (switch vs. stay) as a within-subject factor and *"experiment"* (rIFC-M1 switch, rIFC-M1 stay and M1 control experiment) as a between-subject factor showed a main effect of *"trial type"* ($F_{2,25}$= 78.598, p<0.001 for RTs; $F_{2,25}$= 33.352, p<0.001 for error rates), but no main effect of *"experiment"* and no interaction between *"experiment"* and *"trial type"* (p>0.45). Two similar ANOVAs were conducted just considering the two rIFC-experiments for RTs and error rates, respectively. These ANOVAs with *"trial type"* as a within-subject factor and *"experiment"* (rIFC-M1 switch, rIFC-M1 stay experiment) as a between-subject factor again showed a main effect of *"trial type"* ($F_{1,18}$= 64.493, p<0.001 for RTs; $F_{1,18}$= 27.135, p<0.001 for error rates), but no main effect of *"experiment"* and no interaction between *"experiment"* and *"trial type"* (p>0.25). A post-hoc paired-samples t-test including behavioural data of all three experiments confirmed subjects were significantly slower on switch trials than on stay trials (RT 395.8 ms on switch vs. 290.6 ms on stay trials, t_{27}=9.16, p<0.001, see Figure 20) and made significantly more mistakes (error rate 23.24% on switch trials vs. 2.29% on stay trials, $t_{2,27}$=5.89, p<0.001, see Figure 20). This confirmed the effectiveness of the task manipulation. However independent samples t-tests testing differences between the two rIFC-experiments (switch and stay) in RTs, RTs in switch trials, RTs in stay trials, error rates, error rates in switch trials and error rates in stay trials did not yield any differences in these behavioural measures between the two experiments (p>0.2). RT switching costs were 1.3896 (+/- 0.04479 SEM) and

error switching costs were 20.3252 (+/- 4.48933 SEM). Again these behavioural effects did not differ between the experiments.

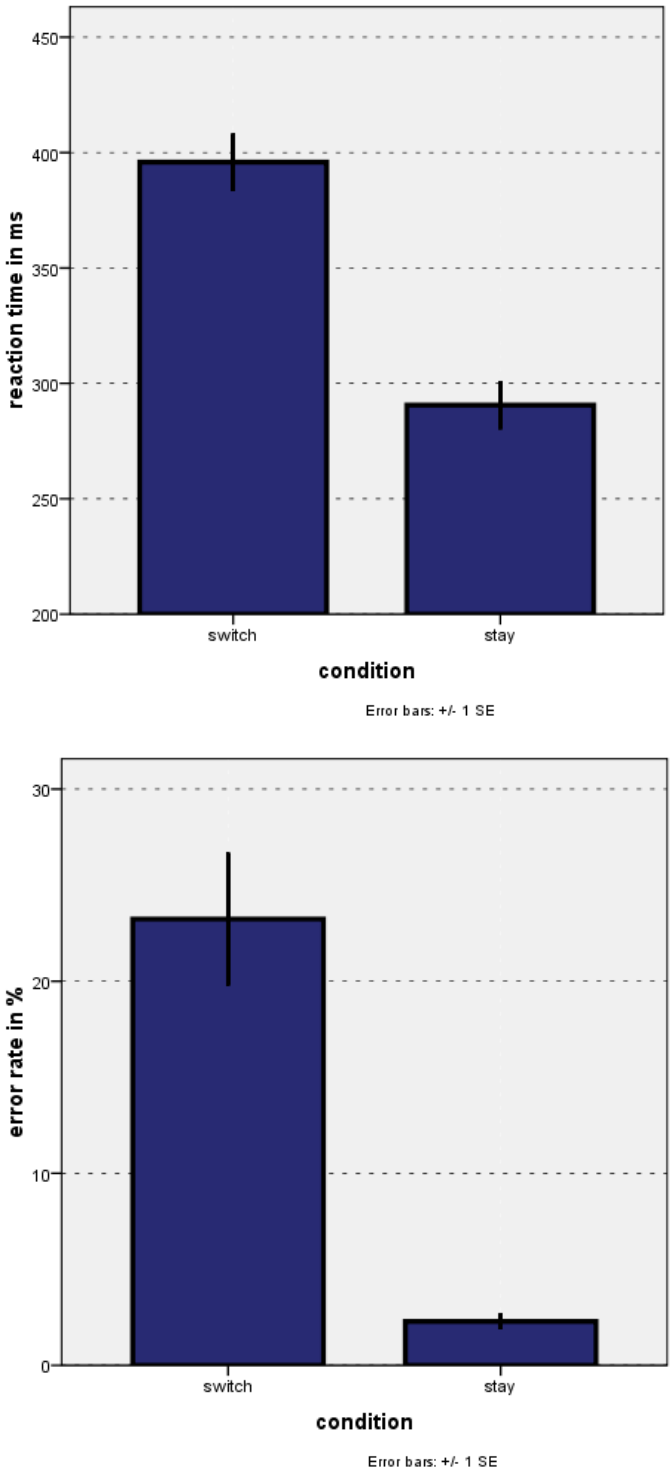


Figure 20: Behavioural data across the two experiments. Subjects were significantly slower on switch trials than on stay trials (top) and made significantly more mistakes (bottom).

Influence of rIFC over M1 during action reprogramming and response inhibition (rIFC-M1 switch experiment). First we would like to present results of the switch experiment (switch trials). The aim of this experiment was to investigate functional interactions between rIFC and M1 during response switching on a sub-second timescale. Therefore we examined M1-corticospinal excitability with TMS pulses over the motor cortex recording EMG activity and measuring peak-to-peak amplitudes of MEPs in a finger involved in the task and examined whether a TMS pulse over rIFC occasionally preceding the M1 pulse by 8 ms would change M1 excitability. Hence single-pulse MEPs served as a baseline, paired-pulse trial MEPs reflected the change in this baseline excitability due to an "activity burst" in rIFC. Again M1 TMS test pulses were only applied to the *left* M1 and MEPs only recorded from the *right* hand FDI and ADM. Hence left hand response trials should be considered as "switch away from the prepared response" trials, i.e. inhibition of the prepared right hand (=recorded hand) response was necessary to avoid performing the prepared but inappropriate response. Right hand trials should be considered as "switch towards the unprepared response" trials, i.e. facilitation of the unprepared right hand response was necessary to successfully complete response switching. To elucidate whether rIFC influences M1 excitability during the time-course of action reprogramming and response switching we conducted an ANOVA test on the single and paired-pulse TMS trial MEPs for every condition (see Figure 21)with within-subjects contrasts of *"pulse"*(single vs. paired), *"SOA"*(75, 125, 175 ms), and *"hand"*(left hand response vs. right hand response). We found a significant effect of *"pulse"* ($F_{1,9}$= 8.381, p=0.018), and significant *"SOA"* × *"pulse"* ($F_{2,18}$= 8.154, p=0.005) interaction. An ANOVA test on the paired-pulse / single-pulse TMS ratios with within-subjects contrasts of *"SOA"*, and *"hand"* revealed a significant effect of

"SOA" ($F_{2,18}$=9.568, p=0.001). One sample two-tailed t-tests of the MEP ratios against baseline (ratio of 1.0 or 100%) showed that the paired-pulse/single-pulse MEP ratio for the left hand-response trials 175 ms after centre colour cue onset was significantly smaller than 1 (t_9=-3.563, p=0.006; mean=0.678 +/- 0.0904 SEM). Post-hoc paired sampled t-tests of the MEP ratios revealed significant differences between 75 ms and 175 ms SOA (t_9=2.642, p=0.027), 125 and 175 ms (t_9=2.608, p=0.028) for right hand response trials and a significant difference between 75 ms and 175 ms SOA (t_9=2.360, p=0.043) for left hand response trials, indicating strong inhibition of M1 excitability by rIFC 175 ms after cue onset on left hand trials. Similar but less prominent effects were observed for 175 ms SOA on right hand response trials (see Figure 21). This is interesting as it could suggest that (1) rIFC exerts inhibitory influence on M1 excitability during response switching and action reprogramming, (2) this inhibitory influence is most prominent in the hand that needs to be inhibited, as it was prepared based on predictions about stimulus identity, but turned out to be wrong after occurrence of the unpredicted stimulus (i.e. the right hand in "switch to the left hand" trials), (3) some inhibition of the unprepared but appropriate response (i.e. the right hand in "switch to the right hand" trials) was observed as well, although it was much weaker than inhibition of the prepared but incorrect response, (4) although this inhibitory influence of rIFC over M1 occurs relatively late in time, compared to functional connectivity between dorsal premotor (PMd) and M1 during action selection (Koch et al., 2006; O'Shea et al., 2007b) and connectivity between pre-SMA and M1 during response switching (Mars et al., 2009), it is still much earlier than average response time in stay trials (median RT= 290.6 ms), indicating that it might play a crucial role in response inhibition and action reprogramming.

Functional specificity of the observed effects. To investigate whether the influence of rIFC on M1 excitability were functionally specific and restricted to the muscles involved in the task (e.g. FDI) or whether these processes could be observed in other nearby hand muscles that do not functionally contribute to the task (e.g. ADM), we analysed MEPs recorded from the ADM muscle (see Figure 21). Again it is noteworthy that this setup was not optimal as TMS pulses were delivered at the M1-representation of the FDI muscle and stimulus intensities were determined based on FDI MEPs. Therefore ADM MEP results should be interpreted with caution. An ANOVA test on the single and paired-pulse trial ADM MEPs for every condition with within-subjects contrasts of *"pulse"*, *"SOA"*, and *"hand"* and an ANOVA test on the ADM MEP ratios with within-subjects contrasts of *"SOA"*, and *"hand"* revealed no significant effects (all $p>0.2$). Hence inhibitory influence of rIFC on M1 might be specific to the movements that are relevant in a certain task. However the experimental setup does not allow strong claims about the effects in ADM, as the whole experiment was designed to investigate effects in the muscles controlling the button presses (i.e. FDI).

Behavioural relevance of the rIFC-M1 physiological interactions. As the inhibitory influence of rIFC on M1 occurred well before the average RT in stay trials, rIFC input could be important for successfully inhibiting premature responses. To test whether processes of rIFC-M1 interaction during response switching would be behaviourally relevant we re-analysed some of the behavioural data (see Figure 22). We obtained switch trial RTs and error rates for every TMS trials type (single vs. paired-pulse) and SOA (75 ms, 125 ms and 175 ms) condition. An ANOVA test on the switch trial median RTs in every condition with within-subjects contrasts of *"pulse"* (single vs. paired) and *"SOA"* (75, 125,175 ms) revealed a significant effect of *"pulse"* ($F_{1,9}$=

8,441, p=0.017), and *"SOA"* ($F_{2,18}$= 4.601, p=0.045). Post-hoc paired samples t-test revealed significant differences between median RTs in single and paired pulse trials at 175 ms SOA (t_9=-2.388, p=0.041, paired-pulse>single-pulse), a significant median RT difference between 75 ms SOA and 175 ms SOA in paired-pulse trials (t_9= -3.337, p=0.009). This is interesting as it suggests that one pulse given over the rIFC at a certain time point (i.e. 175 ms SOA) slows down RTs. Moreover this is exactly the same time point when we found the biggest rIFC-M1 inhibitory influence. An ANOVA test on the switch trial error rate in every condition with within-subjects contrasts of *"pulse"* (single vs. paired) and *"SOA"* (75, 125,175 ms) did not show any significant effects.

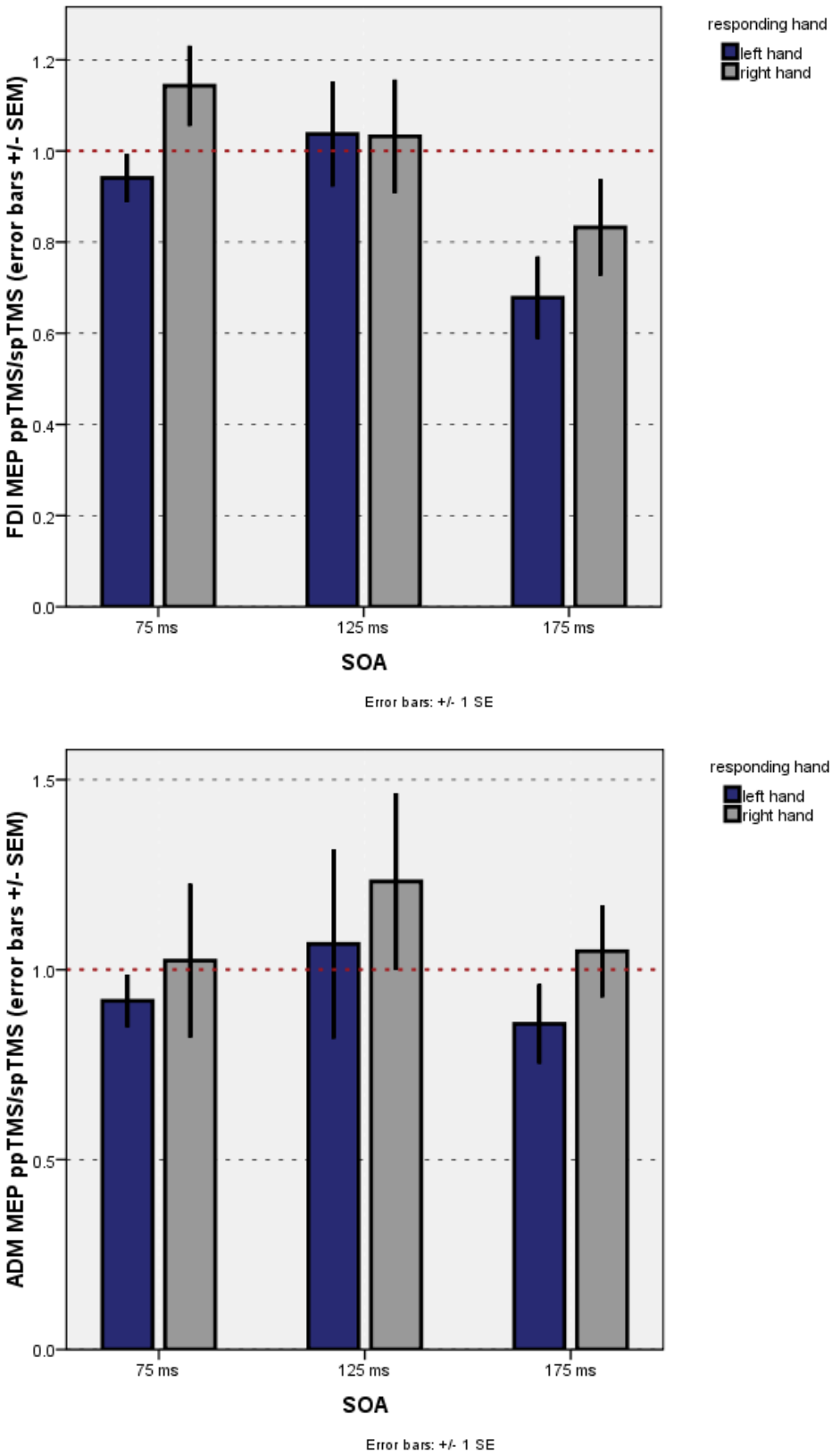


Figure 21: Time course of rIFC-M1 interaction during action reprogramming (switch trials). Single-pulse / paired-pulse TMS MEP ratios are plotted for every condition (SOA and hand). MEP data recorded from the FDI (top) and ADM (bottom) muscle.

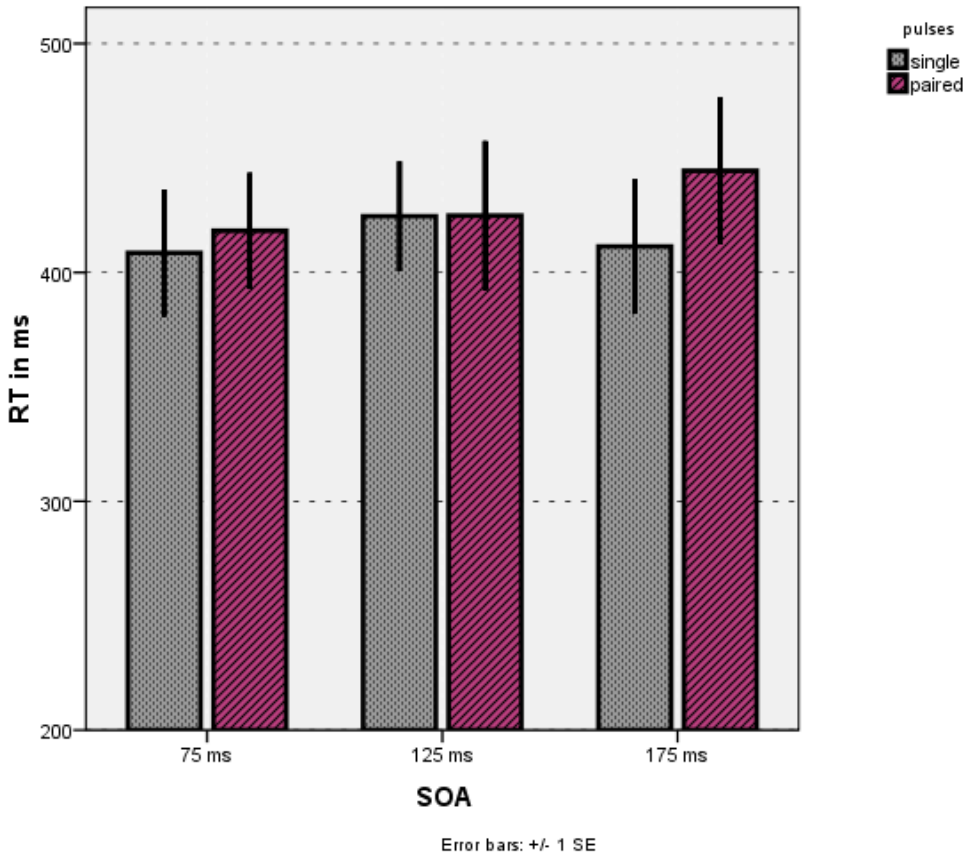


Figure 22: Behavioural relevance of the rIFC-M1 physiological interactions during action reprogramming (switch trials). Median RTs plotted for every condition (SOA, single vs. paired- pulse TMS).

Influence of rIFC over M1 during action execution (rIFC-M1 stay experiment). In a separate experiment we investigated the influence of rIFC on M1 excitability during action execution trials, i.e. when the action, that was prepared before the centre cue colour onset was to be executed. Additionally we wanted to compare the time-course of functional rIFC-M1 connectivity in action reprogramming and action execution trials (in a between-subjects contrast). To investigate rIFC-M1 connectivity associated with the execution of an already prepared response, we analysed MEPs in single-pulse and paired-pulse TMS trials and calculated MEP ratios (see Figure 23). An ANOVA test on the single and paired-pulse trial MEPs for every condition (hand: left vs. right, SOA: 75, 125, 175 ms) with within-subjects contrasts of *"pulse"*, *"SOA"*, and *"hand"* revealed significant effects of *"SOA"* ($F_{2,18}$= 10.195, p=0.001), *"hand"* ($F_{1,9}$= 23.329, p=0.001) and a significant "*hand"* × *"SOA"* ($F_{2,18}$= 9.445, p=0.002) interaction. An ANOVA test on the paired-pulse / single-pulse TMS ratios with within-subjects contrasts of *"SOA"*, and *"hand"* revealed a significant effect of *"hand"* ($F_{1,9}$= 12.247, p=0.01), *"SOA"* ($F_{2,18}$= 7.796, p=0.011), and a significant "*hand"* × *"SOA"* ($F_{2,18}$= 3.973, p=0.037) interaction.

One sample two-tailed t-tests of the MEP ratios against baseline (ratio of 1.0 or 100%) showed that the paired-pulse/single-pulse MEP ratio for right-hand-response trials was significantly facilitated 175 ms after cue onset (t_9= 3.189, p=0.011). Post-hoc paired sampled t-tests of the MEP ratios revealed a significant difference between left and right-hand response trials at 175 ms SOA (t_9= -2.408, p=0.039, right hand>left hand response) and significant differences for right hand response trials between 75 ms and 175 ms SOA (t_9= -4.254, p=0.002, 75 ms<175 ms) and 125 ms and 175 ms SOA (t_9= -2.346, p=0.044, 125 ms<175 ms). This could be interpreted as the appropriate response being facilitated by rIFC 175 ms after centre cue colour

onset in action execution trials. As rIFC has always been associated with response inhibition it seems somehow surprising that rIFC exerts facilitatory influences on the motor system as well. However facilitatory influences of PMv over M1 have already been observed during object grasping (Davare et al., 2009; Buch et al., submitted). Interestingly this facilitatory effect during action execution seems to be hand-specific.

Functional specificity of the observed effects. To investigate whether the influence of rIFC on M1 excitability was functionally specific and restricted to the muscles involved in the task, we again analysed MEPs recorded from the ADM muscle (see Figure 23). An ANOVA test on the single and paired-pulse trial ADM MEPs for every condition with within-subjects contrasts of *"pulse"*, *"SOA"*, and "*hand"* and an ANOVA test on the ADM MEP ratios with within-subjects contrasts of *"SOA"*, and *"hand"* revealed no significant effects ($p>0.25$). Hence the facilitatory influence of rIFC on M1 observed in action execution trials might be specific to the finger that is engaged in a certain task. However as mentioned before the experimental setup limits the conclusions that can be drawn from these results, as the whole experiment was designed to investigate effects in the muscles controlling the button presses (i.e. FDI).

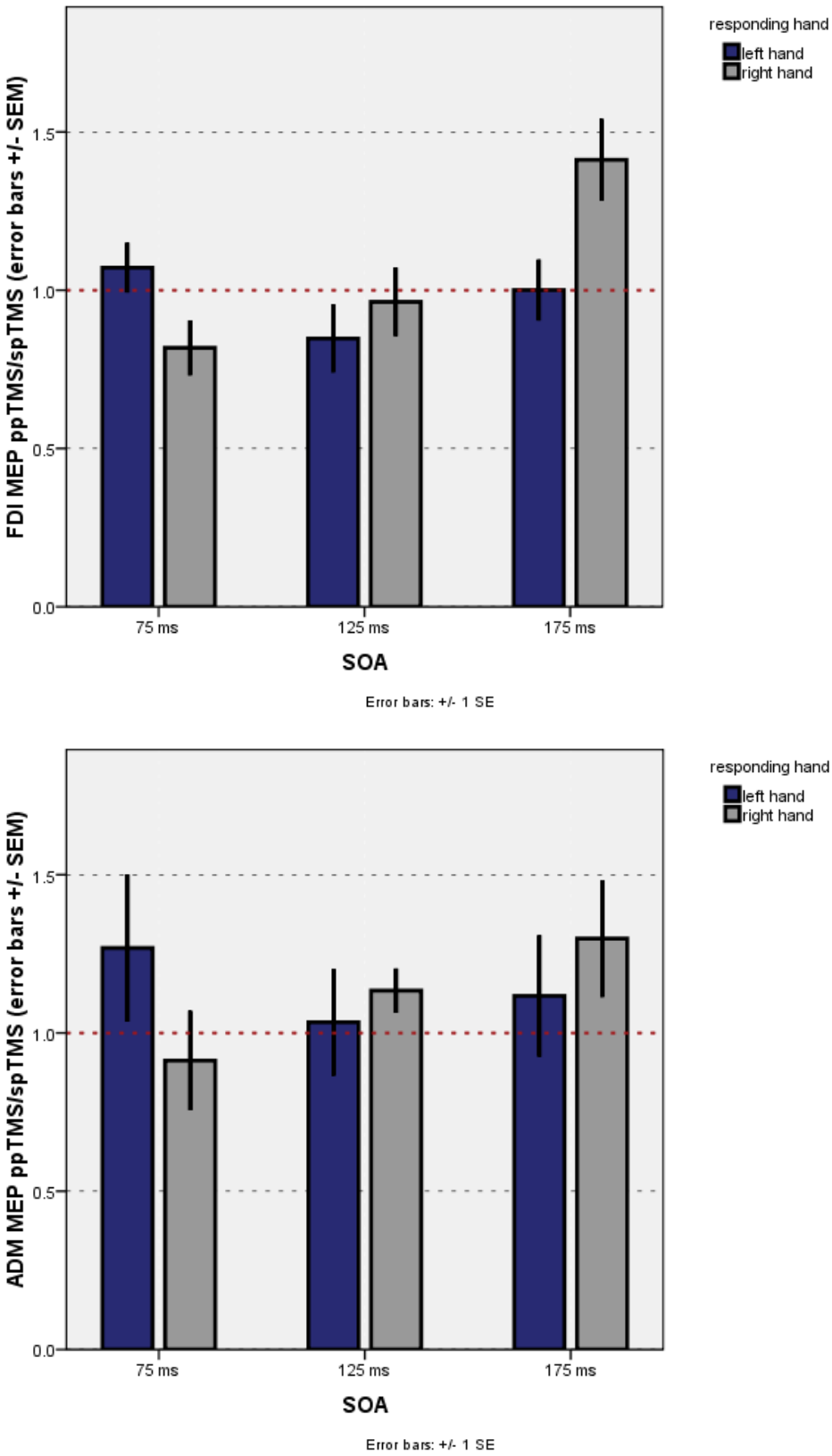


Figure 23: Time course of rIFC-M1 interaction during action execution (stay trials). Single-pulse / paired-pulse TMS MEP ratios are plotted for every condition (SOA and hand). MEP data recorded from the FDI (top) and ADM (bottom) muscle.

Action reprogramming vs. action execution. One aim of this experiment was to compare rIFC-M1 functional connectivity during action reprogramming with rIFC-M1 connectivity during action execution. It seems as if rIFC influences M1 excitability in both, switch and stay trials. We now wanted to test whether these influences differed depending on the trial type (switch vs. stay). We therefore compared paired-pulse TMS MEP results from the two experiments, the rIFC-M1 switch and the rIFC-M1 stay experiment. We thus had to concentrate on a between-subjects design.

Note that the two experiments were exactly the same and behavioural data showed no difference in performance between the two groups. To infer whether rIFC-M1 influence differed depending on whether the subjects were just executing the action they had prepared or whether they had to update their prediction, reprogramme their action plan and inhibit the prepared but incorrect response, an ANOVA test on FDI single and paired-pulse trial MEPs with the within-subjects factors *"pulse", "hand"*, "*SOA*" (75, 125 and 175) and the between subject factor *"condition"* (switch vs. stay) was conducted (see Figure 24). We found an effect of *"hand"* ($F_{1,18}$= 6.938, p=0.017), of *"SOA"* ($F_{2,36}$= 7.062, p=0.003), a significant "*condition*" × *"hand"* interaction ($F_{1,18}$= 17.420, p=0.001), a significant "*condition*" × *"SOA"* interaction ($F_{2,36}$= 6.320, p=0.004), a significant "*pulse*" × *"SOA"* interaction ($F_{2,36}$= 6.668, p=0.019), a significant "*hand*" × *"SOA"* × *"condition"* interaction ($F_{2,36}$= 4.556, p=0.017), and a significant "*hand*" × *"SOA"* × *"pulses"* interaction ($F_{2,36}$= 8.759, p=0.003). An ANOVA test on paired-pulse / single pulse MEP ratios with the within-subjects factors *"hand"*, "*SOA*" (75, 125 and 175) and the between subject factor *"condition"* (switch vs. stay) revealed a significant "*condition*" × *"SOA"* interaction ($F_{2,36}$= 17.015, p<0.001), and a significant "*condition*" × *"hand"* × *"SOA"*

interaction ($F_{2,36}$= 3.386, p=0.045), indicating that the time-course of rIFC-M1 connectivity differed between switch and stay trials. Post-hoc independent samples t-tests not assuming equal variances revealed a significant difference between switch and stay trials for left-hand-response trials at 175 ms SOA (t_{18}= -2.438, p=0.025, switch<stay), and for right-hand-response trials at 175 ms SOA (t_{18}= -3.472, p=0.003, switch<stay).

M1 control experiment. To test whether changes in M1 excitability due to a conditioning pulse over rIFC depending on (1) trial type (switch vs. stay), (2) response (left vs. right hand button press) and (3) time point after instruction cue onset (75 ms, 125 ms and 175 ms SOA) could be explained by processes internal to M1, we analysed single and paired-pulse TMS trial MEP data from a M1 control experiment. This experiment focused on 175 ms SOA but investigated single and paired-pulse TMS MEP effects during switch and stay trials. Experimental setup and stimulation parameters (pulse intensity, inter-pulse interval) were exactly the same as in the previous two experiments, with the only difference that both, test pulses and conditioning pulses were applied to the same M1 (left M1, Figure 25).

An ANOVA test on the FDI single and paired-pulse MEPs with within-subjects contrasts of *"pulse"* (single vs. paired), *"hand"* (left vs. right hand response), and *"condition"* (switch vs. stay trial) revealed a significant effect of *"pulse"* ($F_{1,7}$= 24.742, p=0.003). An ANOVA test on the FDI MEP ratios with within-subjects contrasts of *"hand"* (left vs. right hand response), and *"condition"* (switch vs. stay trial) revealed no significant effect. An ANOVA comparing FDI switch trial MEP ratios between the rIFC-M1 switch experiment and the switch trials from the M1 control experiment with the within subject contrast *"hand"* and the between subjects contrast *"conditioning pulse"* (conditioning pulse over rIFC vs. conditioning pulse

over M1) revealed a significant between subjects effect for *"conditioning pulse"*($F_{1,16}$=14.491, p=0.002). An ANOVA comparing FDI stay trial MEP ratios between the rIFC-M1 stay experiment and the stay trials from the M1 control experiment with the within subject contrast *"hand"* and the between subjects contrast *"conditioning pulse"* (over rIFC vs. over M1) again revealed a significant between subjects effect for *"conditioning pulse"*($F_{1,16}$= 14.065, p=0.002). Post-hoc one samples t-tests of the M1 control experiment MEP ratios against a baseline of 1.0 (or 100%) showed that the conditioning pulse over M1 led to facilitation of the test pulse MEP amplitude in every condition (switch vs. stay, left vs. right hand response). As these effects of M1 test pulse facilitation by conditioning pulses applied to the same M1 were not condition and hand specific, the rIFC-M1 connectivity effects during action reprogramming and action execution trials cannot be explained by M1 internal mechanisms.

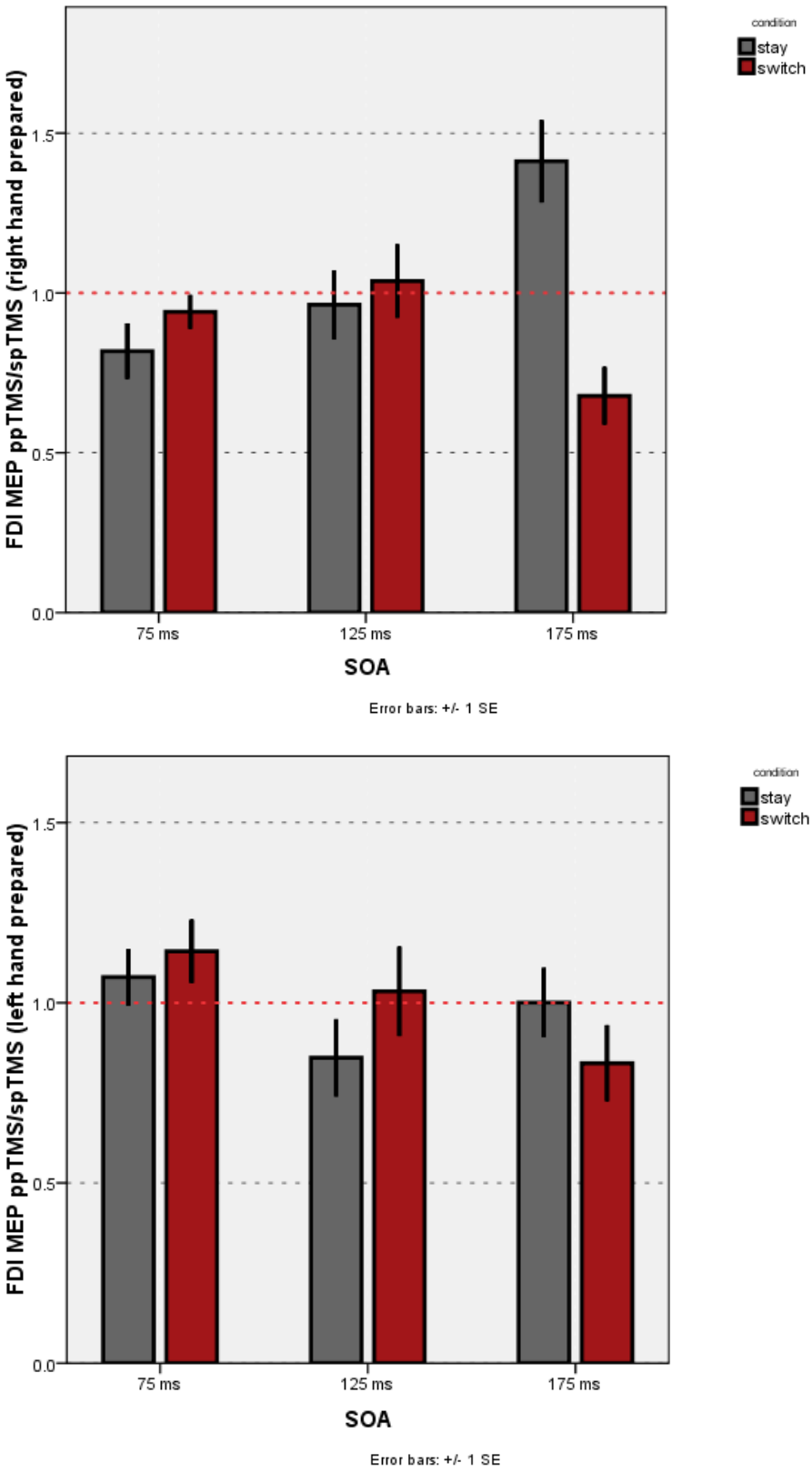
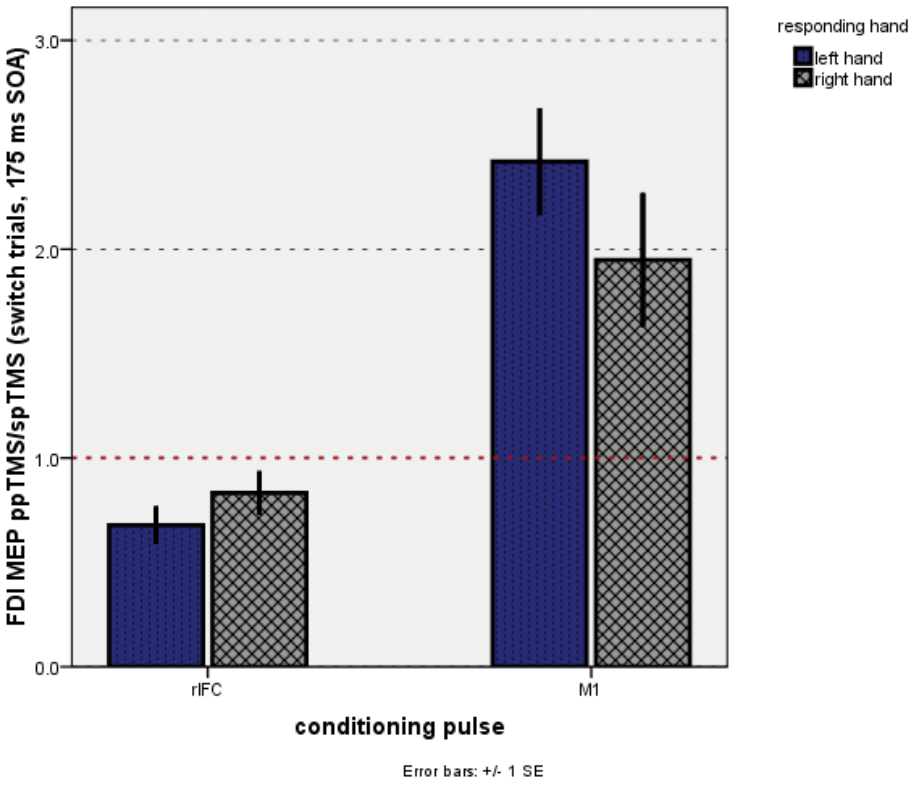


Figure 24: Comparison between functional rIFC-M1 connectivity during action reprogramming (switch trials, red) and action execution (stay trials, grey). Single-pulse / paired-pulse TMS MEP ratios are plotted for every SOA and switch vs. stay. MEP data recorded from the right hand FDI muscle (top) and left hand FDI muscle (bottom).



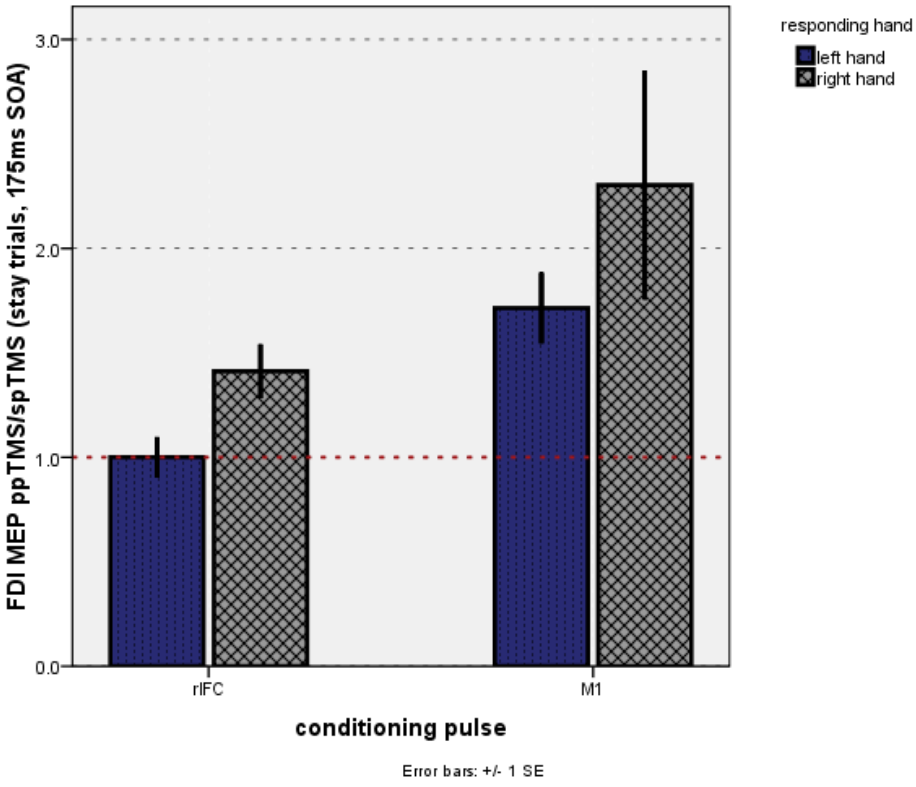


Figure 25: RIFC-M1 interactions (left) during switch (top) and stay trials (bottom) at 175 ms SOA compared to M1 internal (right) paired-pulse effects (exactly the same TMS protocol). Single-pulse / paired-pulse TMS MEP ratios are plotted for every hand and 175 ms SOA at switch (top) and stay (bottom) trials.

Influence of Pre-SMA on M1 excitability and rIFC on M1 excitability during action reprogramming. The exact same task and experimental setup was used in a study by Mars and colleagues (see Chapter 4.2, (Mars et al., 2009)) investigating functional connectivity between pre-SMA and M1 during action execution and action selection, respectively (see Figure 26). As they applied paired-pulse TMS with the exact same protocol and experimental task (see Chapter 4.2, (Mars et al., 2009) and the supplementary information of this article at www.jneurosci.org) it is possible to compare the paired-pulse / single-pulse TMS MEP ratios for action-reprogramming trials (i.e. switch trials) in the "Pre-SMA-M1 switch experiment" with those in the "rIFC-M1 switch experiment" (see Figure 27). An ANOVA comparing FDI switch trial MEP ratios between the pre-SMA-M1 switch experiment and the rIFC-M1 switch experiment with the within subject contrasts *"hand"* (left vs. right hand) and "*SOA*" (75, 125 and 175 ms) and the between subject factor *"conditioning pulse area"* (pre-SMA vs. rIFC) revealed a significant between subjects effect of *"conditioning pulse area"*($F_{1,19}$=6.410, p=0.02), and a significant within-subject effect of *"SOA"* ($F_{2,38}$=8.049, 0.002), a significant "*conditioning pulse area"* × *"hand"* interaction ($F_{1,19}$=4.640, p=0.044) and a *"conditioning pulse area"* × *"SOA"* interaction ($F_{2,38}$=3.398, p=0.047). This indicates that although both areas, pre-SMA and rIFC, seem to exert an influence on M1 excitability during action reprogramming and response inhibition, the influence differs in time course and character (see Figure 27). Whereas pre-SMA seems to facilitate the unexpected and unprepared but correct response 125 ms after instruction cue onset, rIFC could inhibit a premature response tendency 175 ms after cue onset until response conflict is solved.

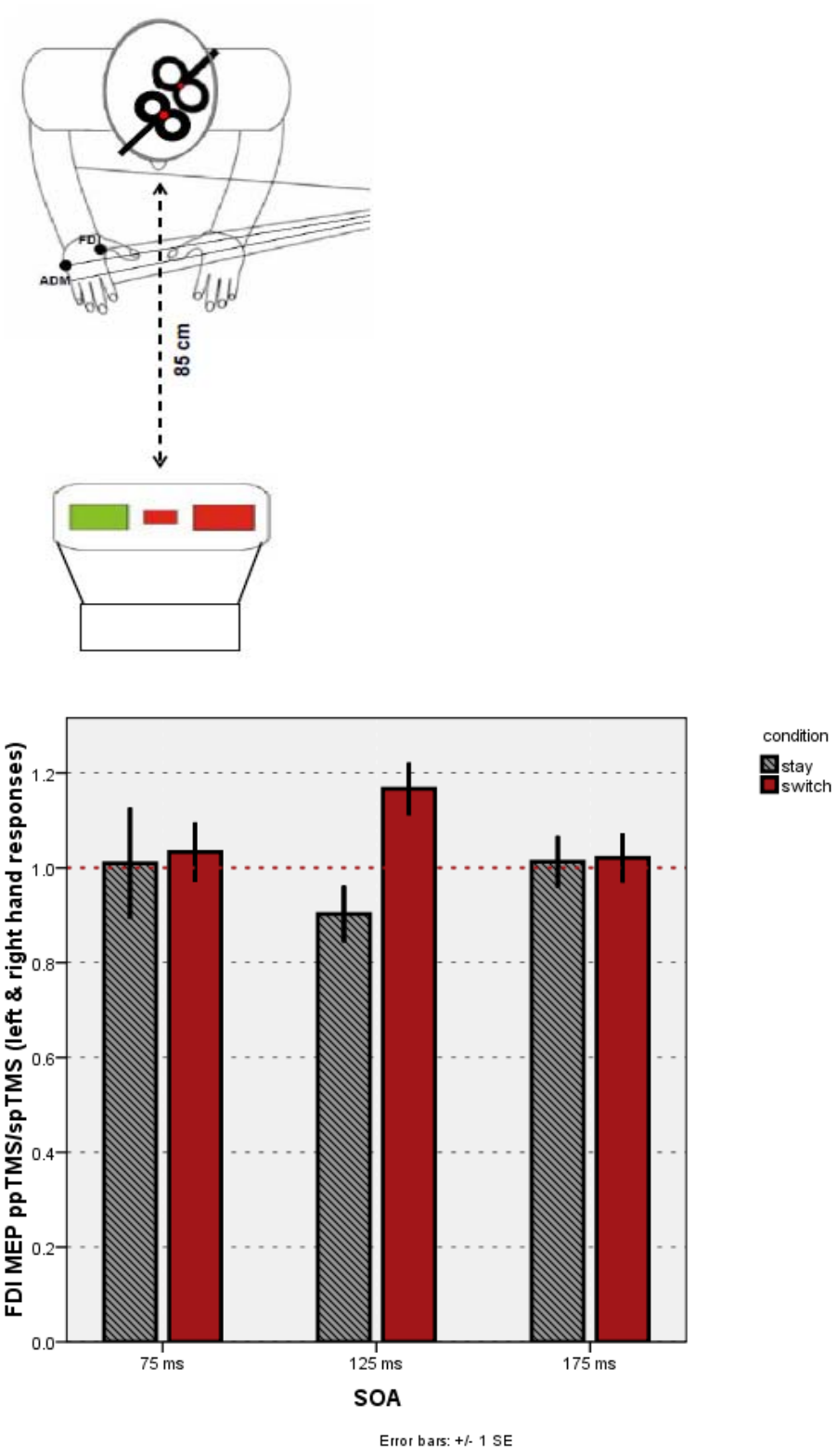


Figure 26: Pre-SMA- M1 functional connectivity during action reprogramming as presented in (Mars et al., 2009). Experimental setup (top) and pre-SMA-M1 functional connectivity results (bottom) plotted as single-pulse / paired-pulse TMS MEP ratios for every condition (switch vs. stay) and SOA (75, 125, 175 ms).

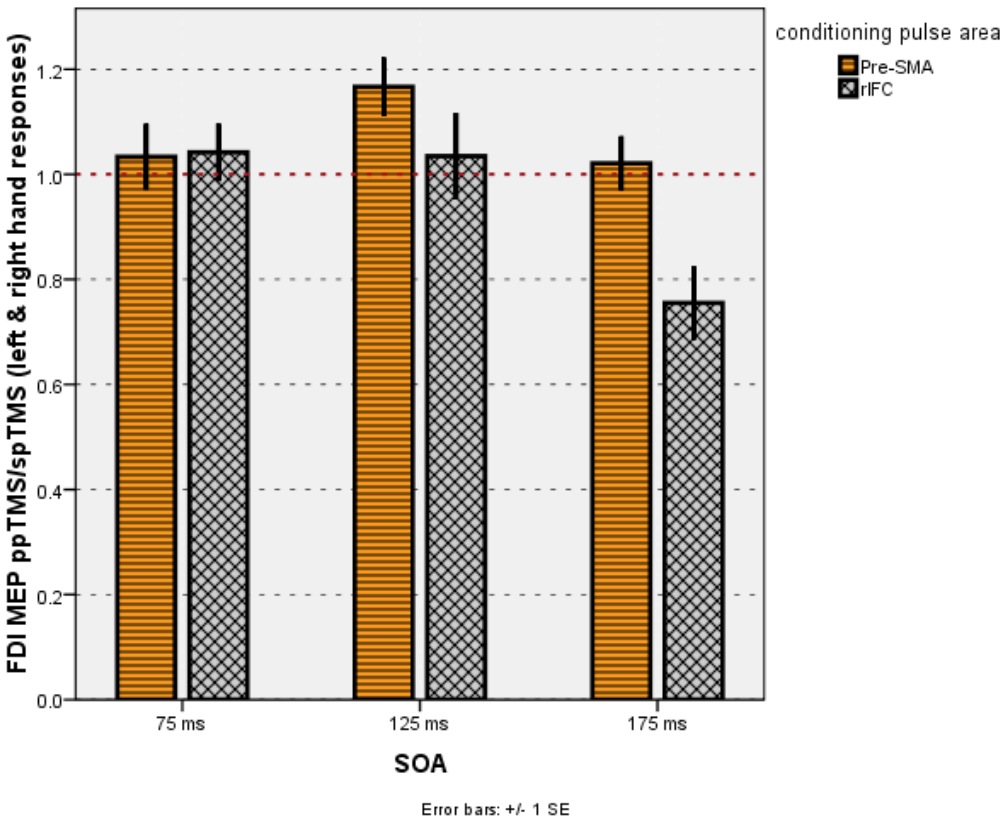


Figure 27: Time course of functional connectivity between pre-SMA- M1 (yellow) and rIFC- M1 (grey) during action reprogramming. Single-pulse / paired-pulse TMS MEP ratios are plotted across hands (left and right hand responses) for 75, 125, 175 ms SOA.

Discussion

Our results indicate that rIFC influences M1 corticospinal excitability in both, action execution and action reprogramming situations at about 175 ms after instruction cue onset at an inter-pulse interval of 8 ms. Whereas rIFC facilitates the correct movement in action execution trials, it inhibits the wrong movement in action reprogramming trials. These effects are anatomically specific to rIFC and differ from processes internal to M1 and from the pre-SMA M1 functional connectivity findings during action execution and action reprogramming (Mars et al., 2009). This is interesting as both areas – pre-SMA and rIFC – have been proposed to be involved in processes of action reprogramming, response inhibition and task switching (Rushworth et al., 2002a; Rushworth et al., 2004; Aron and Poldrack, 2006; Aron et al., 2007a; Mars et al., 2007b; Taylor et al., 2007; Nachev et al., 2008; Chambers et al., 2009; Duann et al., 2009) but the distinct role of these two areas was not yet entirely clear. TMS over rIFC seemed to have an effect on reaction times during action reprogramming only 175 ms after cue onset. (Buch et al., submitted) reports that TMS over PMv slows down RTs in a task that required reprogramming of grasping movements. Additionally the effects of rIFC-M1 connectivity during action reprogramming occur earlier than the average RT in action execution trials, indicating that the influence of rIFC on M1 could play a causal role in inhibiting prepared but incorrect responses. Moreover the estimates for the duration of the stopping processes in go/no-go paradigms based on SSRTs (150 to 200 ms) (Aron et al., 2007b) are very similar to the observed rIFC-M1 inhibitory effects, suggesting that stopping could be mediated by the observed inhibitory physiological mechanism. These stopping mechanisms were proposed to be mediated via a rIFC- STN "hyperdirect pathway" (Nambu et al.,

2000; Nambu et al., 2002; Aron and Poldrack, 2006; Aron et al., 2007a). Neurophysiological recordings in the STN in rhesus monkeys solving the same behavioural task as we used in our experiment, observed increased activity in neurons associated with stopping and reprogramming behaviour at 174 ms after centre colour cue onset (Isoda and Hikosaka, 2008). However this experiment was the first to show an inhibitory influence of rIFC activity on M1 excitability during action reprogramming and although it has been suggested that rIFC may exert an inhibitory physiological influence over other brain areas, including M1 (Aron et al., 2004b), the degree to which inhibition of actions on a behavioural level can be related to inhibition on a physiological level remained to be clarified (Waldvogel et al., 2000; Aron, 2007).

It is interesting that we did not only find inhibition of M1 corticospinal output during response inhibition and action reprogramming trials, but also facilitation of the correct and expected response in normal action execution trials. These findings are similar to the results of paired-pulse TMS experiments investigating PMv-M1 connectivity during grasping movements (Davare et al., 2008; Davare et al., 2009; Buch et al., submitted). These studies suggested that *PMv* as an area within the rIFC might exert facilitatory and inhibitory influences over the M1 to control complex action sequences such as grasping. PMv is widely held to exert an important influence over the activity in M1 when hand movements are made. It is strongly connected with ventral and opercular frontal regions, and via STN with M1. Therefore it might be well placed to mediate both, facilitatory and inhibitory influences over M1, depending on current goals and environmental demands. Hence "inhibition" might only be one of the multiple functions implemented by rIFC/PMv to organise complex movement sequences and action plans (Koechlin and Jubault, 2006). This would suggest that

action reprogramming might well be a complex process recruiting a whole network of brain areas (such as rIFC and pre-SMA) additionally to those active during action execution, but furthermore one brain region might as well exert both, inhibitory and facilitatory influences at the same time (Isoda and Hikosaka, 2007; Buch et al., submitted). Moreover the idea of response inhibition as it is investigated in go/no-go tasks could be considered as being relatively artificial as in real-life situations both, inhibition and reprogramming are often required at the same time. If we wait with our car at an intersection for the traffic lights (Aron et al., 2007b) and we are about to press the gas pedal as they turn green, we do have to stop the car and prevent it from moving, when a cyclist suddenly swerves into our lane as it was pointed out by (Aron et al., 2007b). But stopping the car does not necessarily mean stopping the movement! Preventing the car from moving could mean that we have to reprogramme our plan to press the gas pedal towards the plan to press the breaks as quick as we can. Hence action reprogramming as a whole although shown to involve inhibitory processes, might be a better model for investigating mechanisms used to implement executive control in situations of higher cognitive conflict.

Further research could elucidate neuronal pathways that mediate these physiological processes associated with action reprogramming and should try to illuminate interactions between single prefrontal areas in this “network of inhibition and action reprogramming”.

2.2. Anatomical Networks of Inhibition and Action Reprogramming

Introduction

Paired-pulse TMS is a non-invasive research technique that allows us to investigate functional connectivity between two brain areas while participants engage in behavioural tasks, make decisions, grasp objects or reprogramme responses (Oliveri et al., 2003; Koch et al., 2006; O'Shea et al., 2007b; Davare et al., 2008; O'Shea et al., 2008; Davare et al., 2009; Mars et al., 2009; Buch et al., submitted). Diffusion MRI is a non-invasive research technique that has been used to investigate anatomical connectivity in the brain and to elucidate anatomical networks of white matter tracts. The relation between brain structure and function is of great interest in neuroscience and comparisons between behavioural and structural measures suggest that brain structure correlates with specific skills and critically determines performance (Gaser and Schlaug, 2003; Bengtsson et al., 2005; Johansen-Berg and Behrens, 2009). Although we can image functional activity in certain brain areas non-invasively using modern imaging techniques such as PET and fMRI, it is difficult to investigate functional connectivity between brain areas with these techniques and moreover the anatomical pathways mediating functional connectivity are difficult to elucidate. There is no "imaging technique" for functional connectivity and for the rapid changes in functional connectivity during cognitive tasks to date.

However the effects of functional connectivity can be measured using a paired-pulse TMS paradigm. The individual effect size of functional connectivity can be correlated

with individual anatomical connectivity. Anatomical areas where white matter micro-structural integrity correlates with physiological effects of functional connectivity are likely to mediate these effects (Boorman et al., 2007). If white matter micro-architecture has an effect on behaviour and physiology we would expect that differences in anatomical connectivity in a certain tract would influence functional connectivity between areas connected via this tract. Hence correlating the physiological effect size of functional connectivity with measures of anatomical connectivity and integrity (such as FA) could "image" the structural white matter pathways of certain functional connectivities.

As mentioned before converging evidence from imaging, EEG, TMS and neurophysiological studies suggests that Pre-SMA and rIFC are crucially important in situations involving direct competition, inhibition, reprogramming and updating of actions, action plans and response strategies. However it is not yet entirely clear, what the difference between the influences exerted by pre-SMA and rIFC on the motor system is. Several pathways have been suggested to mediate these interactions (Nambu et al., 2002; Aron and Poldrack, 2006; Aron et al., 2007a; Isoda and Hikosaka, 2007, 2008; Duann et al., 2009; Mars et al., 2009).

Pre-SMA and rIFC could influence M1 excitability and motor output via the basal ganglia. Neuronal tracer studies in monkeys have shown that medial frontal cortex, PMv and PMd directly project to the STN (Nambu et al., 2000; Nichols and Holmes, 2002). It has been proposed that rIFC as well as pre-SMA exert an inhibitory influence on the motor system when prepared responses have to be inhibited and ongoing movements have to be stopped. Additionally it has been proposed that this inhibitory influence is mediated via the STN and a so called hyper-direct frontal cortex → STN → globus pallidus internus → thalamus → M1 pathway. However

action reprogramming and response inhibition occurring earlier in time and well before the onset of the inappropriate movements might act on a cortical level. Therefore "breaking" and inhibiting an already initiated response could engage a frontal cortex -basal ganglia pathway such as the "hyper-direct pathway", whereas reprogramming and inhibiting action plans would employ direct cortico-cortical projections. The former process is likely to be faster but less specific than the latter one. Pre-SMA and rIFC could play a role in both processes. Many situations in every day life are likely to require both, quick but unspecific inhibition of prepared but inappropriate movements and a more specific reprogramming of action plans.

We used a paired-pulse TMS paradigm in combination with the same behavioural task that has already been described in the previous two experiments to investigate different pathways of functional pre-SMA-M1 connectivity and rIFC-M1 connectivity during action reprogramming and response inhibition. We investigated functional connectivity between pre-SMA and M1 in one TMS experiment (subsequently called the pre-SMA-M1 experiment) and connectivity between rIFC and M1 in a second experiment (rIFC-M1 experiment). The order of the two experiments was counterbalanced. Single and paired-pulse TMS was delivered only at one time point after instruction cue onset but with variable IPIs. Thus we were able to measure effects of functional connectivity depending on the IPI. We hypothesised that we would be able to depict different pathways of functional connectivity between pre-SMA and M1 and between rIFC and M1 with different IPIs. To relate these different pathways of functional connectivity to anatomical white matter tracts mediating these various effects we correlated paired-pulse TMS effect sizes with diffusion MRI derived FA maps of each subject's brain. Hence the aim of this experiment was to show different pathways of functional pre-SMA-M1 and rIFC-M1 connectivity during

action reprogramming and to localise these pathways within the brain's white matter. Additionally we aimed to elucidate whether pre-SMA, rIFC and M1 belong to a bigger network involved in action reprogramming, conflict resolution and response inhibition.

Methods

Participants. Sixteen healthy volunteers (age range 18 – 40 years, mean age = 25.5 +/- 5.65 SD, 10 females) with no personal or familial history of neurological or psychiatric disease participated in two successive paired-pulse TMS experiments and a diffusion weighted MRI scan. One paired-pulse TMS experiment aimed to investigate pre-SMA M1 functional connectivity during action reprogramming. The other paired-pulse TMS experiment investigated functional connectivity between rIFC and M1 during action reprogramming. The order of the two experiments was counterbalanced, with eight subjects starting with the pre-SMA- M1 experiment and eight subjects starting with the rIFC-M1 experiment. There was at least one week in-between the two experimental sessions. The experiment was approved by the Oxfordshire Research Ethics Committee and conducted in accordance with the declaration of Helsinki. The same behavioural task as in the pervious two experiments was used in two separate experimental sessions to investigate functional and anatomical pathways mediating the observed functional connectivity effects during response inhibition and action reprogramming (1) between pre-SMA and M1 (subsequently called the "pre-SMA-M1 experiment") and (2) between rIFC and M1 (referred to as the "rIFC-M1 experiment"), respectively. All participants were right-handed and gave written informed consent. They were all screened for adverse reactions to TMS and risk factors by means of a safety questionnaire.

Experimental setup. Participants were seated in a darkened room and wore a tight-fitting EEG cap, on which TMS sites were marked and earplugs to protect against TMS noise. A chin rest was used to minimise head movements.

Behavioural Task. The behavioural task used in the two paired-pulse TMS experiment was exactly the same as in the previous two experiments and as in the experiment conducted by (Mars et al., 2009)(see Figure 28, Table 4). It was modelled on the paradigm developed by (Isoda and Hikosaka, 2007) and required participants to respond with the left or right index finger in response to visual stimuli presented on a computer screen. The participants had to respond to one of two flanker stimuli on the screen. A centre cue following the flankers instructed them whether they should respond to the left or the right flanker. However the centre cue took the same identity for trains of 3-7 trials. Hence each trial subjects could prepare a movement based on their knowledge of the identity of the centre cue on the previous trial. However after taking the same colour for a series of trials the centre cue colour changed. This manipulation meant that there were two types of trials: stay trials, in which the fixation square turned into the same colour as in the previous trial, thus allowing the participants to exert the already prepared response, and switch trials, in which the fixation square turned into a different colour as in the previous trial, thus requiring participants to inhibit an already prepared response and to reprogramme their action plans. Stimuli were pseudo-randomly generated and a different stimulus order was used for each block. Custom software written in Presentation (version 0.53) controlled the experiment. As in the previous two experiment participants were familiarised with the task before the actual experiment in one behavioural training block (without TMS pulses, 30 trials) and with the whole experimental setup including paired-pulse TMS in a second training block (with TMS pulses, 30 trials). Both, the pre-SMA-M1 experiment and the rIFC-M1 experiment consisted of 7 experimental blocks, each containing 30 switch and 150 stay trials. Reaction times were recorded, defined as the

duration between the centre square colour occurrence and the index finger response button press.

TMS. As in the previous experiment we aimed to investigate the influence exerted by pre-SMA on M1 and rIFC on M1 during action reprogramming and response inhibition. We therefore decided to use a paired-pulse TMS paradigm with one TMS coil over M1 and another TMS coil over pre-SMA (in the pre-SMA-M1 experiment) and over rIFC (in the rIFC-M1 experiment), respectively. In both experiments TMS pulses were delivered on 30 out of 180 trials per block. As in the previous experiment there were two types of TMS trials. On half of the trials, so-called *"single-pulse" trials*, a single TMS "test pulse" was delivered over the left M1-representation of the right FDI. Intensity of this TMS test pulse was such that an MEP of 1 – 1.5 mV was evoked in the relaxed, contralateral FDI. This intensity was 42.35% (SEM +/- 1.994) of the maximum stimulator output in the pre-SMA-M1 experiment, and 43.88% (SEM+/- 1.948) for the rIFC- M1 experiment. On the other half of the trials, so-called *"paired-pulse" trials*, the M1 test pulse was preceded by a "conditioning pulse" over the pre-SMA in the "pre-SMA-M1 experiment" and over rIFC in the "rIFC-M1 experiment". Intensity of the M1 test pulse was exactly the same as in "single-pulse trials". The intensity of the preceding conditioning pulse was set at 120% of the resting motor threshold (RMT) for TMS pulses over pre-SMA in the pre-SMA-M1 experiment. This value was derived from findings by (Civardi et al., 2001; Mars et al., 2009). The intensity for the conditioning pulse over rIFC in the rIFC-M1 experiment was set at 110% RMT as in the previous study. Average RMT was 37.059% (+/-1.459 SEM) of maximum stimulator output in the pre-SMA-M1 experiment and 37.82% (+/-1.724 SEM) in the rIFC-M1 experiment. The inter-pulse interval was varied using five different IPIs to investigate different pathways of pre-SMA-M1 and rIFC-M1

interaction. IPIs in both experiments were 3 ms, 6 ms, 9 ms, 12 ms and 18 ms. We applied pulses only at one time point after centre cue colour presentation (i.e. only one SOA). Test pulses were delivered 125 ms after cue onset in the pre-SMA-M1 experiment and 175 ms after cue onset in the rIFC-M1 experiment (see Table 4). These were the time-points where previous experiments had found the strongest pre-SMA-M1 effects and rIFC-M1 effects, respectively (Mars et al., 2009). We hypothesised that the preceding conditioning pulse would change the MEP amplitude elicited by the test pulse, depending on (1) the area over which the conditioning pulse was applied (pre-SMA vs. rIFC) and (2) the IPI (3, 6, 9, 12, 18 ms). Pulses were delivered through two 55 mm diameter figure-of-eight coils directly connected to two high-power Magstim 200 MonoPulse machines (The Magstim Company ®). The magnetic stimulus had monophasic pulse configuration, with a rise time of ~ 100 μs, decaying back to zero over ~ 800 μs. TMS coil applying test pulses to the left M1 was placed tangentially to the scalp, inducing posterior-to-anterior current flow perpendicular to the central sulcus. TMS coil applying conditioning pulses to the pre-SMA was placed tangentially to the scalp with the handle pointing in the anterior direction, as close as possible to a position 4cm anterior to the electrode position Cz, previously shown to be an appropriate location for the stimulation of pre-SMA (Rushworth et al., 2002b; Mars et al., 2009)(see Figure 29). TMS coil applying conditioning pulses to the rIFC was placed tangentially to the scalp with the handle of the coil pointing forwards and upwards, perpendicular to the sylvian fissure (see Figure 29). MRI scans were acquired for every subject. The location for the conditioning coil in the pre-SMA-M1 experiment was assessed using an MRI-aligned frameless stereotaxic neuronavigation system (Brainsight, Rogue Research Inc.®). The same neuronavigation system was used to determine rIFC location in the rIFC-

M1 experiment. Areas of TMS (left M1, pre-SMA and rIFC) were evaluated with respect to the Montreal Neurological Institute (MNI) standard space using subject's individual MRI scans and the linear registration tool "FLIRT" of FMRIB's Software Library "FSL" (Smith et al., 2004). The mean MNI location across subjects for the pre-SMA conditioning coil location in the pre-SMA experiment was x= 1.02 (+/- 1.388 SEM), y=22.97 (+/- 1.897) and z=60.26 (+/- 1.713). The mean MNI location of the rIFC in the rIFC-M1 experiment was x=58.48 (+/- 0.945), y=15.48 (+/- 1.066) and z=30.48 (+/-1.369) (see Figure 30). The mean M1 test coil MNI location was x= -43.69 (+/- 2.535), y= -10.42(+/- 3.389) and z= 60.02 (+/- 2.2) in the pre-SMA-M1 experiment, and x= -44.57 (+/- 2.89), y= -11.36 (+/-1.78) and z= 58.67 (+/-3.288) in the rIFC -M1 experiment (see Figure 30). "Pre-SMA-M1 experiment" and "rIFC-M1 experiment" were separate experimental sessions involving the same participants. During "pre-SMA-M1 experiment" and "rIFC-M1 experiment" pulses were almost exclusively delivered on switch trials (24 TMS trials per block delivered on switch trials, 6 per block on stay trials). Only switch-trial MEPs were analysed. TMS was delivered 125 ms after cue onset in the pre-SMA-M1 experiment and 175 ms after cue onset in the rIFC-M1 experiment. For both, the pre-SMA-M1 experiment and the rIFC-M1 experiment a total of 14 TMS trials per condition (hands, single and paired pulse TMS trials, IPIs) were obtained and used for the analysis. TMS trials were presented at least 7 seconds apart, to ensure that pulses on adjacent trials did not influence each other. In each block, TMS trials were distributed evenly over response hands, respective IPIs and single- or paired-pulse TMS.

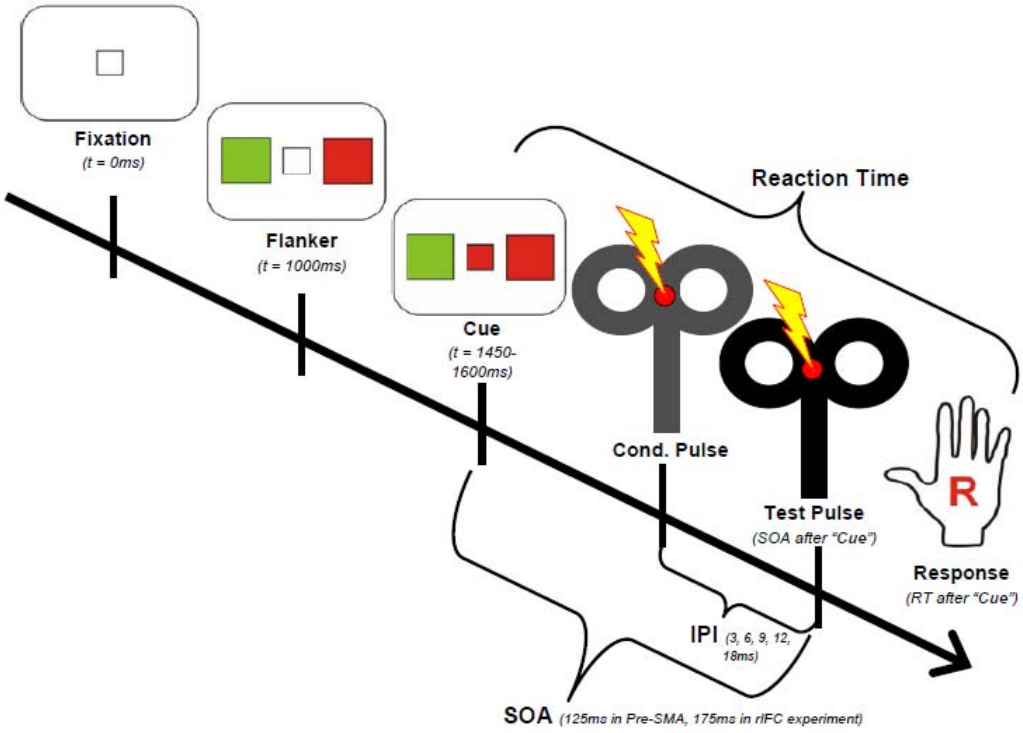


Figure 28: Experimental setup. Pulses were delivered through two 55 mm diameter figure-of-eight coils. The conditioning coil was placed either over pre-SMA or over rIFC, the test coil was placed over left M1. One experimental block contained 180 trials, 30 switch trials and 150 stay trials. Single and paired-pulse TMS was delivered at 125 ms SOA in the pre-SMA-M1 experiment and 175 ms SOA in the rIFC-M1 experiment. IPIs varied between 3, 6, 9, 12 and 18 ms.

	pre-SMA-M1 experiment	**rIFC-M1 experiment**
SOA	125 ms	175 ms
IPIs	3 ms, 6 ms, 9 ms, 12 ms, 18 ms	3 ms, 6 ms, 9 ms, 12 ms, 18 ms
muscles recorded	FDI, ADM (→ Figure 8)	FDI, ADM
conditioning pulse intensity	120% RMT	110% RMT
test pulse intensity	intensity eliciting a 1mv peak-to-peak MEP in FDI muscle	intensity eliciting a 1mv peak-to-peak MEP in FDI muscle
experimental blocks	7 blocks	7 blocks

Table 4: The table summarises the experimental and TMS setup of the two experiments investigating pre-SMA-M1 and rIFC-M1 functional connectivity.

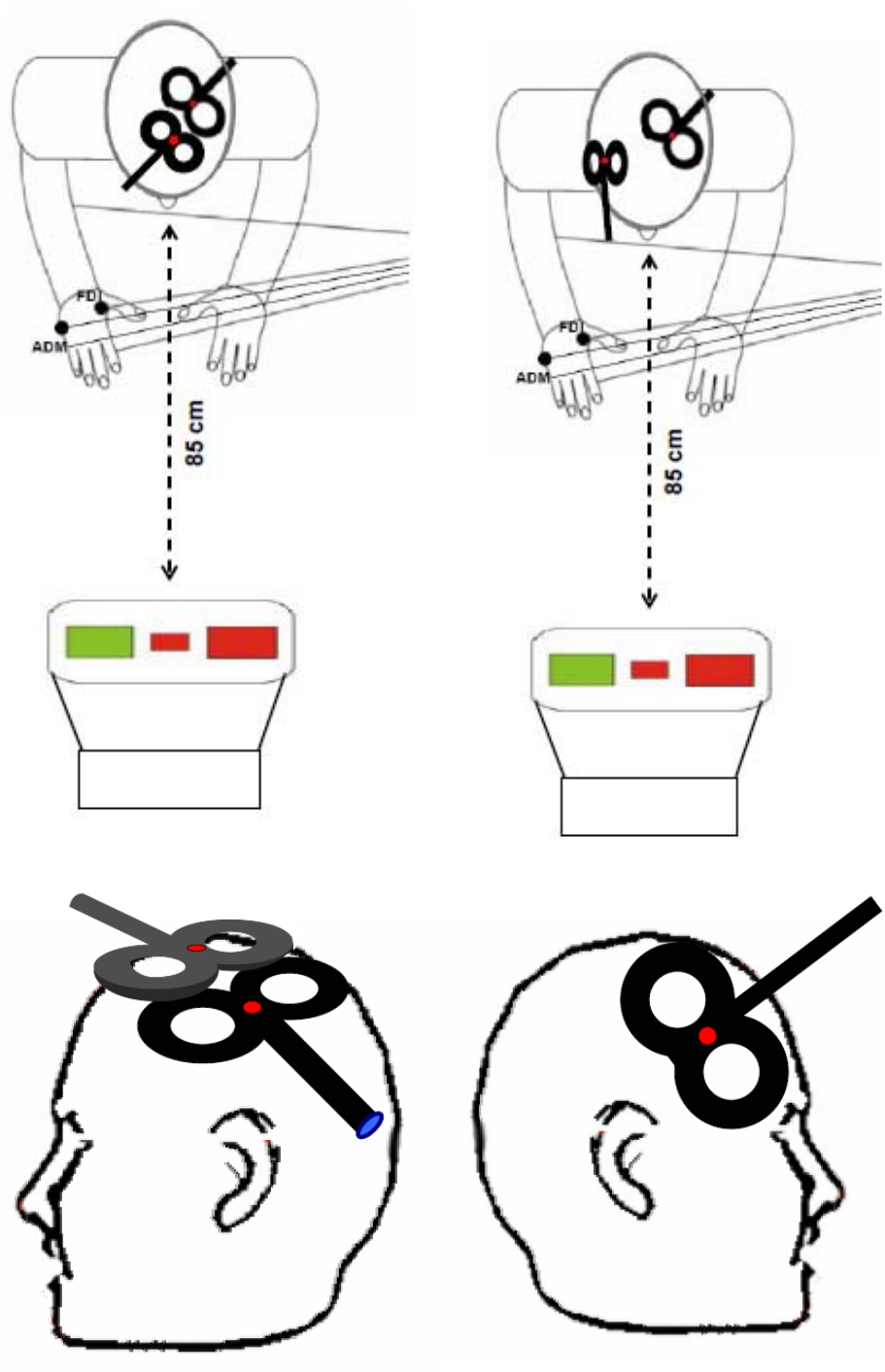


Figure 29: Experimental setup. TMS coil applying test pulses to left M1 was placed tangentially to the scalp, with the handle oriented posteriorly. TMS coil applying conditioning pulses to the pre-SMA (left) was placed tangentially to the scalp with the handle pointing in the anterior direction, as close as possible to a position 4cm anterior to the electrode position Cz. TMS coil applying conditioning pulses to the rIFC was placed tangentially to the scalp with the handle of the coil pointing forwards and upwards, perpendicular to the sylvian fissure.

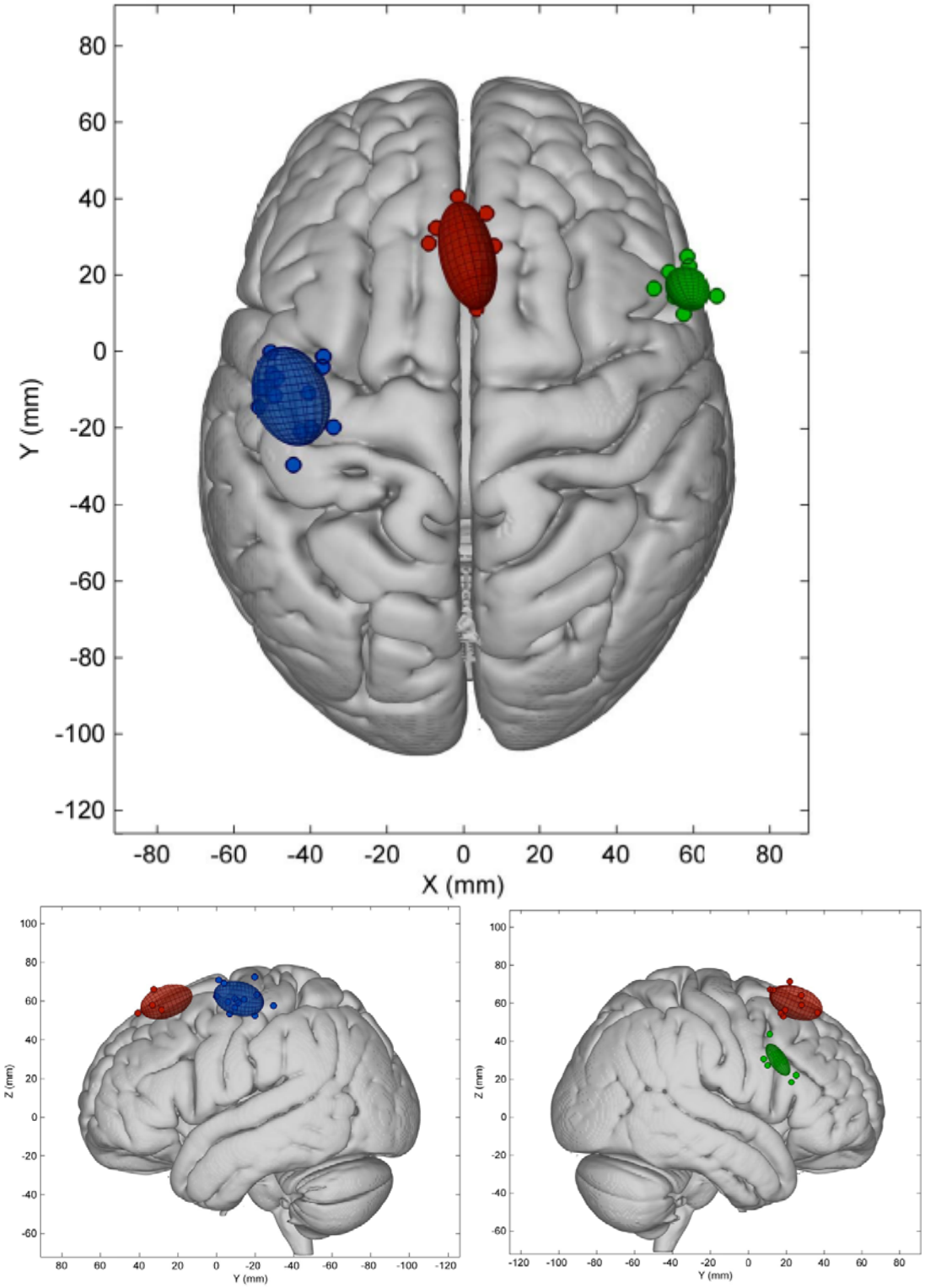


Figure 30: MNI coordinates for TMS targets. Circular symbols indicate individual subjects' stimulation locations in MNI152 space. Ellipsoids represent 95% confidence limits of the mean group stimulation location for each area. The mean MNI location across subjects for the pre-SMA conditioning coil location (red) in the pre-SMA experiment was x= 1, y=22 and z=60. The mean MNI location of the rIFC (green) in the rIFC experiment was x=58, y=15 and z=30.

Electrophysiological recordings. As in the previous experiment MEPs were recorded from two muscles, the FDI and the ADM, in the right hand using two surface Ag-AgCl electrodes in tendon-belly montage. An earth electrode was placed on the right elbow. EMG responses were band-pass filtered between 10-1000 Hz, with an additional 50 Hz notch filter, sampled at 5000 Hz, and recorded using a CED 1902 amplifier, a CED micro 1401 Mk.II A/D converter, and a PC running Spike2 (Cambridge Electronic Design, Cambridge, UK).

Analysis. For the analysis of pre-SMA- M1 functional connectivity and rIFC-M1 functional connectivity during action reprogramming we analysed MEP data from the switch trials in both experiments (24 out of 30 TMS trials per block). As in previous experiments analysis of electrophysiological data concentrated on peak-to-peak amplitudes of the MEPs measured on "switch trials" with TMS. Trials with incorrect responses, trials with premature (RT<150) responses, trials in which the test pulse failed to elicit a reliable MEP (amplitude < 0.1 mV), and trials in which participants pre-contracted the FDI muscle prior to application of the TMS pulse (EMG amplitude > 0.1 mV in the 80 ms before the pulse) were discarded from the analysis. Following this pre-processing, on average 2.78 (SEM± 0 .371) trials per condition (of a total of 14 trials per condition) in the pre-SMA-M1 experiment and 2.87 (± 0.496) trials per condition (of a total of 14 trials) in the rIFC-M1 experiment had to be excluded. Two paired-samples t-tests contrasting the number of excluded trials in single-pulse and paired-pulse TMS trials in each experiment (pre-SMA-M1 and rIFC-M1) could not find any difference in the number of MEPs excluded from the analysis between single-pulse and paired-pulse TMS trials in any of the two experiments. To account for differences in coil placement between experimental blocks, MEP sizes were median- normalised within each block. Analyses of MEPs were carried out on the

mean of the normalized MEP amplitudes in each condition and the ratio [paired-pulse TMS MEP / single-pulse TMS MEP] was calculated. Analyses of both behavioural and electrophysiological data were conducted using ANOVA tests, using repeated measures where possible. Significant effects were identified based on Huynh-Feldt corrected ANOVA values, using SPSS 16.0. Post-hoc two-sided t-tests were used to further investigate significant effects in the ANOVAs. Correlations between different IPI paired-pulse TMS MEP effects were determined using bivariate correlation tables and Pearson Correlation coefficients.

Diffusion MRI. To further explore different routes by which stimulation of pre-SMA or rIFC influenced M1 corticospinal excitability we used diffusion-weighted imaging (DWI), which is sensitive to apparent water diffusion properties in tissue. Diffusion can be orientation-dependent in tissue characterised by a high degree of directional organisation, such as white matter in the brain, and therefore become "anisotropic". Orientational dependency can be quantified by "fractional anisotropy" (FA). FA reflects functionally relevant micro-structural properties of the white matter, such as axonal architecture, density and myelination. Correlations between functional connectivity measured with paired-pulse TMS and anatomical white-matter microstructure across subjects have been shown (Boorman et al., 2007; Mars et al., 2009; Buch et al., submitted). Therefore the idea of this experiment is to at least partly explain variability in the paired-pulse TMS MEP change (paired-pulse TMS MEP / single-pulse TMS MEP ratio) by differences in the underlying white matter tracts. According to this logic, a region of white matter where FA values correlate with the size of the MEP change (i.e. stronger facilitation or stronger inhibition) might be a possible route mediating these effects of functional connectivity. Diffusion-weighted magnetic resonance imaging data (three acquisitions of 60 directions, b-value 1000 s/

mm^2, 2×2×2 mm^3 voxels, 60 slices) were acquired from all 16 participants taking part in the pre-SMA-M1 and the rIFC-M1 experiment on a 3.0 T Siemens Trio MR scanner. Image analysis was carried out and FA values were calculated with FMRIB's diffusion toolbox from FSL (Smith et al., 2004).

TBSS analysis. There has been much recent interest in using magnetic resonance diffusion imaging to provide information about individual differences in anatomical connectivity and white matter tract architecture. FA is one of the measures most commonly derived from diffusion data. Using FA images in a voxelwise statistical analysis, in order to localise FA changes related to development, disease or physiological measurements, such as paired-pulse TMS MEPs has proven to be fruitful. However, in order to compare individual subject's FA images, it is necessary to solve the "correspondence problem", i.e. to determine which location in each subject's FA image corresponds to the equivalent location in the other subjects (Johansen-Berg and Behrens, 2009). A recently proposed method, termed "tract-based spatial statistics" (TBSS) aims to solve issues of alignment and smoothing (Smith et al., 2006; Smith et al., 2007; Johansen-Berg and Behrens, 2009). TBSS was used to carry out a cross-subject comparison of the whole brain white matter in order to find local changes in FA that can be attributed to the variability in the paired-pulse TMS MEP changes in the pre-SMA-M1 experiment and the rIFC-M1 experiment, respectively. TBSS is part of FSL ("TBSS - Tract-Based Spatial Statistics - v1.2"). Individual FA images were aligned into standard space using the "FMRIB 58 FA" image as a target image and a nonlinear registration (step 2 "tbss_2_reg" of the FSL TBSS protocol). These nonlinear transforms were subsequently used to bring the individual images into standard space (step 3 "tbss_3_postreg"). All the standardised FA images were merged into a single 4D image and a mean FA image was created

which then underwent "skeletonisation" in which only the centres of tracts (i.e. the peak FA values) are spared and voxels with lower FA values are suppressed (the threshold was 0.2) creating a "mean FA skeleton" (step 3 "tbss_3_postreg"). Each participant's FA image was then projected onto this mean skeleton, which enabled statistical comparison of FA values from homologous regions of the FA map across participants (step 4 "tbss_4_prestats"). To test whether there was a relationship between functional connectivity effects at different IPIs as found in the paired-pulse TMS experiment and FA values across participants, we followed a procedure outlined by (Boorman et al., 2007; Mars et al., 2009; Buch et al., submitted). We calculated the ratio [paired-pulse TMS MEP / single-pulse TMS MEP] for each IPI (3, 6, 9, 12, 18 ms) for each hand (left and right) in each experiment (pre-SMA-M1 experiment and rIFC-M1 experiment). We used the 6 ms and 12 ms IPI MEP ratios in right hand response trials from the pre-SMA-M1 and rIFC-M1 experiment as TBSS regressors in a four-regressor multiple regression analysis as these four MEP ratios were significantly different from baseline (i.e. a significant MEP change due to the conditioning pulse) and not correlated to one another. The MEP ratios were de-meaned before they were entered into the regression. To test for local correlations between MEP effect size and FA values, we used permutation testing (Nichols and Holmes, 2002) as implemented in FSL ("Randomise v2.1"). We hypothesised that the size of the changes in paired pulse TMS MEPs (effect size) would correlate with the FA in a given area (i.e., a bigger MEP effect size reflects a higher FA value). Therefore, as we found facilitation for both IPIs (i.e. 6 and 12 ms) in the pre-SMA-M1 experiment and inhibition for both IPIs (6 and 12 ms) we were only interested in positive correlations for the pre-SMA-M1 experiment (i.e. stronger facilitation associated with bigger FA) and negative correlations for the rIFC-M1 experiment (i.e.

stronger inhibition associated with bigger FA). Effects were reported as significant at a one-tailed statistical threshold of $P \leq 0.001$ (uncorrected) ((Boorman et al., 2007; Mars et al., 2009; Buch et al., submitted) have used an uncorrected threshold of $P < 0.005$). This threshold is similar to that used in many functional MRI studies, where the number of voxels—and thus the possibility of a false positive—is an order of magnitude greater. In addition, we note that it is not possible to use the correction for multiple comparisons that is standard in functional neuroimaging studies due to the skeletal nature of the FA maps. As an extra precaution against false positives, we only report clusters with an extent of > 10 voxels (Boorman et al., 2007; Mars et al., 2009; Buch et al., submitted). Additionally we rejected clusters that were not significantly correlated to the paired-pulse TMS MEP ratios on the cluster level. All significant correlations between FA value and MEP effect were also tested after partitioning out variance related to the possible confounding factors of participants' age and intensity of the test coil stimulation. All correlations reported remained significant.

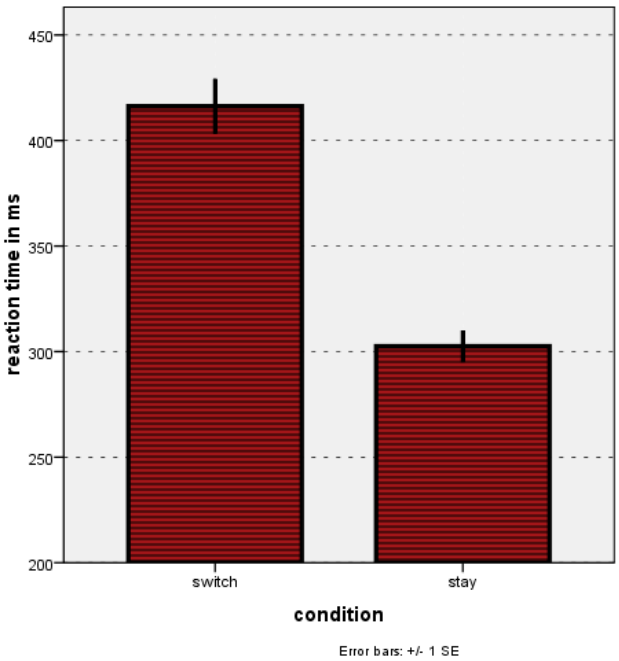
Probabilistic Diffusion Tractography. To elucidate the white matter tracts in which local clusters of FA correlation were found and the grey matter target to which they project we used pre-SMA and rIFC masks as seed masks and the respective clusters of FA correlation as waypoints for multifibre probabilistic diffusion tractography (PDT) (Behrens et al., 2007). To test the clusters of significant FA correlation with the pre-SMA-M1 experiment paired-pulse TMS MEP effects we used a mask of right pre-SMA derived from a study by (Johansen-Berg et al., 2004) and kindly provided by Johansen-Berg and colleagues as a seed mask (see Figure 1) and the respective clusters as waypoints for the PDT. To test the clusters of significant FA correlation with the rIFC-M1 experiment paired-pulse TMS MEP effects we used a mask of right PMv derived from a study by (Tomassini et al., 2007) and kindly provided by

Johansen-Berg and colleagues as a seed mask (see Figure 1) and the respective clusters as waypoints. PDT estimates a probability distribution function (pdf) on fibre direction at each voxel. A multifibre model was fit to the diffusion data at each voxel, allowing for the tracing of fibres through regions of fibre crossing or complexity. Here, we drew 1,000 streamline samples from our seed masks via the waypoint clusters to form an estimate of the probability distribution of connections from the masks via each individual waypoint cluster. The masks (pre-SMA and PMv) and the correlated clusters identified with TBSS were transformed into individual subject's space using the FSL nonlinear registration tool "FNIRT". PDT was run using the FSL PDT toolbox "Probtrackx" with 1,000 streamline samples, 2,000 steps per sample, a step-length of 0.5mm, and a Curvature threshold of 0.2. When these streamlines reach a voxel in which more than one direction is estimated, they follow the direction that is closest to parallel with the direction at which the streamline arrives (if it does not exceed the curvature threshold). Tracts generated by PDT are volumes wherein values at each voxel represent the number of samples (or streamlines) that passed through that voxel. For the elimination of spurious connections, tractography in individual subjects was thresholded to include only voxels through which at least 10 samples had passed (out of 1,000, similar to (Boorman et al., 2007)). These individual tracts were then binarised, transformed back into MNI standard space using "FNIRT" and summed across subjects to produce group probability maps for each pathway. In these maps, each voxel value represents the number of subjects in whom the pathway passes through that voxel.

Results

Behavioural results. ANOVAs of median RTs on correct trials and of error rates (incorrect responses/total number of trials) with "trial type" (switch vs. stay) and "experiment" (pre-SMA -M1 experiment vs. rIFC-M1 experiment) as within-subject factors showed a main effect of "trial type" ($F_{1,15}$= 111.659, $p<0.001$ for RTs; $F_{1,15}$= 46.828, $p<0.001$ for error rates), but no main effect of "experiment" and no interaction between "experiment" and "trial type" ($p>0.45$). A post-hoc paired-samples t-test on the behavioural data confirmed subjects were significantly slower on switch trials than on stay trials (RT 416.2 ms on switch vs. 302.5 ms on stay trials, t_{15}= 9.96, $p<0.001$) and made significantly more mistakes (error rate 22.1% on switch trials vs. 2.1% on stay trials, t_{15}= 6.65, $p<0.001$) (see Figure 31). This confirmed the effectiveness of the task manipulation. However paired samples t-tests testing differences between the pre-SMA-M1 experiment and the rIFC-M1 experiment in RTs, RTs in switch trials, RTs in stay trials, error rates, error rates in switch trials and error rates in stay trials did not yield any differences in these behavioural measures between the two experiments ($p>0.2$). This indicates that performance did not differ between the two experimental sessions. RT switching costs were 1.39 (+/- 0.041 SEM) and error switching costs were 20.3831 (+/-7.152 SEM). Again these behavioural effects did not differ between the experiments. To evaluate behavioural consistency across the two experimental sessions we conducted bivariate correlations and calculated Person Correlation coefficients for RTs, RTs in switch trials, RTs in stay trials, error rates, error rates in switch trials and error rates in stay trials, RT switching costs, and error switching costs. We found significant correlations for RTs (Person= 0.891, $p<0.001$), RTs in stay trials (Pearson= 0.840, $p<0.001$), RTs in switch trials (Pearson= 0.899, $p<0.001$), RT switching costs

(Pearson= 0.898, $p<0.001$), and error switching costs (Pearson= 0.637, $p=0.008$) (see Figure 32), indicating very strong "test retest reliability" of behavioural measurements between the two experimental sessions, although the order of the experiments was counterbalanced and at least 1 week was between experimental sessions. This suggests individual "behavioural patterns" of response switching and action reprogramming possibly due to individual anatomical and functional networks of executive control and action reprogramming.



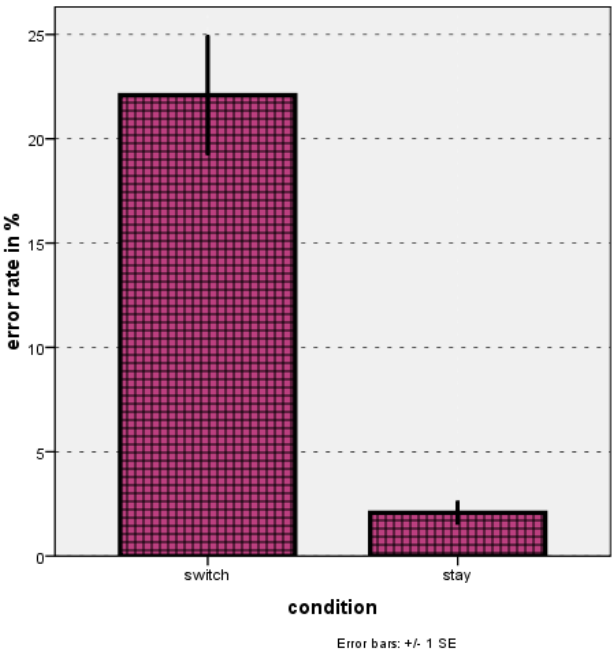


Figure 31: Behavioural data across the two experiments. Subjects were significantly slower on switch trials than on stay trials (top) and made significantly more mistakes (bottom).

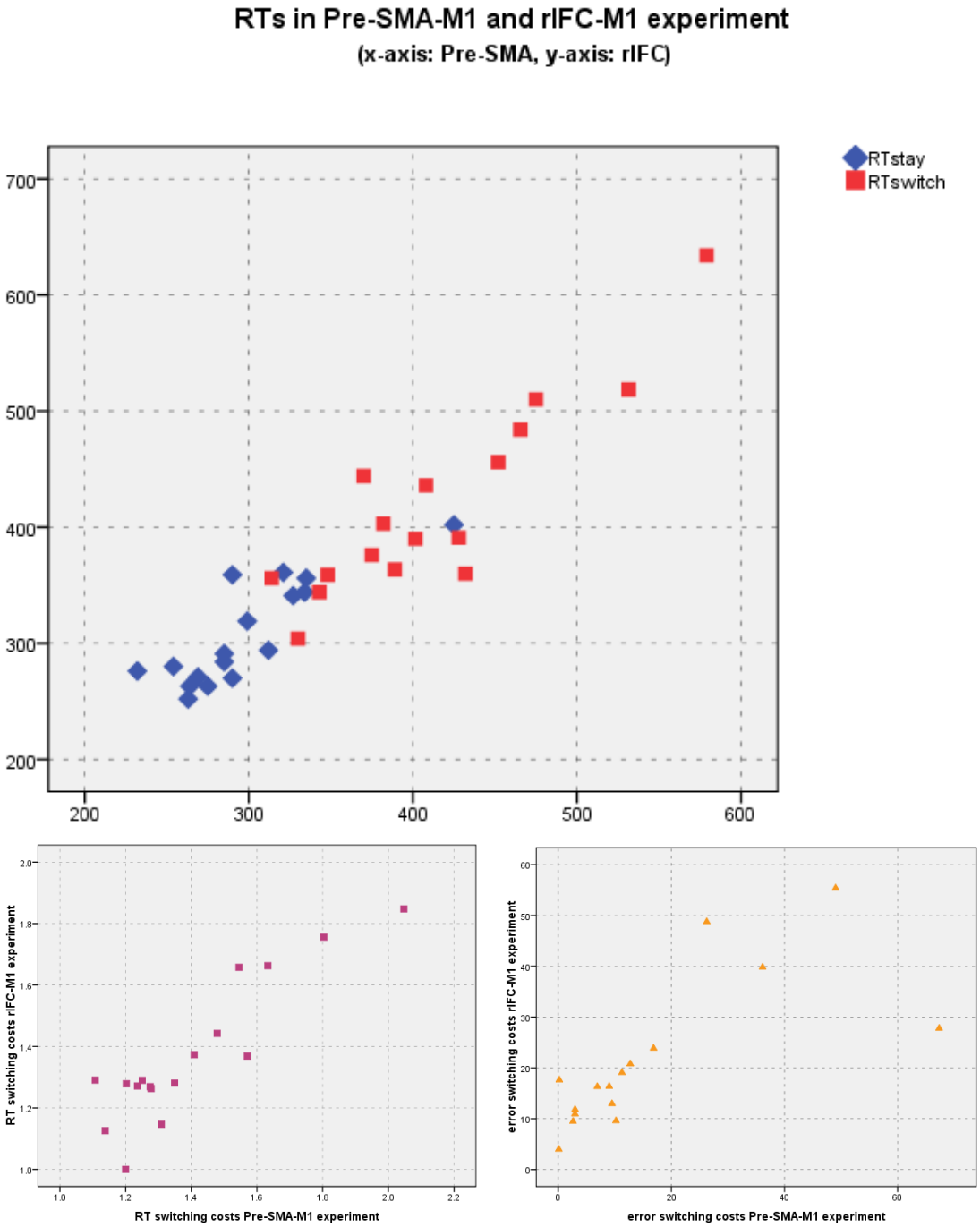


Figure 32: Consistency of behavioural measures across experimental sessions. A significant correlation for RTs at stay and switch trials (top) as well as RT switching costs (bottom left, magenta) and error rates (bottom right, yellow) between the two experimental sessions (pre-SMA experiment, rIFC experiment, order counterbalanced) was observed.

Pre-SMA-M1 functional connectivity during action reprogramming and response inhibition. The aim of this experiment was to investigate functional interactions between pre-SMA and M1 during action reprogramming and response inhibition as reported by (Mars et al., 2009). Single and paired-pulse TMS was delivered only at one time point after cue onset (125 ms SOA) based on the results reported in (Mars et al., 2009). However the IPI was varied to investigate differences in paired-pulse TMS MEP ratios associated with differences in IPIs due to distinct pathways mediating the influence of pre-SMA on M1. M1-cortico-spinal excitability was assessed with TMS pulses over the motor cortex recording EMG activity and measuring peak-to-peak amplitudes of MEPs in a finger involved in the task (FDI). To investigate whether a TMS pulse over pre-SMA occasionally preceding the M1 test pulse by variable durations (3, 6, 9, 12, 18 ms) would change M1 excitability we calculated the ratio [paired-pulse TMS MEP / single-pulse TMS MEP]. Hence singe-pulse TMS MEPs served as a baseline, paired-pulse trial MEPs reflected the "change" in this baseline excitability due to an "activity burst" in pre-SMA. Again M1 TMS test pulses were only applied to the *left* M1 and MEPs only recorded from the *right* hand FDI and ADM (see Figure 33). Hence left hand response trials should be considered as "switch away from the prepared response" trials. Right hand trials should be considered as "switch towards the unprepared response". To elucidate if pre-SMA influences M1 excitability during action reprogramming via different pathways we conducted an ANOVA test on the paired-pulse TMS / single-pulse TMS MEP ratios for every condition with within-subjects contrasts of *"IPI"* (3, 6, 9, 12, 18 ms), and *"hand"* (left vs. right). We found a significant effect of *"hand"* ($F_{1,16}$=5.748, p=0.029), and a significant *"hand"* × *"IPI"* interaction ($F_{4,64}$=3.063, p=0.023). Post-hoc two-tailed one-sample t-tests against baseline (ratio of 1.0 or 100%) revealed significant

facilitation of M1 excitability due to pre-SMA conditioning pulses in right hand responses with and IPI of 6 ms (t_{15}=3.077, p=0.007), 9 ms (t_{15}=3.996, p=0.001) and 12 ms (t_{15}=3.286, p=0.005). Paired samples t-tests between left and right hand response trials showed a significant difference for an IPI of 6 ms (t_{15}=-2.576, p=0.02, right>left), 9 ms (t_{15}=-3.105, p=0.007, right>left), and 12 ms (t_{15}=-2.596, p=0.02, right>left). Paired-samples t-tests between different IPIs revealed a significant difference for right hand responses between 3 ms and 6 ms IPI (t_{15}=-4.608, p<0.001, 6 ms>3 ms), 3 ms and 9 ms IPI (t_{15}=-4.070, p=0.001, 9 ms>3 ms), 3 ms and 12 ms IPI (t_{15}=-3.475, p=0.003, 12 ms>3 ms). This replicates the findings by (Mars et al., 2009) and extends them to longer IPIs (i.e. 9 ms and 12 ms).

Functional specificity of the observed effects. To investigate whether the influence of Pre-SMA on M1 excitability were functionally specific and restricted to the muscles involved in the task (e.g. FDI) or whether these processes could be observed in other nearby hand muscles (e.g. ADM), we analysed MEPs recorded from the ADM muscle (see Figure 33). Again it is important to note that this setup was not optimal to answer this question as TMS pulses were delivered at the M1-representation of the FDI muscle and stimulus intensities were determined based on FDI MEPs. An ANOVA test on the paired-pulse TMS / single-pulse TMS MEP ratios for every condition with within-subjects contrasts of *"IPI"* (3, 6, 9, 12, 18ms), and *"hand"* (left vs. right) revealed no significant effects (all p>0.25). Hence facilitatory influences of pre-SMA on M1 might be specific to the movements that are relevant in a certain task.

Behavioural relevance of the pre-SMA-M1 physiological interactions. As the facilitatory influence of pre-SMA on M1 occurs well before the average RT in stay trials (125 ms vs. 302.5 ms), pre-SMA input could be important for successfully reprogramming prepared actions. To test whether processes of pre-SMA-M1

interaction during response switching would be behaviourally relevant we reanalysed some of the behavioural data. We obtained switch trial RTs and error rates for every IPI condition. However an ANOVA test on the switch trial median RTs in every IPI condition with within-subjects contrasts of *"IPI"* (3, 6, 9, 12, 18 ms) revealed no significant effect. Additionally an ANOVA test on the error rates in every IPI condition with within-subjects contrasts of *"IPI"* (3, 6, 9, 12, 18 ms) did not show any significant effect either.

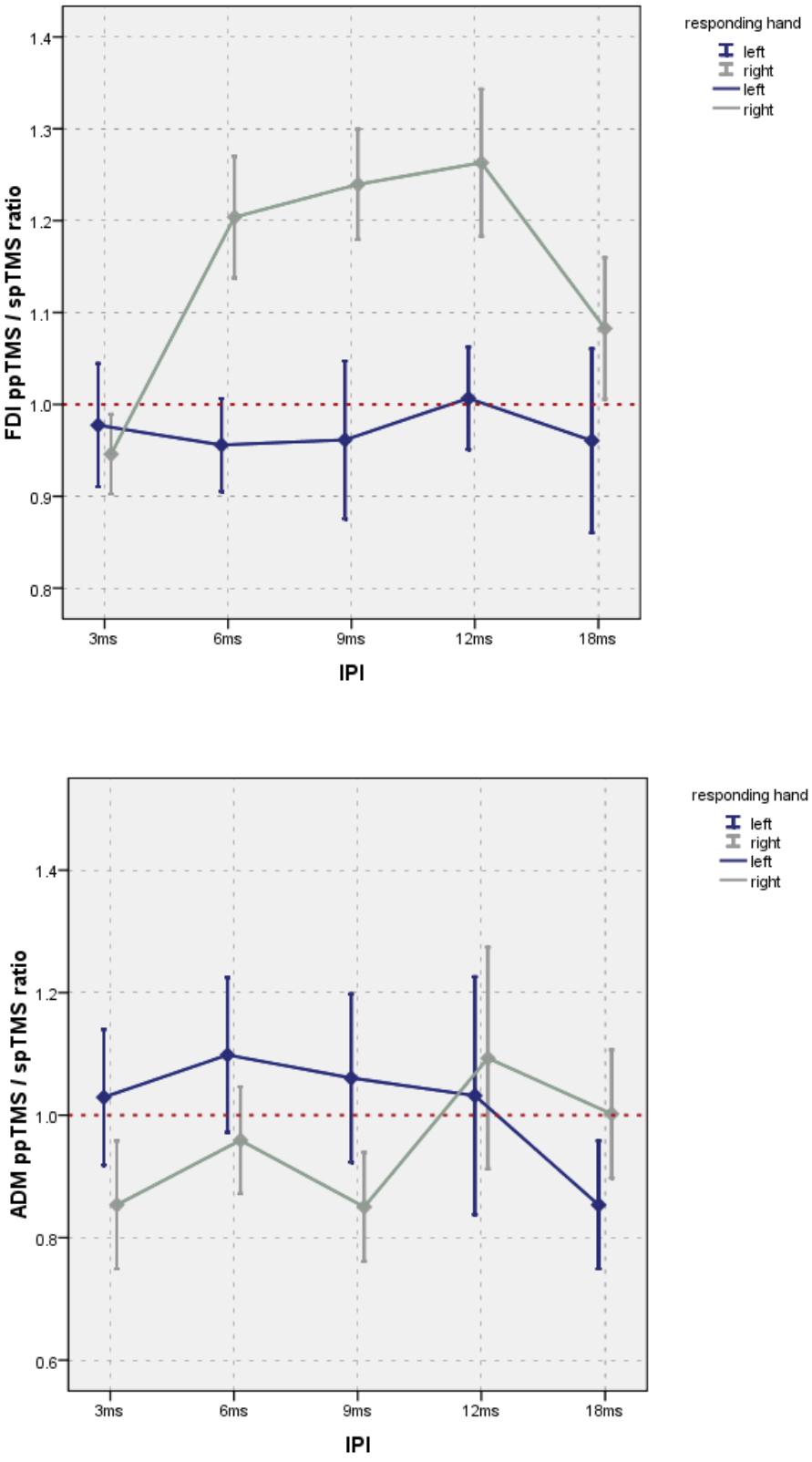


Figure 33: Pre-SMA- M1 functional connectivity during action reprogramming at 125 ms SOA with variable IPIs. Pre-SMA-M1 functional connectivity results plotted as single-pulse / paired-pulse TMS MEP ratios for every hand and IPI (3, 6, 9, 12, 18 ms). MEP data was recorded from the FDI muscle (top) and the ADM muscle (bottom).

RIFC-M1 functional connectivity during action reprogramming and response inhibition. The aim of this experiment was to investigate functional interactions between rIFC and M1 during action reprogramming and response inhibition as reported in the previous experiment (see 2.1.2). Single and paired-pulse TMS was delivered only 175 ms after cue onset as we previously found the strongest rIFC-M1 interaction at this SOA. The IPI was varied to investigate differences in paired-pulse TMS MEP ratios associated with differences in IPIs. Again M1-cortico-spinal excitability was assessed with TMS pulses over left M1 and the change in M1 corticospinal excitability due to the conditioning pulse over rIFC was quantified as a ratio [paired-pulse TMS MEP / single-pulse TMS MEP] (see Figure 34). To elucidate if rIFC influences M1 excitability during action reprogramming via different pathways we conducted an ANOVA test on the paired-pulse TMS / single-pulse TMS MEP ratios for every condition with within-subjects contrasts of *"IPI"* (3, 6, 9, 12, 18 ms), and *"hand"* (left vs. right). We found a significant effect of *"IPI"* ($F_{4,64}$= 6.160, p<0.001). Post-hoc two-tailed one-sample t-tests against baseline (ratio of 1.0 or 100%) revealed significant inhibition of M1 excitability due to rIFC conditioning pulses in right hand responses with an IPI of 6 ms (t_{15}= -2.740, p=0.015), and 12 ms (t_{15}= -4.740, p<0.001) and in left hand responses with an IPI of 6 ms (t_{15}= -2.873, p=0.011), and 9 ms (t_{15}= -3.071, p=0.007). Paired samples t-tests between left and right hand response trials showed no significant differences. Paired-samples t-tests between different IPIs revealed a significant difference for left hand responses between 3 ms and 6 ms IPI (t_{15}=2.171, p=0.045, 6 ms<3 ms), 3 ms and 9 ms IPI (t_{15}=2.679, p=0.016, 9 ms<3 ms), and for right hand response between 3 ms and 6 ms IPI (t_{15}=2.353, p=0.032, 6 ms<3 ms), and between 3 ms and 12 ms IPI (t_{15}=3.255, p=0.005, 12 ms<3 ms). This replicates the findings of our previous experiment and

extends them to different IPIs, i.e. 6 ms and 9 ms in left hand response trials, 6 ms and 12 ms in right hand response trials.

Functional specificity of the observed effects. As in the pre-SMA-M1 experiment we investigated whether the influence of rIFC on M1 excitability was functionally specific and restricted to the muscles involved in the task (e.g. FDI) or whether it this effect could be observed in other nearby hand muscles. Therefore we again analysed MEPs recorded from the ADM muscle (see Figure 34). However an ANOVA test on the paired-pulse TMS / single-pulse TMS MEP ratios for every condition with within-subjects contrasts of *"IPI"* (3, 6, 9, 12, 18 ms), and *"hand"* (left vs. right) revealed no significant effects (all p>0.3). Hence inhibitory influence of rIFC on M1 might be specific to the muscles that are relevant in a certain task.

Behavioural relevance of the rIFC-M1 physiological interactions. The inhibitory influence of rIFC on M1 occurs well before the average RT in stay trials (175 ms vs. 302.5 ms). Hence rIFC input could be important for successfully inhibiting prepared but inappropriate responses. To test whether processes of rIFC-M1 interaction during response switching would be behaviourally relevant we re-analysed some of the behavioural data (see Figure 35). We obtained switch trial RTs and error rates for every IPI condition. An ANOVA test on the switch trial median RTs in every IPI condition with within-subjects contrasts of *"IPI"* (3, 6, 9, 12, 18 ms) revealed a significant effect of *"IPI"* ($F_{1,15}$= 3.708, p=0.017). An ANOVA test on the error rates in every IPI condition with within-subjects contrasts of *"IPI"* (3, 6, 9, 12, 18 ms) showed an effect of IPI approaching significance ($F_{1,15}$=2.353, p=0.071). Post-hoc paired samples t-tests revealed significant differences between single-pulse and paired-pulse TMS trial median RTs for an IPI of 3 ms (t_{15}=-3.212, p=0.005, paired-pulse RT> single-pulse RT), an IPI of 6 ms (t_{15}= -4.258, p=0.001, paired-pulse RT>

single-pulse RT), an IPI of 9 ms (t_{15}= -2.729, p=0.015, paired-pulse RT> single-pulse RT), and an IPI of 12 ms (t_{15}= -5.229, p<0.001, paired-pulse RT> single-pulse RT), and a significant difference for paired-pulse TMS trials between IPIs of 12 ms and 18 ms (t_{15}= 2.808, p=0.013, 12 ms>18 ms).

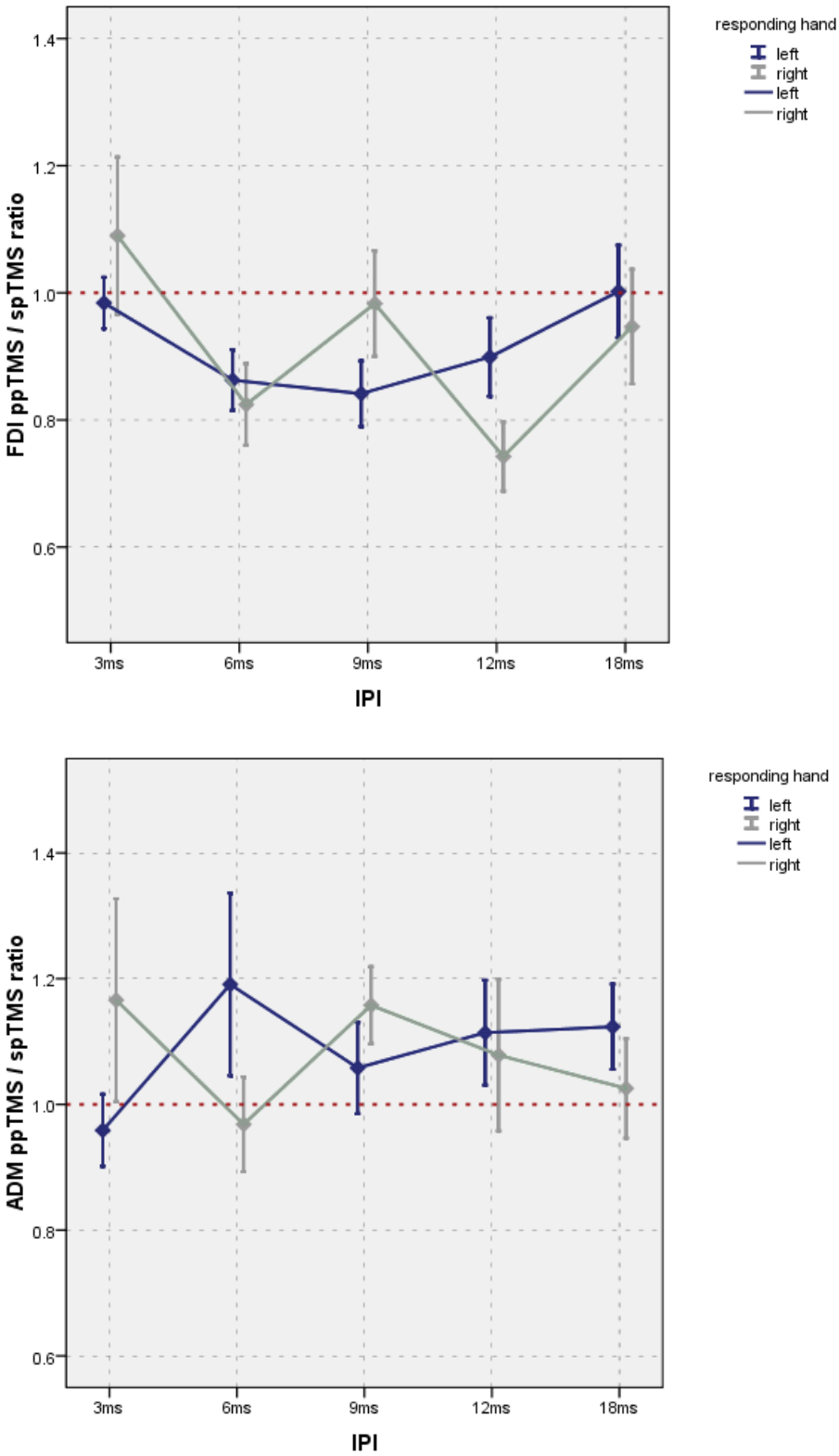
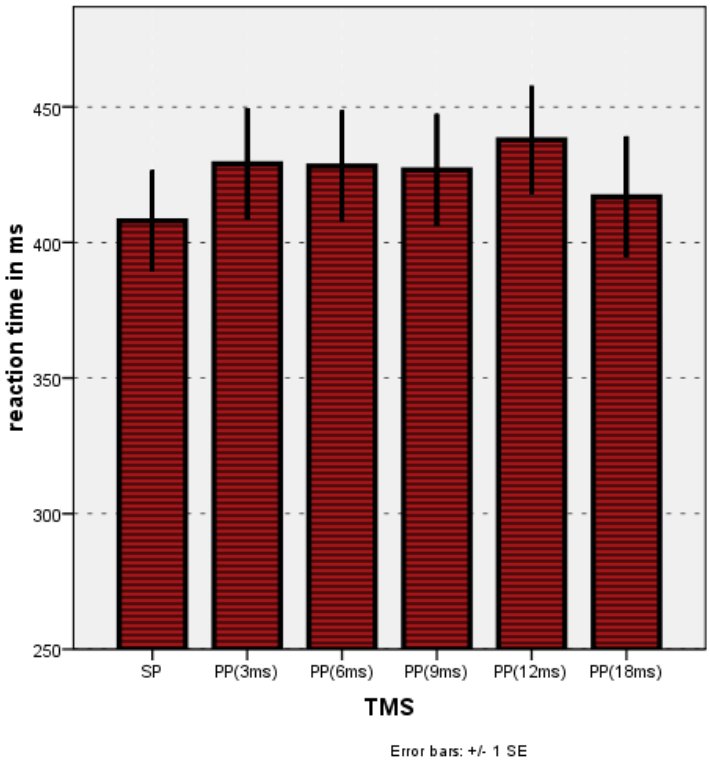


Figure 34: RIFC - M1 functional connectivity during action reprogramming at 175 ms SOA with variable IPIs. Results plotted as single-pulse / paired-pulse TMS MEP ratios for every hand and IPI (3, 6, 9, 12, 18 ms). MEP data was recorded from the FDI (top) and the ADM (bottom).



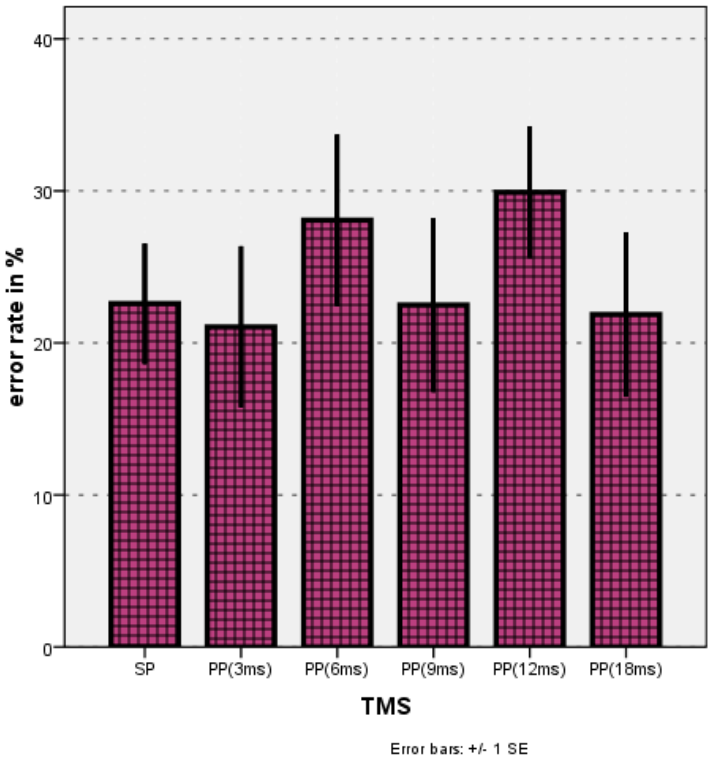


Figure 35: Behavioural relevance of the rIFC-M1 physiological interactions during action reprogramming. Median RTs and error rates plotted for every condition (single vs. paired- pulse TMS, different IPIs).

Functional connectivity between Pre-SMA and M1 vs. functional connectivity between rIFC and M1. To infer whether pre-SMA and M1 functional connectivity would differ significantly from functional connectivity between rIFC and M1 we conducted an ANOVA test with "hand" (left vs. right), "IPI" (3, 6, 9, 12, 18 ms) and "area" (pre-SMA vs. rIFC). Although the "area" contrast was obtained in two separate experimental sessions, as we only tested pre-SMA-M1 functional connectivity in the pre-SMA-M1 experiment and only rIFC-M1 functional connectivity in the rIFC-M1 experiment (order was randomised), we could still test this contrast as a within-subject contrast, because the same group of subjects participated in these two experimental sessions. The ANOVA showed a main effect of "area" ($F_{1,15}=10.233$, $p=0.006$) and significant "area" × "IPI" ($F_{4,60}=6.166$, $p<0.001$) and "area" × "IPI" × "hand" ($F_{4,60}=3.208$, $p=0.018$) interactions. This suggests that both areas differently influence the M1 corticospinal excitability and motor output during action reprogramming and response switching (see Figure 36).

Functional pathways mediating Pre-SMA and M1 connectivity and rIFC-M1 connectivity. One goal of this experiment was to investigate pathways of functional connectivity and relate these to anatomical pathways between these areas. Functional connectivity can be examined using a paired-pulse TMS paradigm (Koch et al., 2006; O'Shea et al., 2007b; O'Shea et al., 2008). In this experiment we varied the interval between the two pulses, assuming that different intervals would depict functional connectivity mediated via different pathways. These differences in functional connectivity associated with different IPIs and therefore different pathways mediating these effects can be related to anatomical white matter pathways represented in diffusion weighted images (Boorman et al., 2007; Mars et al., 2009; Buch et al., submitted). To infer whether paired-pulse TMS effects of functional connectivity

differed depending on the IPI and hence were mediated by different "functional pathways" we evaluated bivariate Pearson correlations between the paired-pulse / single-pulse TMS MEP ratios for the different areas of conditioning pulse application (pre-SMA and rIFC) and the different IPIs (3, 6, 9, 12, 18 ms) (see correlation matrices Figure 37 and Figure 38). Correlation tables for the MEP ratios in the pre-SMA-M1 experiment showed significant correlations between IPIs of 9 ms and 12 ms (Pearson= 0.702, p=0.002), 9 ms and 18 ms (Pearson= 0.792, p<0.001), 12 ms and 18 ms (Pearson= 0 .962, p<0.001). Correlation tables for the MEP ratios in the rIFC - M1 experiment showed significant correlations between IPIs of 3 ms and 6 ms (Pearson= 0.907, p<0.001), 9 ms and 12 ms (Pearson= 0. 983, p<0.001), 9 ms and 18 ms (Pearson= 0 .530, p=0.029), 12 ms and 18 ms (Pearson= 0.496, p=0.043). There were no correlations in paired-pulse / single-pulse TMS MEP ratios between the two experiments (pre-SMA and rIFC) for any given IPI. This is interesting as it could indicate that pre-SMA influence on M1 could be exerted via two distinct routes, one "shorter" and possibly more direct path (6 ms) that has already been reported in a study by (Mars et al., 2009) and one "longer" and indirect path depicted with longer IPIs (9, 12, 18 ms). The same holds for the rIFC-M1 experiment. Correlations between paired-pulse / single-pulse TMS MEP ratios at different IPIs suggest that rIFC influence on M1 during action reprogramming might be exerted via two distinct paths connecting these areas, one direct route (3, 6 ms) and one more indirect route (9, 12, 18 ms). This is important for the subsequent TBSS analysis which aims to relate these measures of functional connectivity to a measure of anatomical white matter micro-architecture (FA) derived from diffusion MRI scans that were acquired for every subject participating in both, the pre-SMA-M1 and the rIFC-M1 experiment. We therefore decided to enter the paired-pulse / single-pulse TMS MEP ratios for

right hand response trials with 6 ms and 12 ms IPI from the pre-SMA-M1 and the rIFC-M1 experiment as four regressors (Pre-SMA-M1: 6 ms and 12 ms, rIFC-M1: 6 ms and 12 ms) into the multiple-regression-TBSS analysis (see Figure 39).

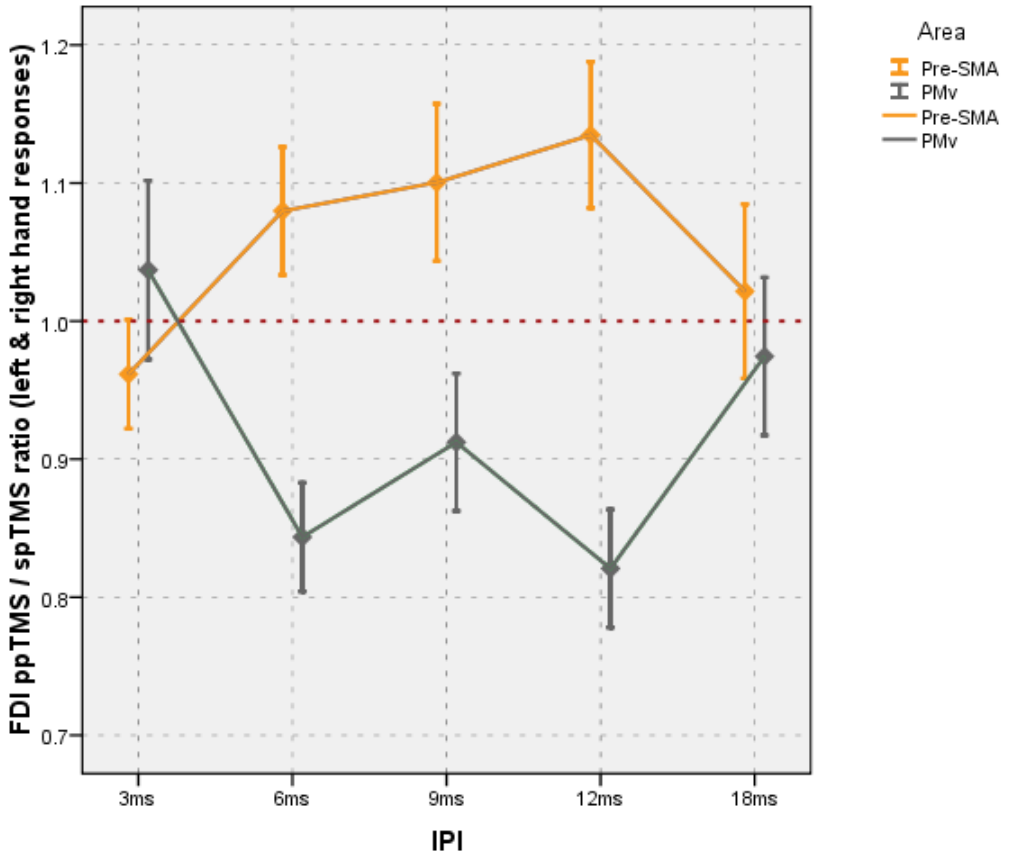


Figure 36: Pre-SMA- M1 (yellow) and rIFC- M1 (grey) during action reprogramming. Single-pulse / paired-pulse TMS MEP ratios are plotted across hands (left and right hand responses together) for different IPIs (3, 6, 9, 12, 18 ms).

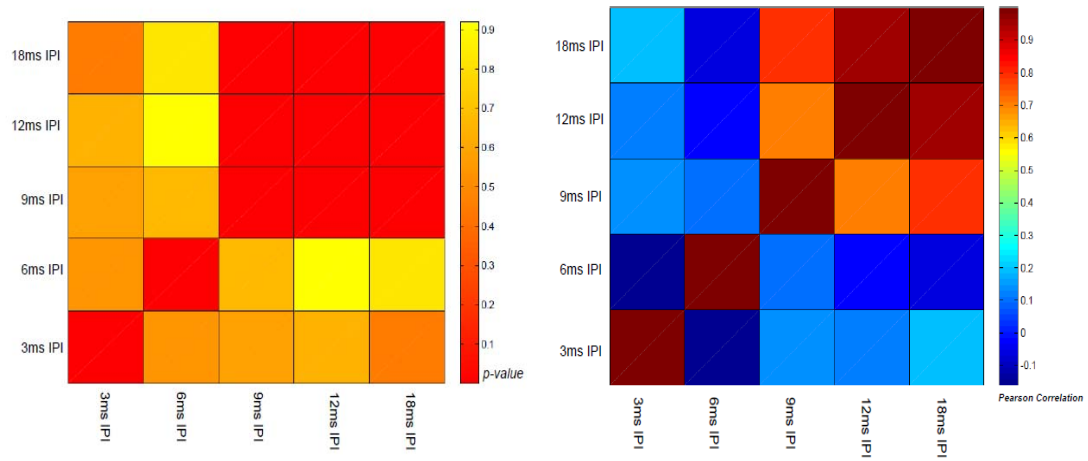


Figure 37: Cross correlation matrices for pre-SMA-M1 functional connectivity effects at different IPIs. Single-pulse / paired-pulse TMS MEP effects are correlated across different IPIs (yellow graph in Figure 36) and plotted with their respective p-value (left) and Pearson correlation coefficient (right).

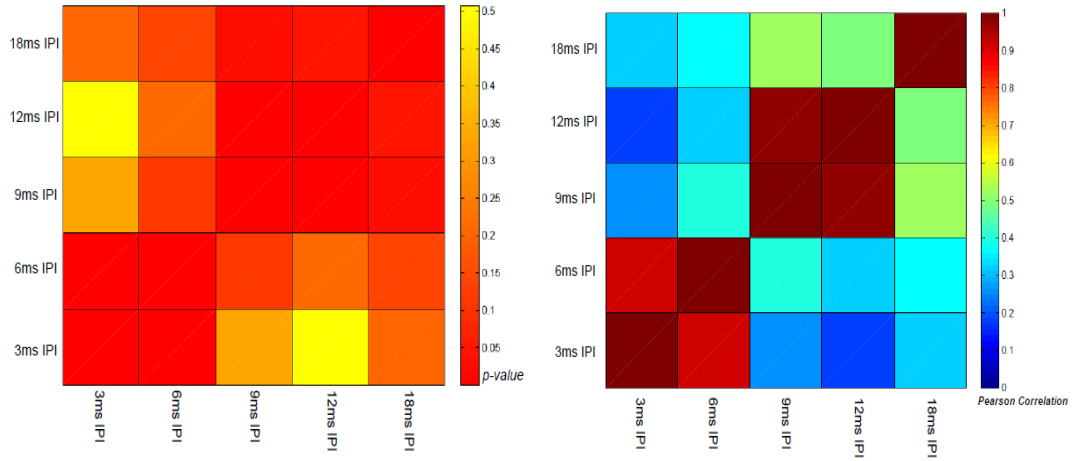


Figure 38: Cross correlation matrices for rIFC - M1 functional connectivity effects at different IPIs. Single-pulse / paired-pulse TMS MEP effects are correlated across different IPIs (grey graph in Figure 36) and plotted with their p-value (left) and Pearson correlation coefficient (right).

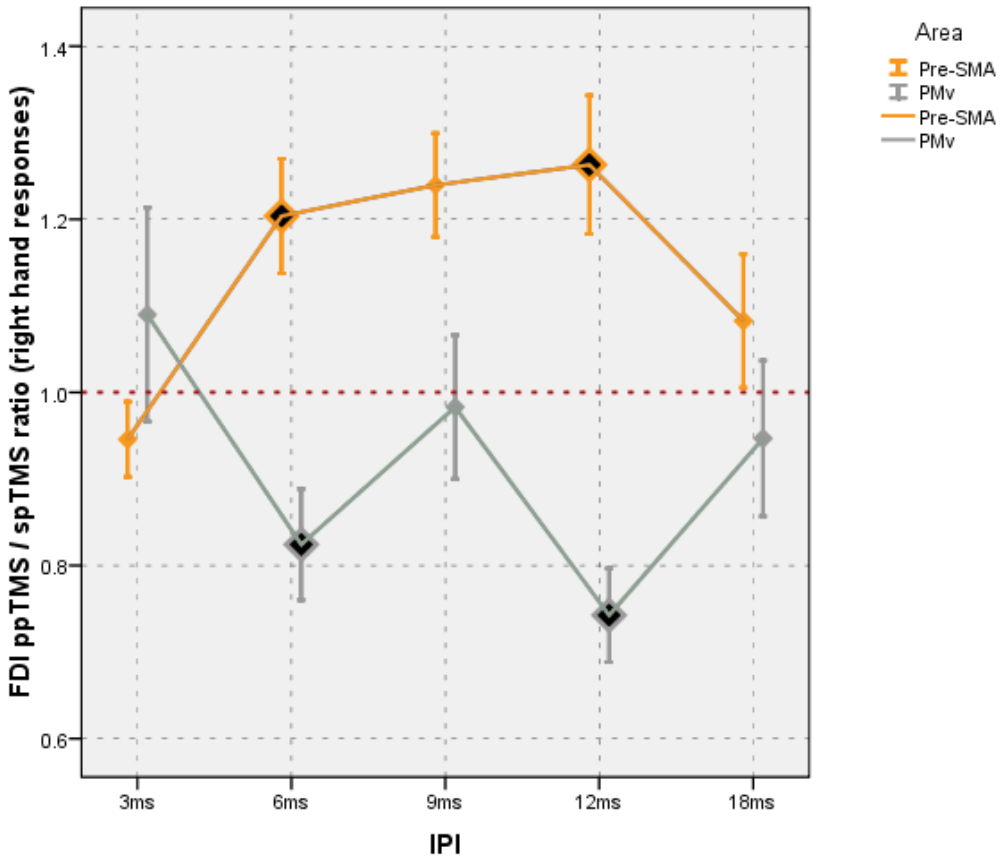


Figure 39: Pre-SMA- M1 (yellow) and rIFC- M1 (grey) during action reprogramming. Single-pulse / paired-pulse TMS MEP ratios are plotted only for right hand responses as these showed effects significantly from baseline in both experiments: M1 was significantly facilitated for right hand response trials in the pre-SMA- M1 experiment at 6, 9 and 12 ms IPI. M1 was significantly inhibited for right hand response trials in the rIFC- M1 experiment at 6 and 12 ms IPI. Effects at 6 and 12 ms IPI from both experiments (filled black) were used as regressor in the TBSS four regressor multible regression analysis.

Correlations between TMS measures of functional connectivity and FA. To infer whether there was a relationship between functional connectivity and white-matter microstructure across subjects, we used TBSS (Smith et al., 2006; Boorman et al., 2007; Smith et al., 2007; Mars et al., 2009; Buch et al., submitted) to investigate local correlations between FA and paired-pulse / single-pulse TMS MEP ratio effect size. We used MEP ratios with 6 ms and 12 ms IPI from right hand response trials in the pre-SMA-M1 experiment and the rIFC-M1 experiment. Therefore we had four different regressors in the TBSS multiple-regression analysis. The regressors (MEP ratios) were de-meaned. Effects were reported as being significant at a one-tailed statistical threshold of $p<0.001$ (uncorrected) and a cluster size of >10 voxels. The correlations remained significant on a cluster-level (average FA values within each cluster) and after controlling for age and TMS test pulse stimulation intensity. We found two clusters significantly correlated with the TMS effect in the pre-SMA-M1 experiment at an IPI of 6 ms. These clusters lie in dorsal white matter tracts connecting premotor, motor and parietal areas, thus replicating the results by (Mars et al., 2009). Additionally we found six clusters significantly correlated with the TMS effect in the pre-SMA-M1 experiment at an IPI of 12 ms. These clusters lie in white matter surrounding the basal ganglia, white matter underlying the pre-SMA and white matter connecting premotor and motor areas. We found three clusters significantly correlated with the TMS effect in the rIFC-M1 experiment at an IPI of 6 ms. These clusters were found in the white matter underlying anterior and posterior rIFC and white matter underlying the motor cortex. Moreover we found 14 clusters significantly correlated with the TMS effect in the rIFC-M1 experiment at an IPI of 12 ms. These clusters were found in the vicinity of the STN, in the white matter

underlying rIFC and ventro-lateral PFC, white matter underlying the pre-SMA and in the superior longitudinal fascicle.

Tractography from areas of conditioning pulse stimulation (pre-SMA or rIFC) via the white matter regions showing local correlations. To elucidate the white-matter tracts in which local clusters of FA correlation were and the grey matter target areas to which they most likely projected, we used the respective areas of conditioning pulse stimulation as seed masks and the clusters as waypoints for multifibre probabilistic tractography. Hence we used a mask of right pre-SMA derived from a study by (Johansen-Berg et al., 2004) as a seed mask for tractography via the clusters significantly correlated with TMS effects in the pre-SMA-M1 experiment. We used a mask of right PMv derived from a study by (Tomassini et al., 2007) as seed mask for tractography via the clusters significantly correlated with TMS effects in the rIFC - M1 experiment. Interestingly clusters significantly correlated with TMS effects in the pre-SMA-M1 experiment with an IPI of 6 ms generated tracts within the SLF connecting pre-SMA, premotor areas with motor and parietal areas. However tracts derived from clusters significantly correlated with TMS effects in the pre-SMA-M1 experiment with an IPI of 12 ms connected pre-SMA with ventral and dorsal premotor areas, areas in the rIFC, M1 and parietal areas and with areas in the vicinity of STN. Similarly clusters significantly correlated with TMS effects in the rIFC -M1 experiment with an IPI of 6 ms generated tracts connecting PMv and other rIFC areas with M1 and temporo-parietal areas, whereas tracts derived from clusters significantly correlated with TMS effects at 12 ms IPI connected PMv with more anterior areas in rIFC, ventro-lateral PFC, medial frontal cortex and pre-SMA, M1 and areas in the parietal lobe and with the STN. These group probabilistic tractography maps can be summed for each area and IPI separately (pre-SMA-M1 vs. rIFC-M1, 6 ms vs. 12 ms)

to construct composite connectivity networks (see Figure 52, Figure 53, Figure 54, and Figure 55). These composite connectivity networks summarise the tractography derived from each TBSS cluster for each IPI (6 and 12 ms) and each area of conditioning pulse stimulation (pre-SMA and rIFC). Tracts derived from the clusters correlated with the 6 ms IPI paired-pulse TMS effects in the pre-SMA experiment produce a complex network of tracts in the dorsal white matter connecting pre-SMA, dorsal premotor and motor areas as well as regions in the parietal lobe and extend into the pyramidal tract. Tracts derived from clusters correlated with the 12 ms IPI TMS effects in the pre-SMA-M1 experiment produce a slightly different network of white matter tracts connecting pre-SMA, dorsal premotor, motor and parietal areas with rIFC, ventro-lateral PFC and the basal ganglia system. This could suggest that effects of functional connectivity observed in the pre-SMA-M1 paired-pulse TMS experiment at different IPIs were mediated by different paths of anatomical connectivity. The facilitatory influence of pre-SMA on M1 during action reprogramming could be exerted via a direct (6 ms IPI) and an indirect (12 ms) pathway (see Figure 37, Figure 52, and Figure 53). Composite connectivity networks derived from clusters correlated with the 6 ms IPI TMS effects in the rIFC-M1 experiment produce paths connecting rIFC, pre-SMA, M1 and parietal areas. However connectivity networks derived from clusters correlated with the 12 ms IPI TMS effects in the rIFC-M1 experiment produce connections between posterior rIFC and anterior rIFC, ventro-lateral PFC, pre-SMA, dorsal premotor, motor and parietal areas and deeper basal ganglia structures. This is interesting as it again suggests two different pathways mediating the effects of functional connectivity observed in the rIFC-M1 experiment. A direct pathway (6 ms IPI) might connect rIFC and M1 via premotor areas, whereas a more indirect pathway (12 ms IPI) could connects rIFC and

M1 via the STN. Moreover it seems somehow surprising that the pre-SMA-M1 and the rIFC-M1 composite connectivity networks derived from the respective clusters correlated with the 12 ms TMS effects in each experiment seem very similar. Both networks connect motor areas, ventral and dorsal premotor areas, more anterior rIFC regions, pre-SMA and structures in the basal ganglia possibly indicating a bigger network of white matter tracts connecting areas involved in action reprogramming, response switching and movement inhibition.

x	y	z	hemisphere	anatomical region	average p-value	correlation (cluster)	size (voxels)
-39	-26	31	left	SLF III	0.001	0.652	15
28	-18	36	right	SLF I	0.001	0.582	15

Table 5: Details of clusters showing significant positive correlation between individual TMS functional connectivity effects in the pre-SMA-M1 experiment with 6 ms IPI and individual FA.

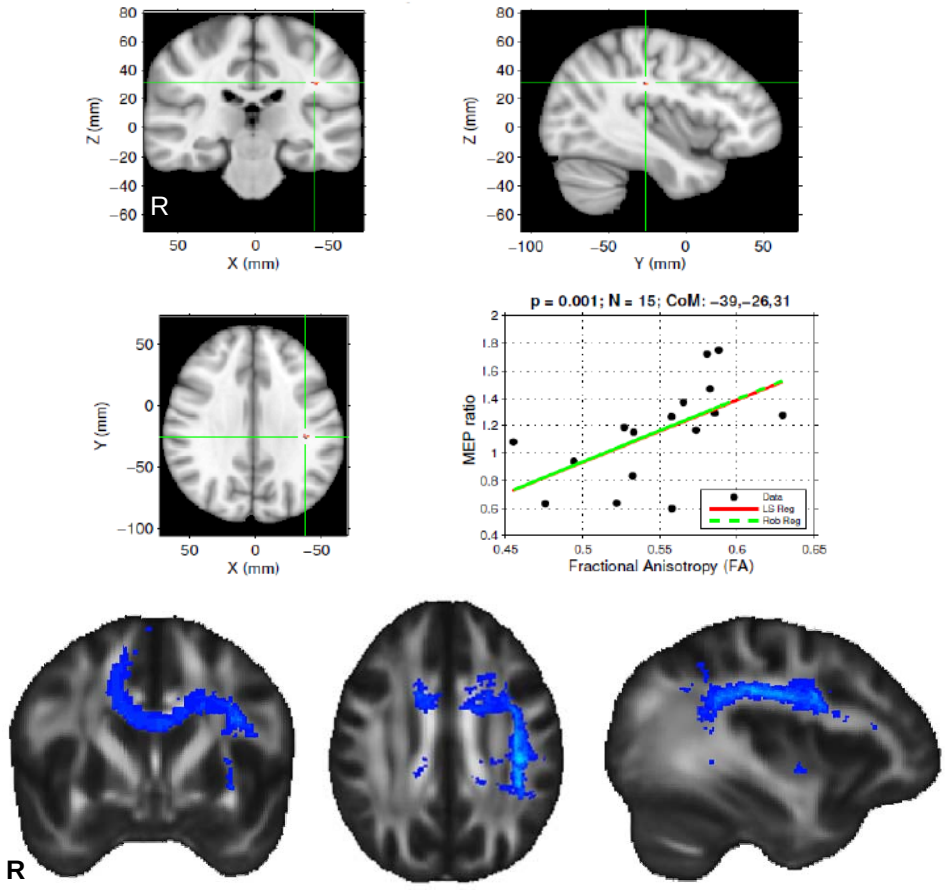


Figure 40: Local correlation between FA and pre-SMA-M1 functional connectivity at 6 ms IPI (top) plotted on the MNI152 brain template. The cluster shows significant positive correlation between individual FA and MEP effects size. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio, an ordinary least square (red) and a robust regression (green). The average p-value of the correlation between FA and MEP ratio, number of voxels in the cluster and the MNI coordinates of the centre of mass is indicated above the scatterplot. Group probability maps of tracts generated by PDT from pre-SMA via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

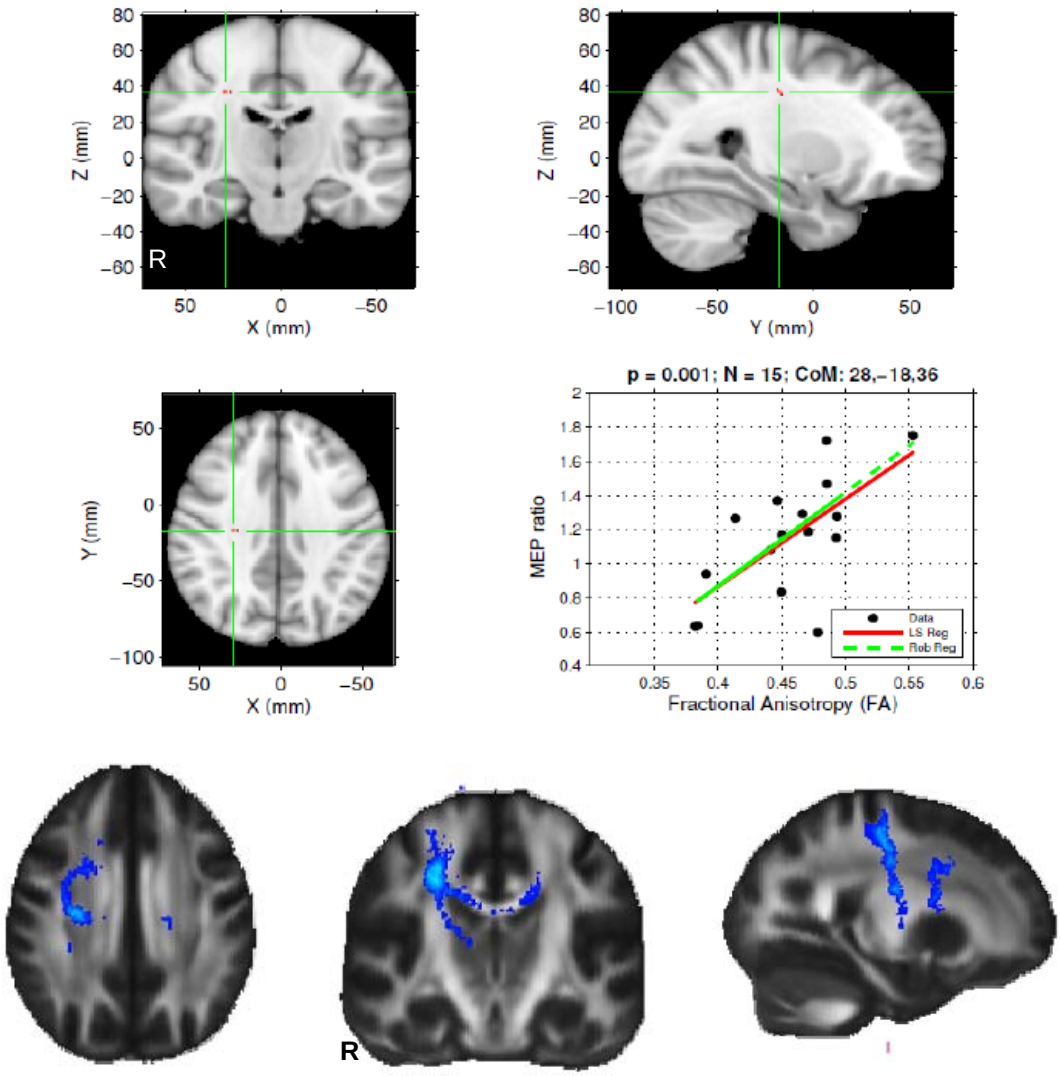


Figure 41: Local correlation between FA and pre-SMA-M1 functional connectivity at 6 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from pre-SMA via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

x	y	z	hemisphere	anatomical region	average p-value	correlation (cluster)	cluster size (voxels)
46	-7	-13	right	Temporal Lobe	<0.001	0.638	17
-28	-20	-7	left	Internal Capsule	0.001	0.698	11
-19	-4	36	left	SLF I	0.001	0.708	18
-21	4	36	left	SLF I, white matter under Pre-SMA	0.001	0.663	12
-23	-48	39	left	SLF III	0.001	0.638	21
18	5	40	right	SLF I, white matter under Pre-SMA	<0.001	0.724	54

Table 6: Details of clusters showing significant positive correlation between individual TMS functional connectivity effects in the pre-SMA-M1 experiment with 12 ms IPI and individual FA.

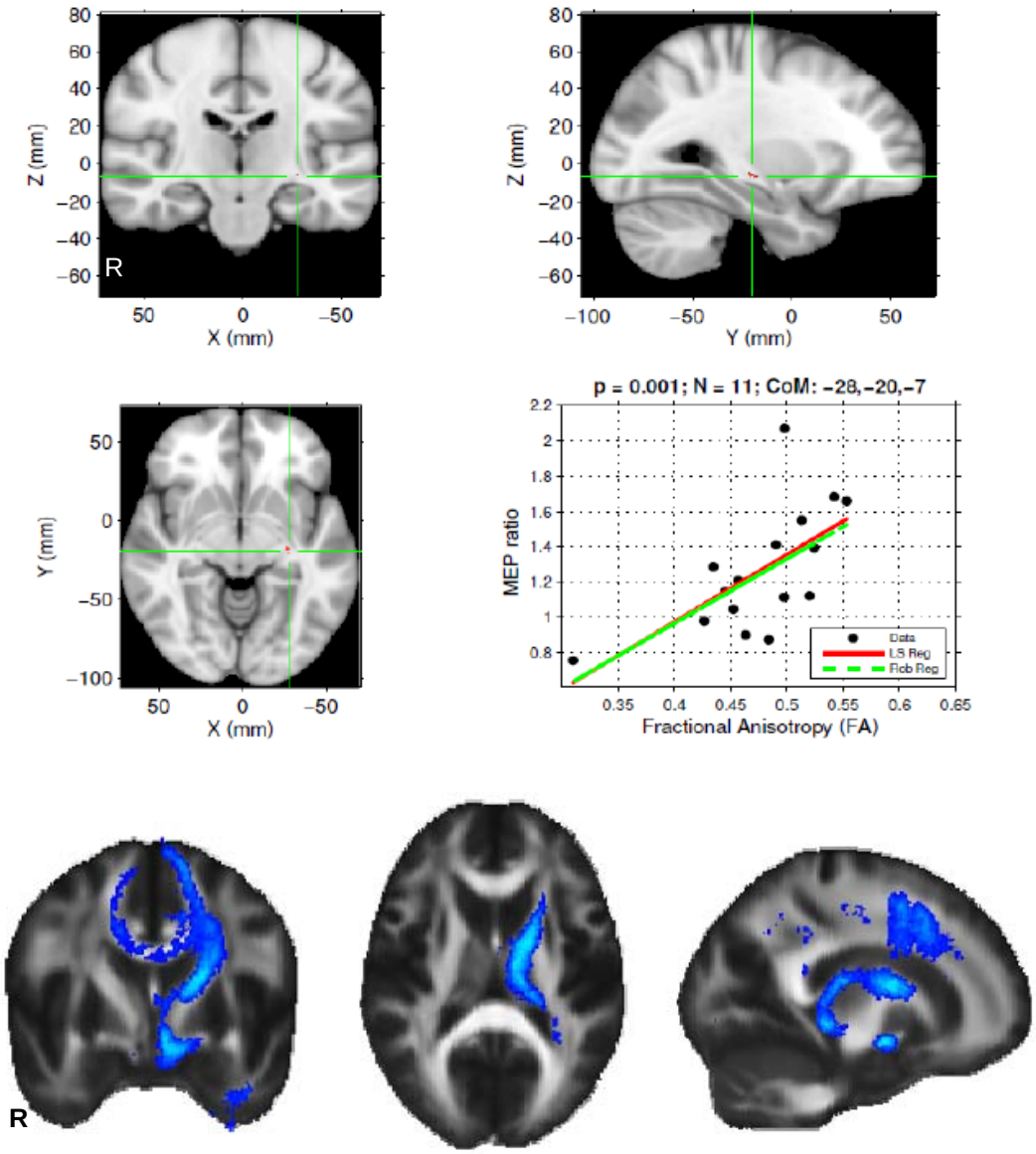


Figure 42: Local correlation between FA and pre-SMA-M1 functional connectivity at 12 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from pre-SMA via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

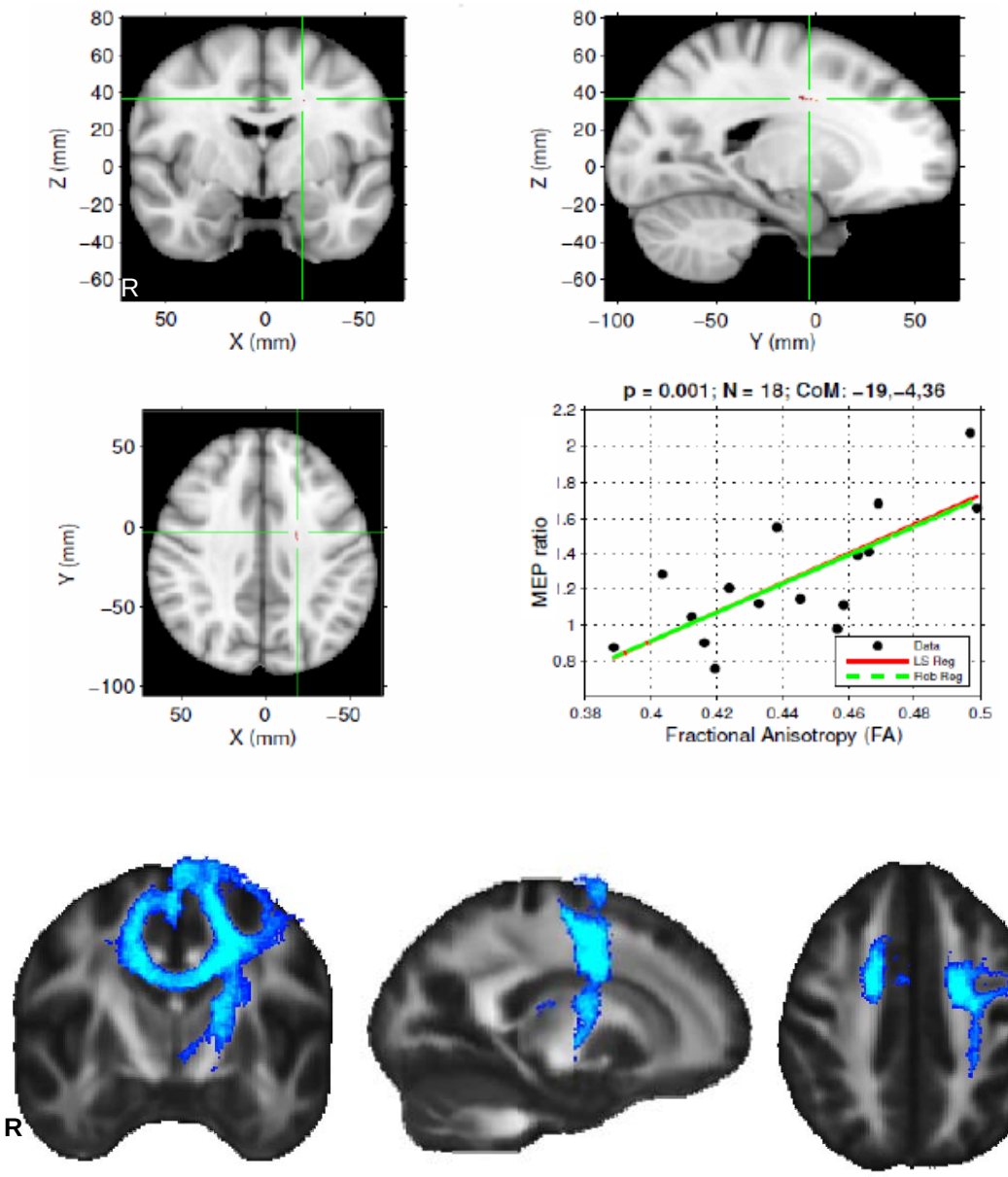


Figure 43: Local correlation between FA and pre-SMA-M1 functional connectivity at 12 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from pre-SMA via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

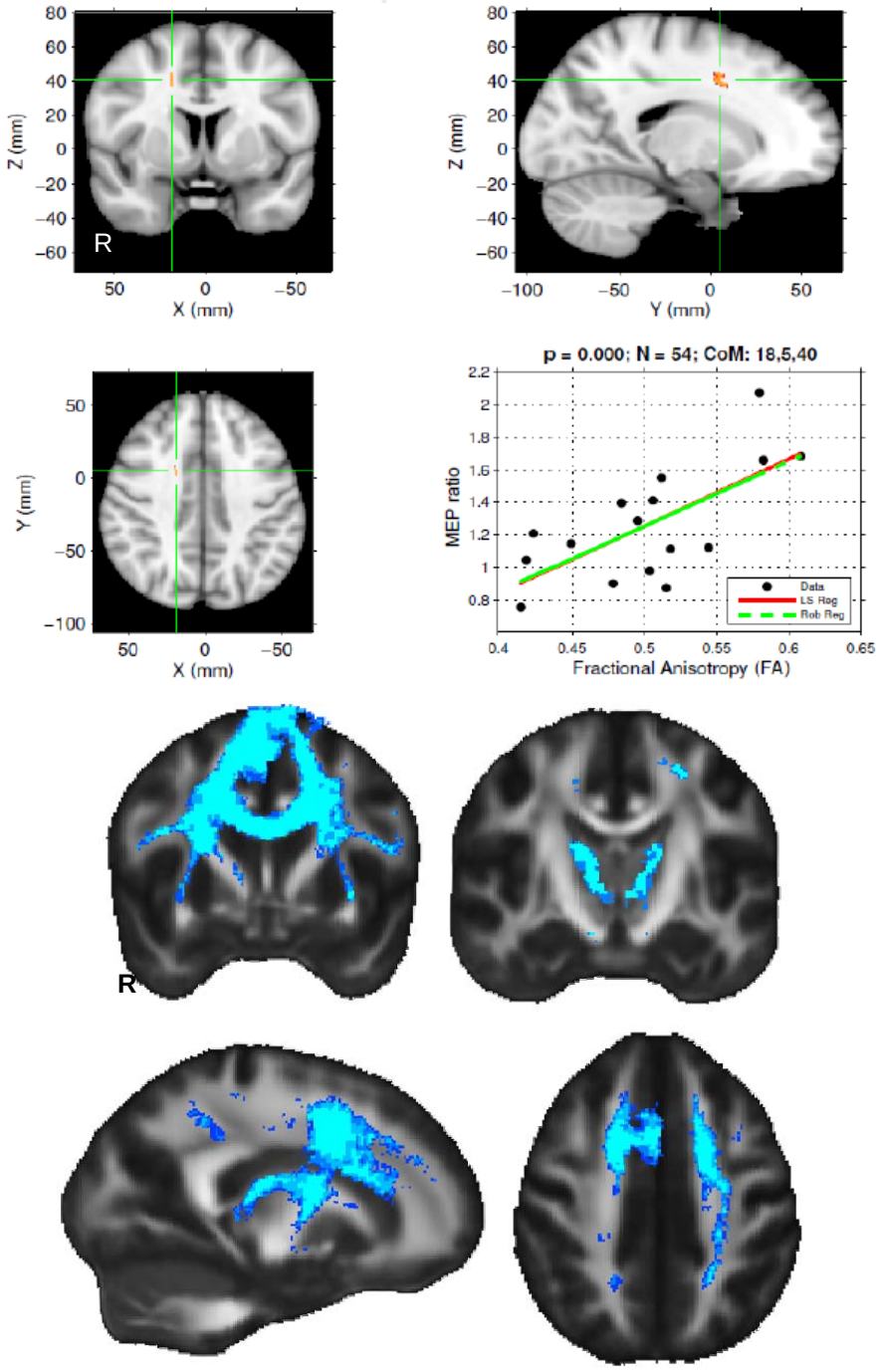


Figure 44: Local correlation between FA and pre-SMA-M1 functional connectivity at 12 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from pre-SMA via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

x	y	z	hemisphere	anatomical region	average p-value	correlation (cluster)	cluster size (voxels)
36	28	-7	right	White matter under rIFC	<0.001	-0.505	10
35	10	18	right	White matter under rIFC / PMv	<0.001	-0.508	11
-14	-27	60	left	SLF I	0.001	-0.720	10

Table 7: Details of clusters showing significant negative correlation between individual TMS functional connectivity effects in the rIFC-M1 experiment with 6 ms IPI and individual FA.

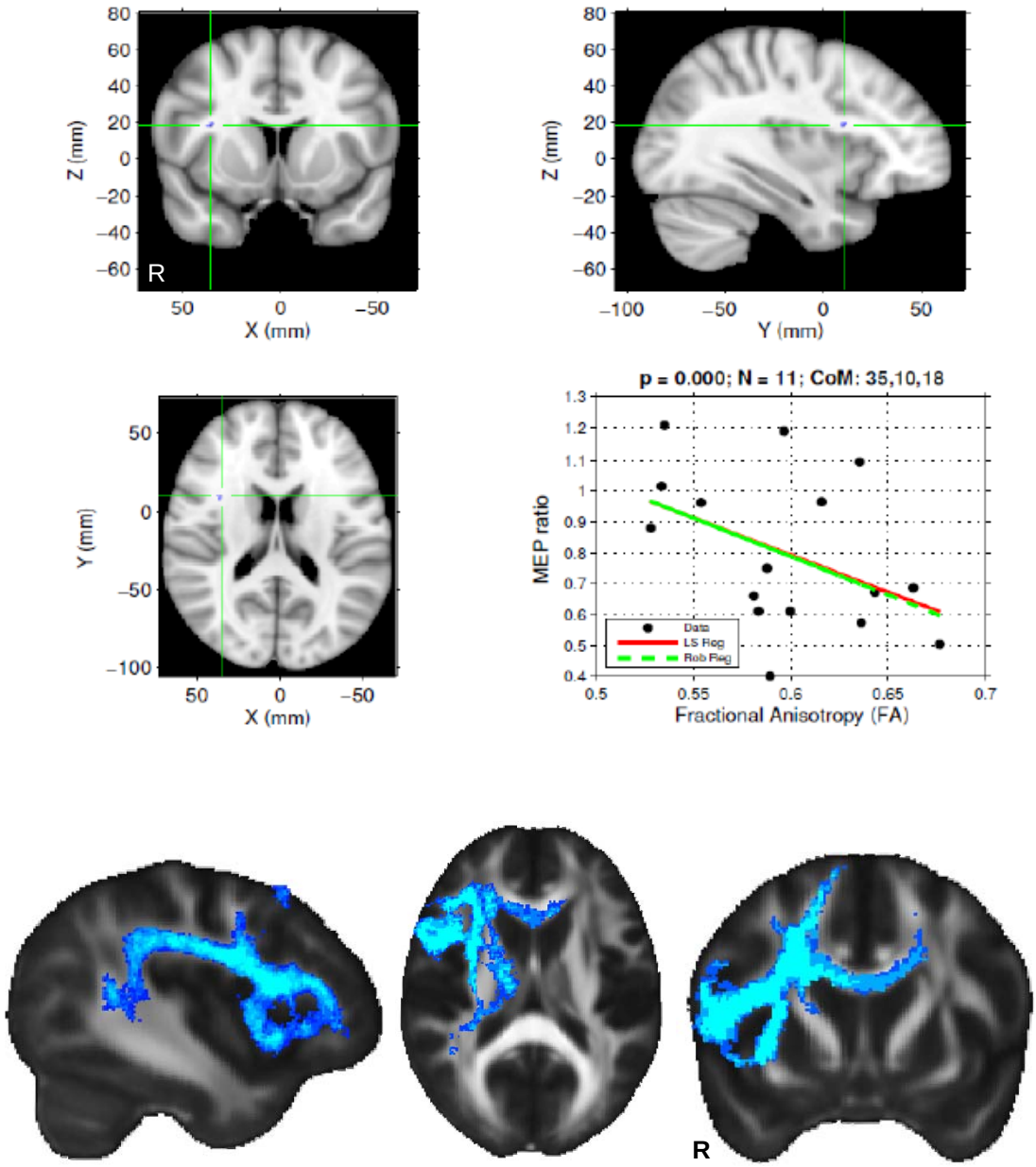


Figure 45: Local correlation between FA and rIFC -M1 functional connectivity at 6 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from rIFC via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

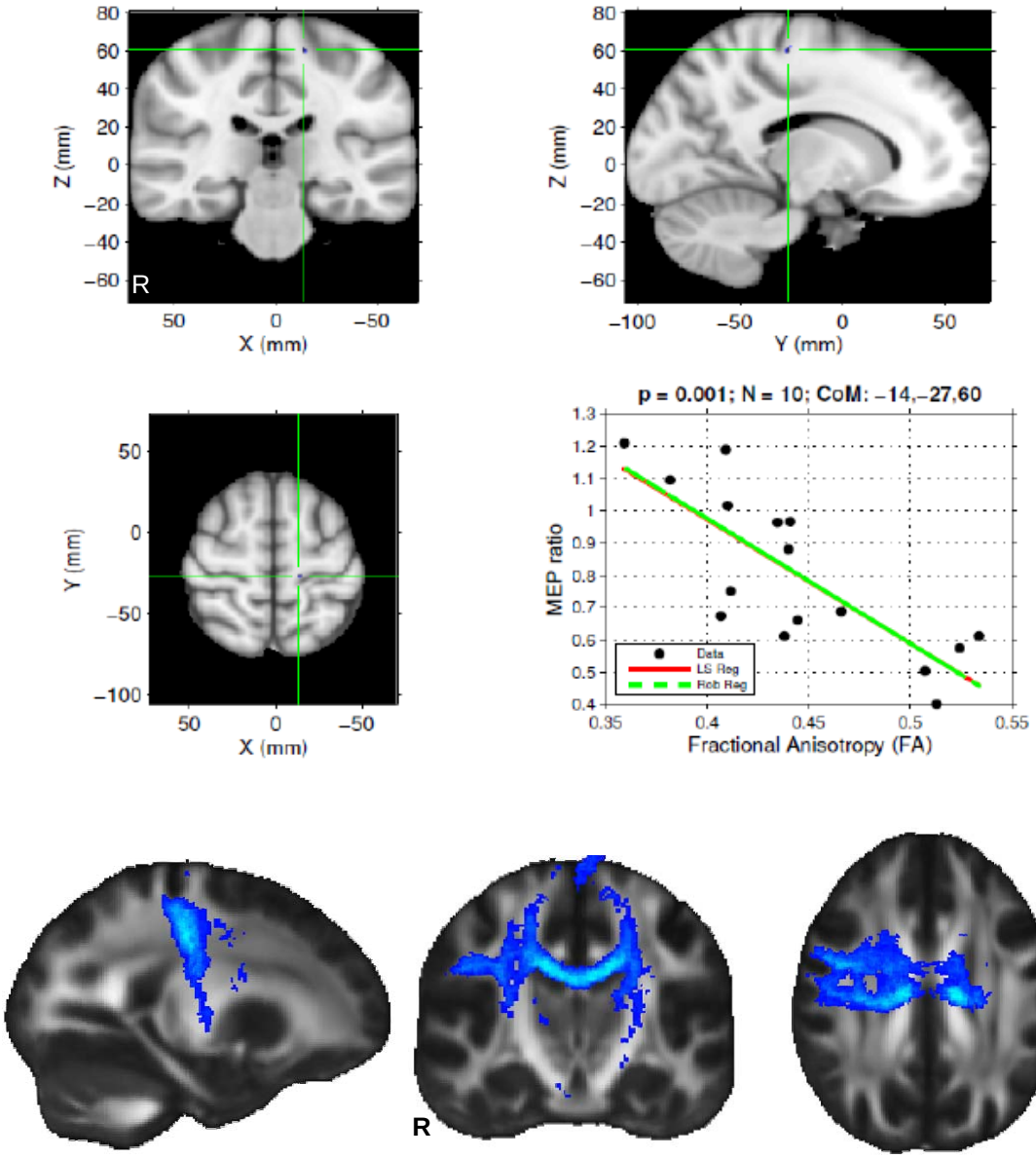


Figure 46: Local correlation between FA and rIFC-M1 functional connectivity at 6 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from rIFC via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

x	y	z	hemisphere	anatomical region	average p-value	correlation (cluster)	cluster size (voxels)
-19	-3	-10	left	White matter in the vicinity of STN	<0.001	-0.588	30
20	-4	-10	right	White matter in the vicinity of STN	0.001	-0.580	14
35	-12	-5	right	Extreme Capsule	0.001	-0.686	19
30	36	0	right	White matter underlying ventro-lateral PFC	<0.001	-0.536	18
-30	35	8	left	White matter underlying ventro-lateral PFC	<0.001	-0.517	13
20	35	27	right	SLF I	<0.001	-0.536	28
-18	33	29	left	SLF I	<0.001	-0.887	32
36	-13	33	right	SLF II	<0.001	-0.872	17
-31	54	34	left	SLF II	0.001	-0.528	11
29	-8	44	right	SLF I, white matter underlying PMd	<0.001	-0.718	25
18	-3	46	right	SLF I, white matter underlying PMd, Pre-SMA	0.001	-0.649	25
-9	29	52	left	White matter underlying pre-SMA	<0.001	-0.620	27
-18	-33	53	left	SLF I, white matter underlying M1	0.001	-0.679	26
21	-20	56	right	SLF I, white matter underlying M1	<0.001	-0.690	14

Table 8: Details of clusters showing significant negative correlation between individual TMS functional connectivity effects in the rIFC-M1 experiment with 12 ms IPI and individual FA.

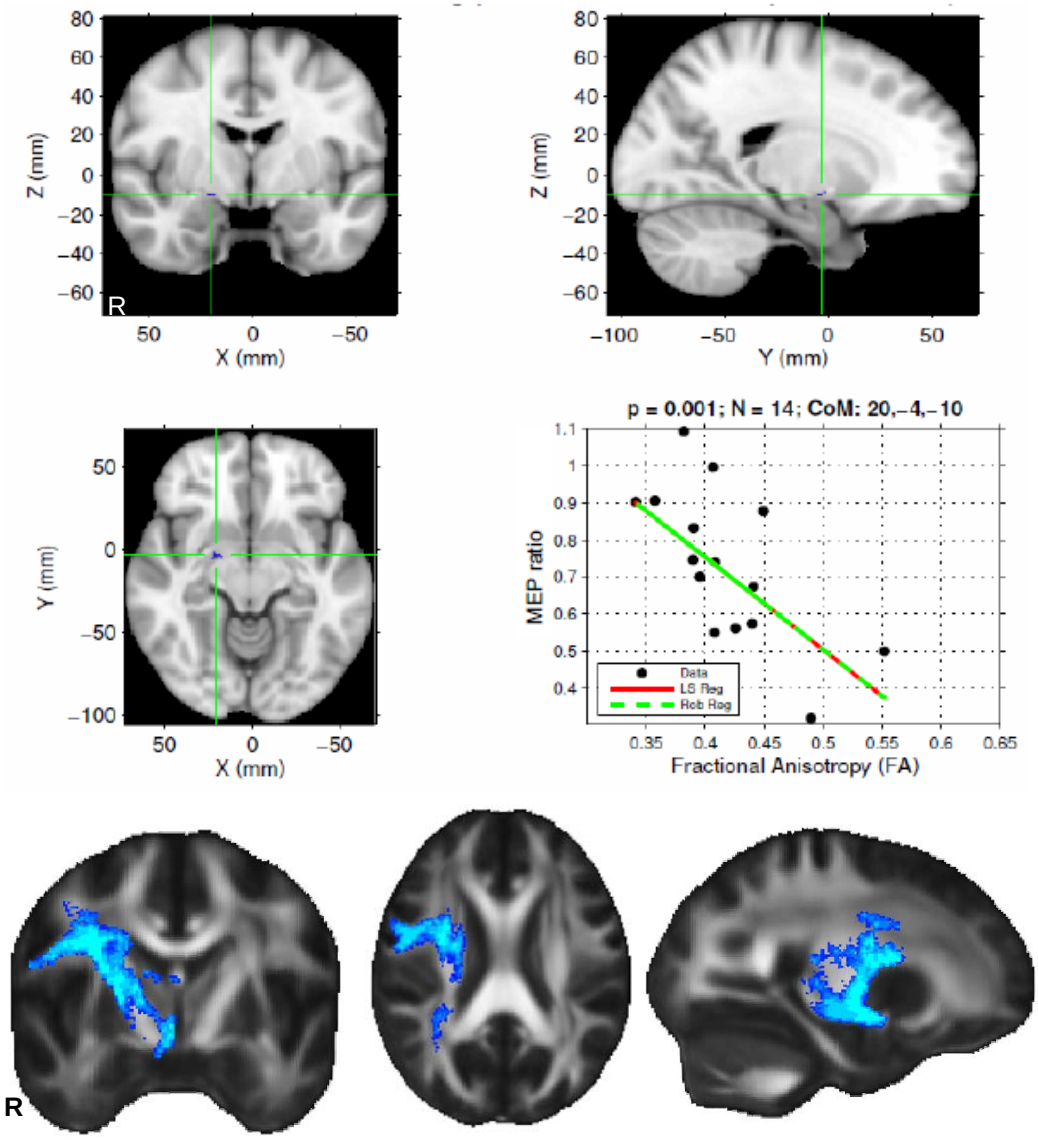


Figure 47: Local correlation between FA and rIFC-M1 functional connectivity at 12 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from rIFC via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

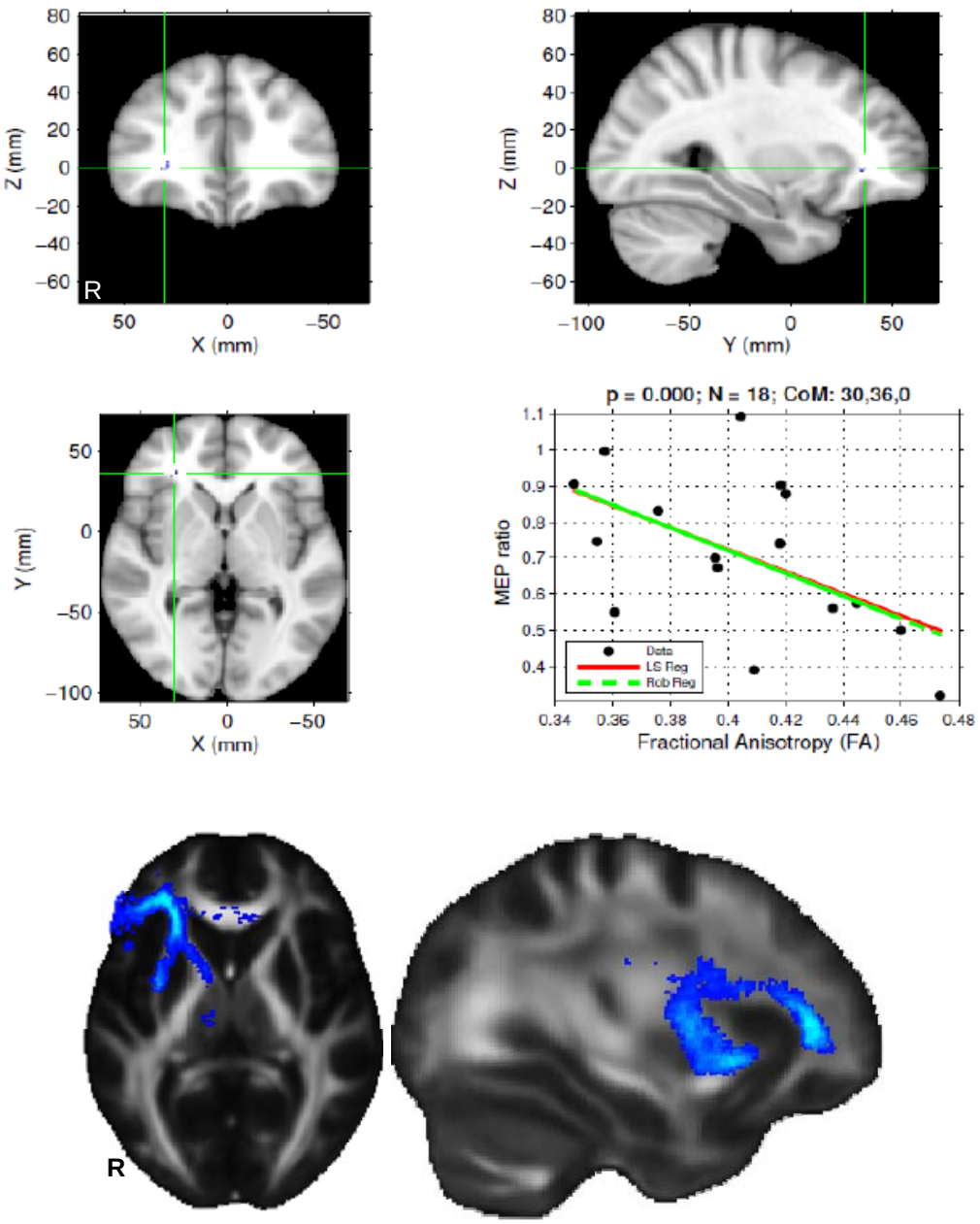


Figure 48: Local correlation between FA and rIFC-M1 functional connectivity at 12 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from rIFC via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

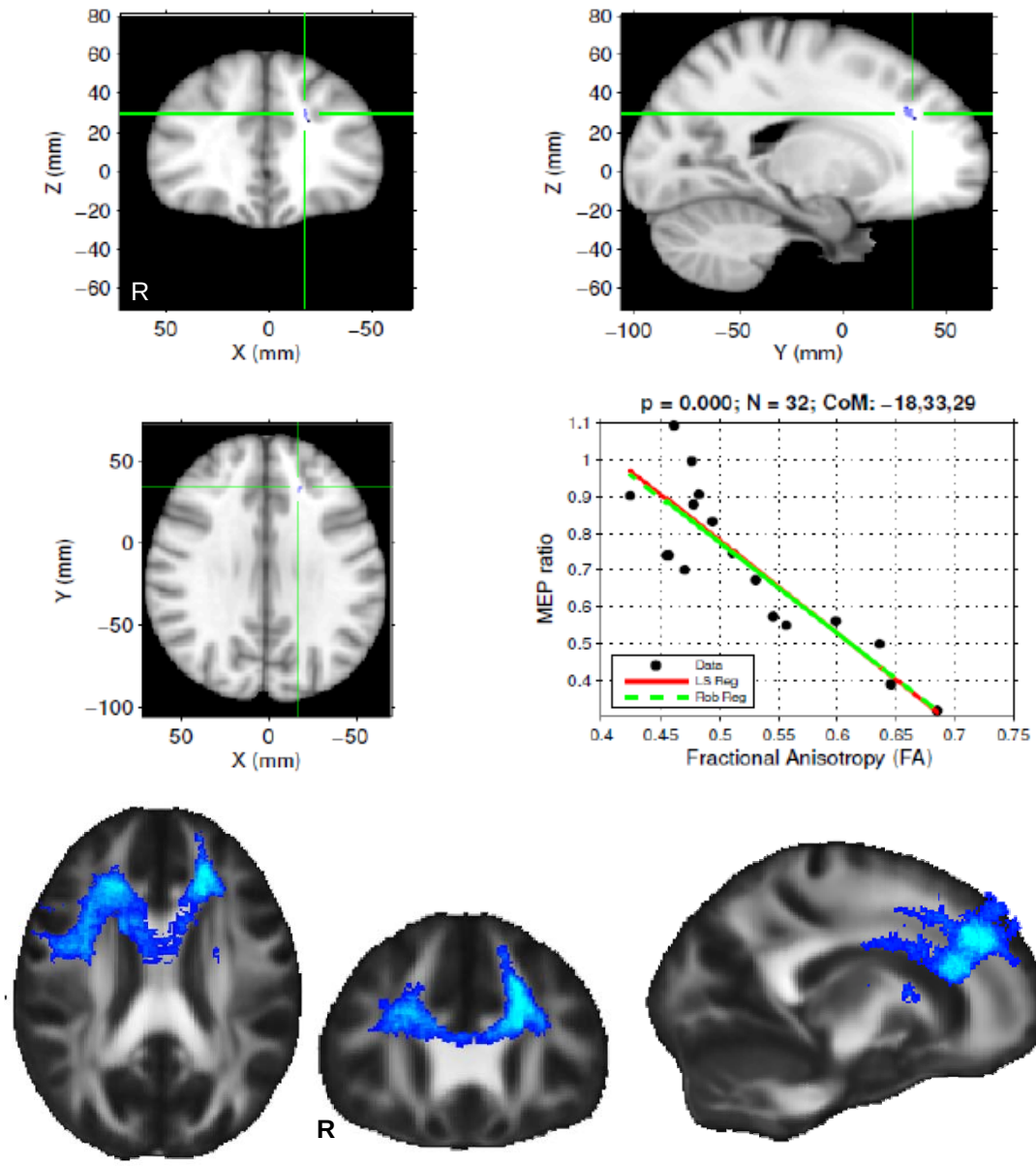


Figure 49: Local correlation between FA and rIFC-M1 functional connectivity at 12 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from rIFC via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

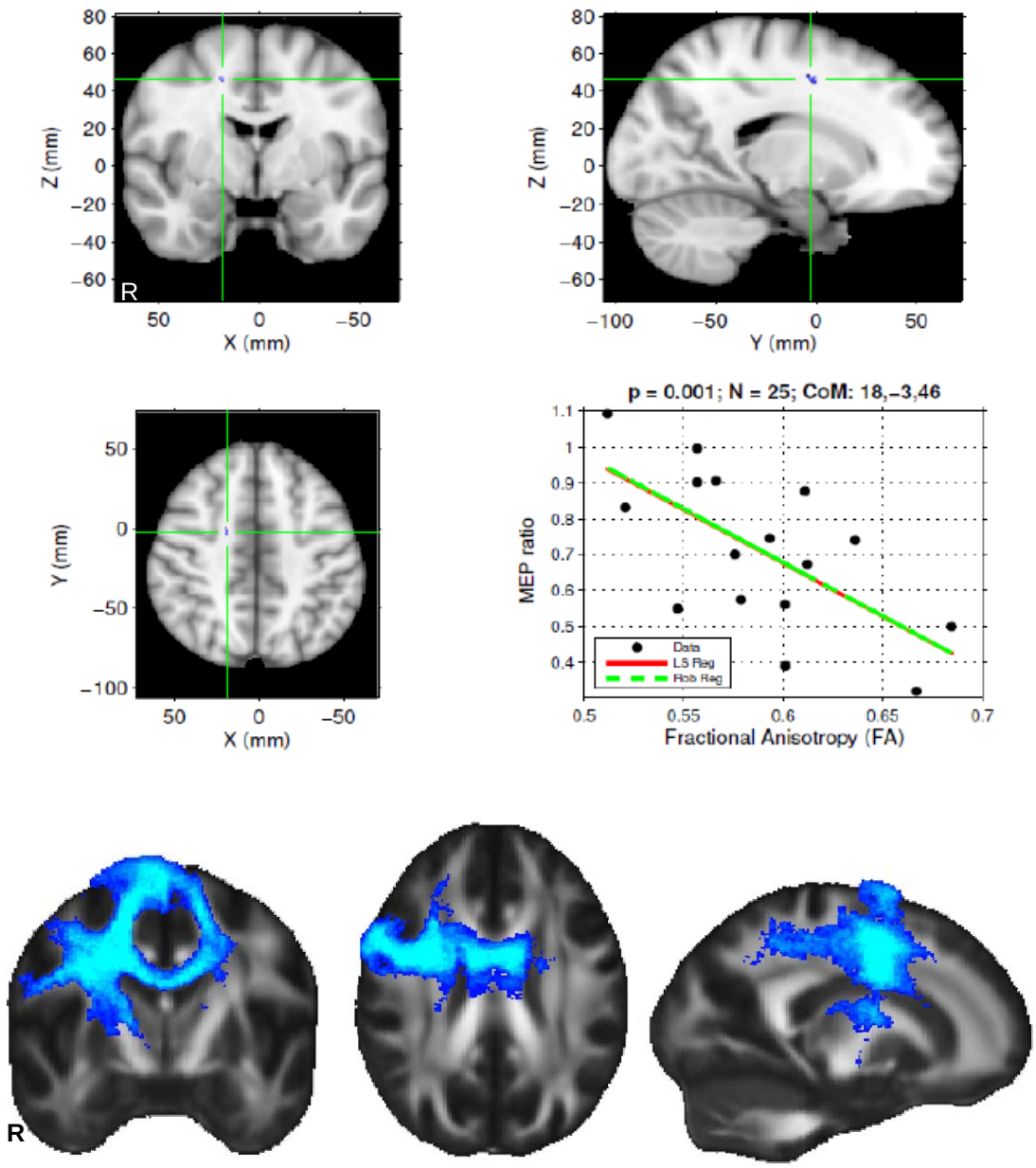


Figure 50: Local correlation between FA and rIFC-M1 functional connectivity at 12 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from rIFC via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

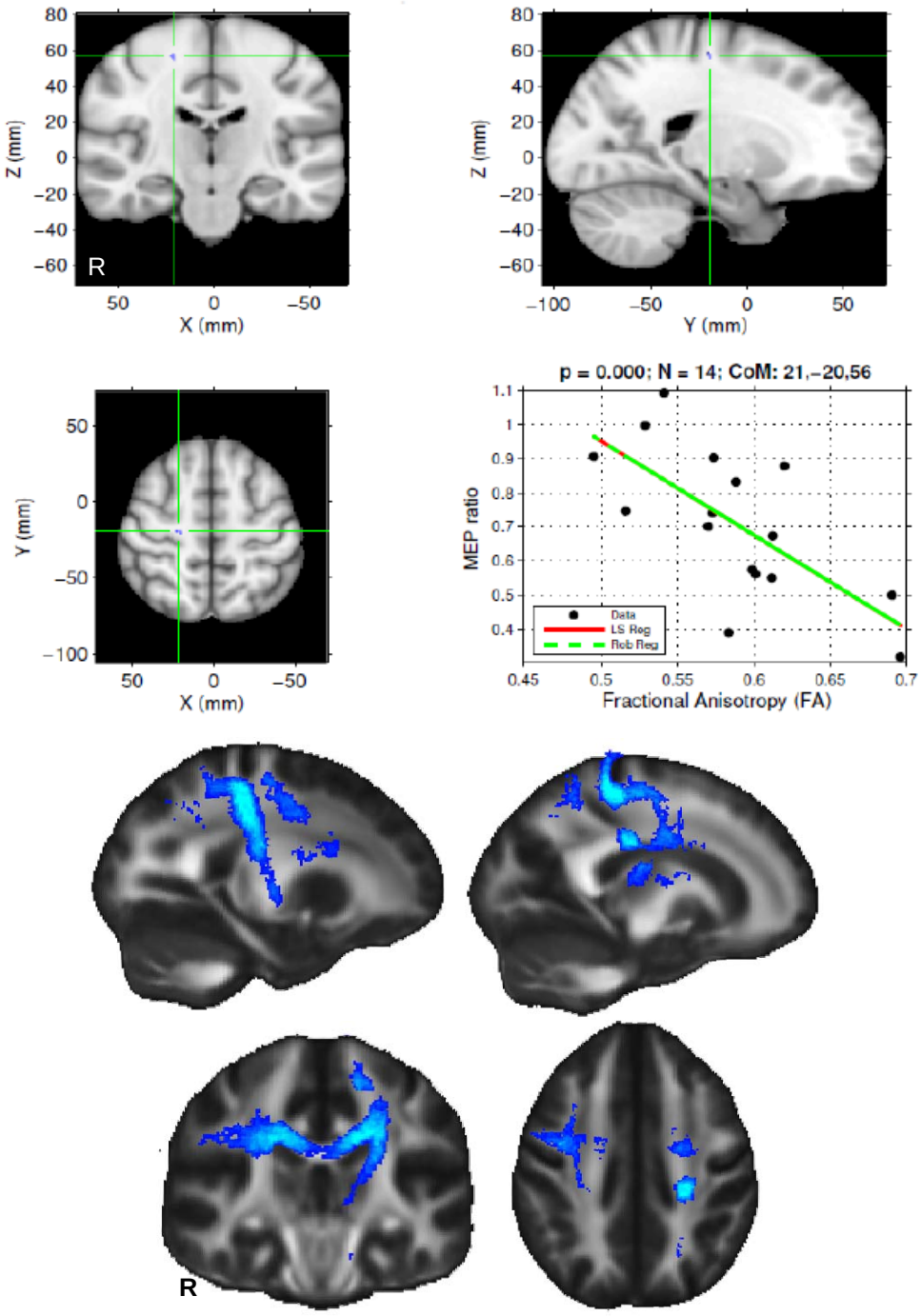


Figure 51: Local correlation between FA and rIFC-M1 functional connectivity at 12 ms IPI (top) plotted on the MNI152 brain template. The scatterplot shows the average FA value within the cluster plotted against the individual MEP ratio. Group probability maps of tracts generated by PDT from rIFC via the correlated FA cluster (bottom) is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

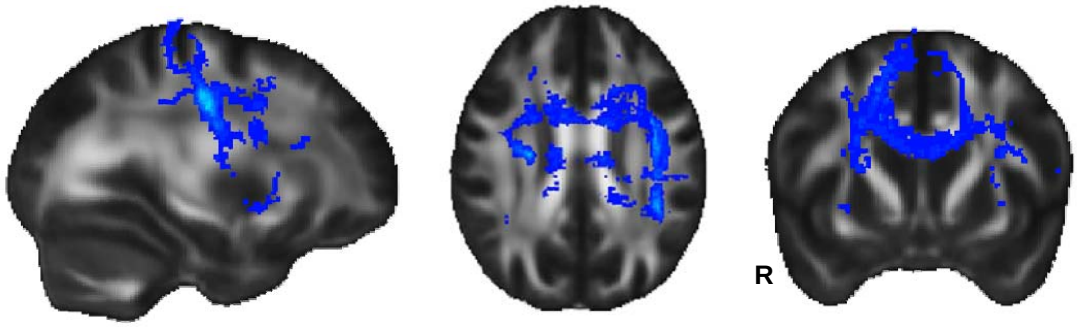


Figure 52: Composite connectivity networks of tracts generated by PDT from the pre-SMA mask via the correlated FA clusters (Figure 40 and Figure 41). The group probability maps of tracts presented above are summed for all tracts derived from clusters significantly correlated with the 6 ms IPI pre-SMA effects to construct this composite connectivity network. This is plotted onto the FMRIB58 FA template. Images are colour-coded according to whether the number of subjects containing that path is high (light blue) or low (dark blue).

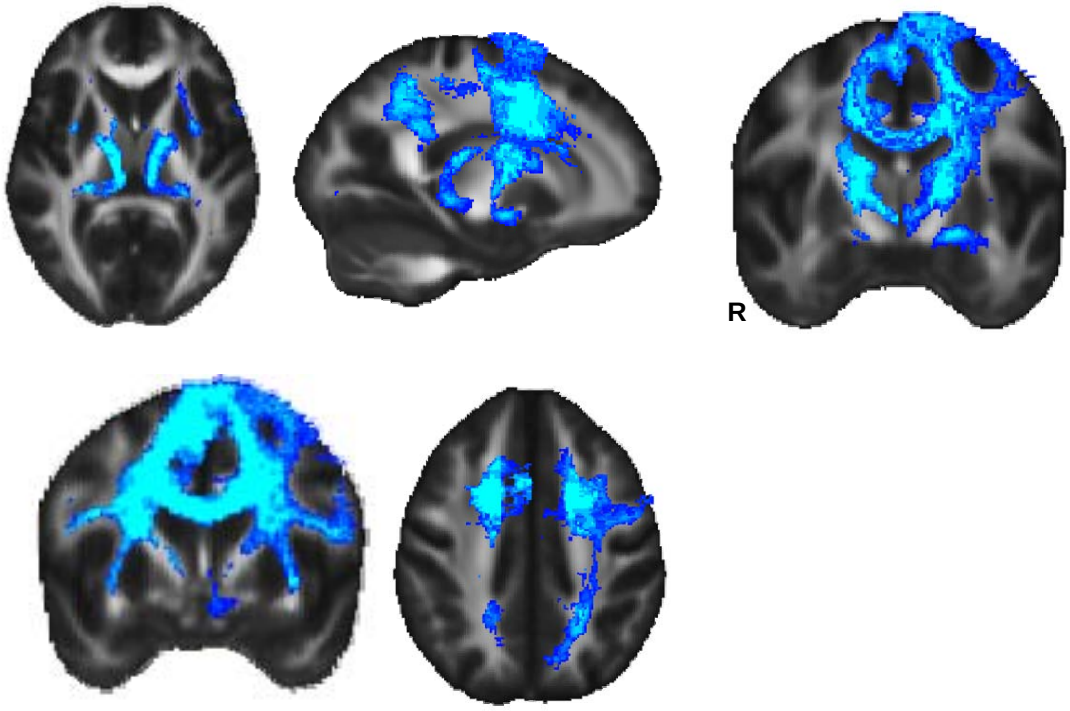


Figure 53: Composite connectivity networks of tracts generated by PDT from pre-SMA via the correlated FA clusters (Figure 42, Figure 43 and Figure 44). Group probability maps are summed for all tracts derived from clusters significantly correlated with the 12 ms IPI pre-SMA effects to construct this composite connectivity network plotted onto the FMRIB58 FA template.

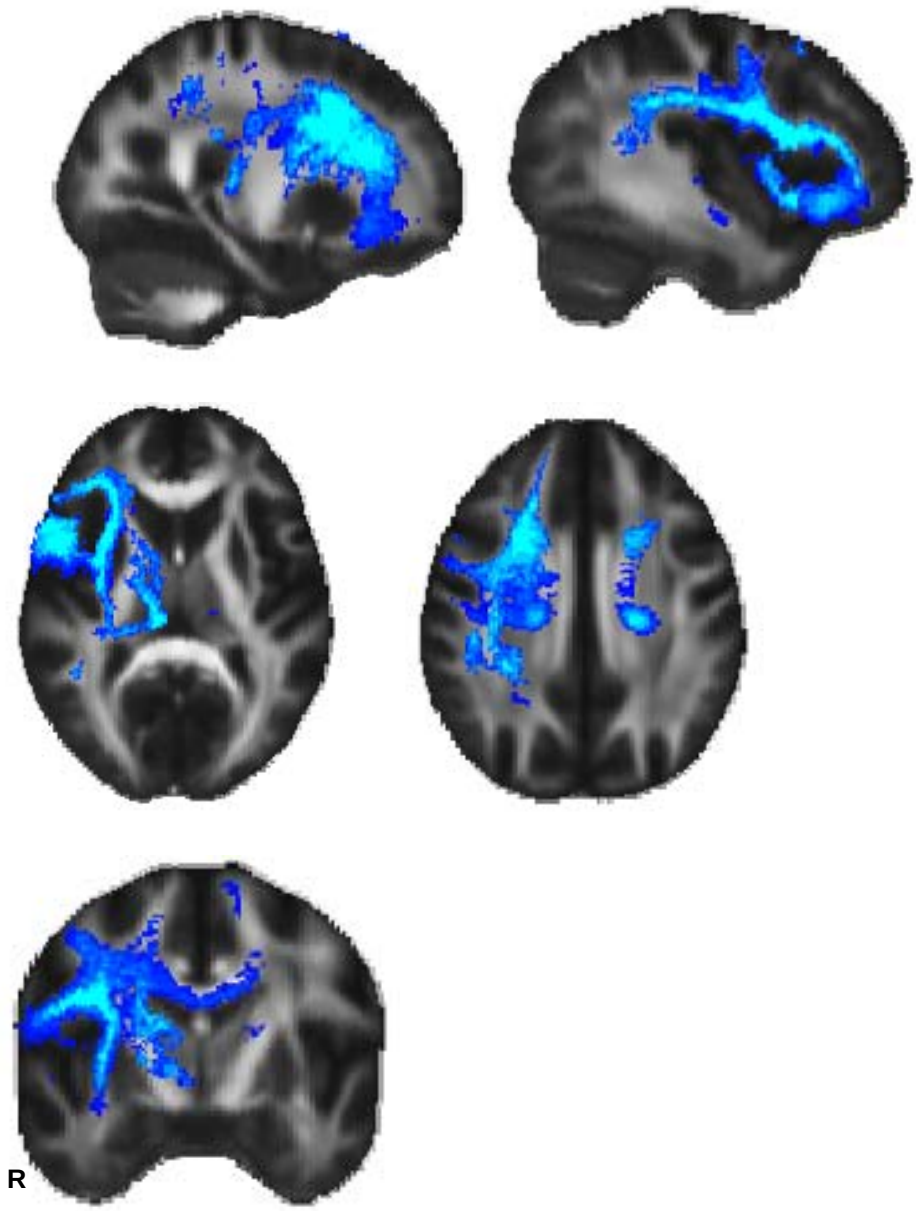


Figure 54: Composite connectivity networks of tracts generated by PDT from the rIFC mask via the correlated FA clusters (Figure 45 and Figure 46). Group probability maps are summed for all tracts derived from clusters significantly correlated with the 6 ms IPI rIFC effects to construct this composite connectivity network plotted onto the FMRIB58 FA template.

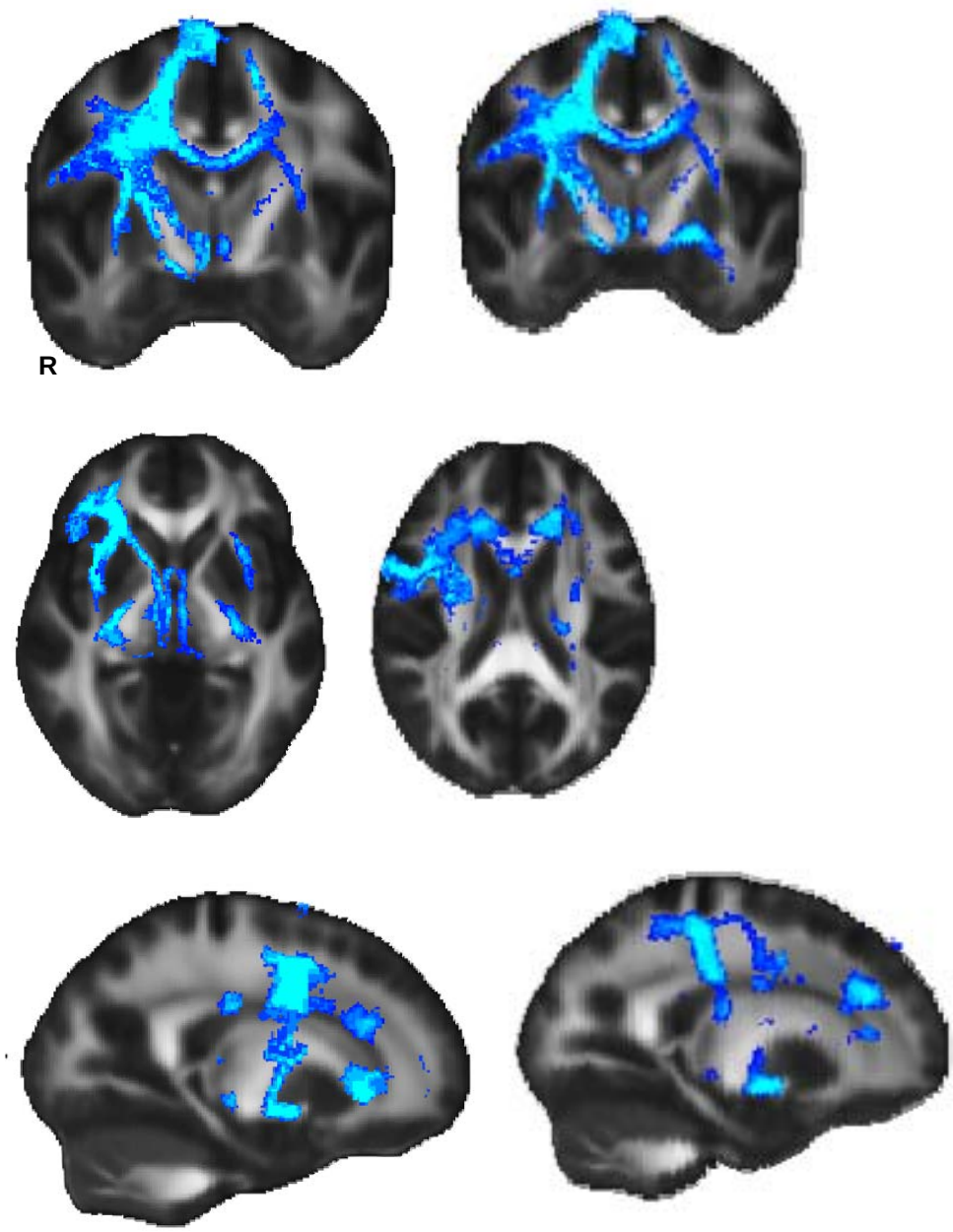


Figure 55: Composite connectivity networks of tracts generated by PDT from the rIFC mask via the correlated FA clusters (Figure 47, Figure 48, Figure 49, Figure 50 and Figure 51). Group probability maps are summed for all tracts derived from clusters significantly correlated with the 12 ms IPI rIFC effects to construct this composite connectivity network plotted onto the FMRIB58 FA template.

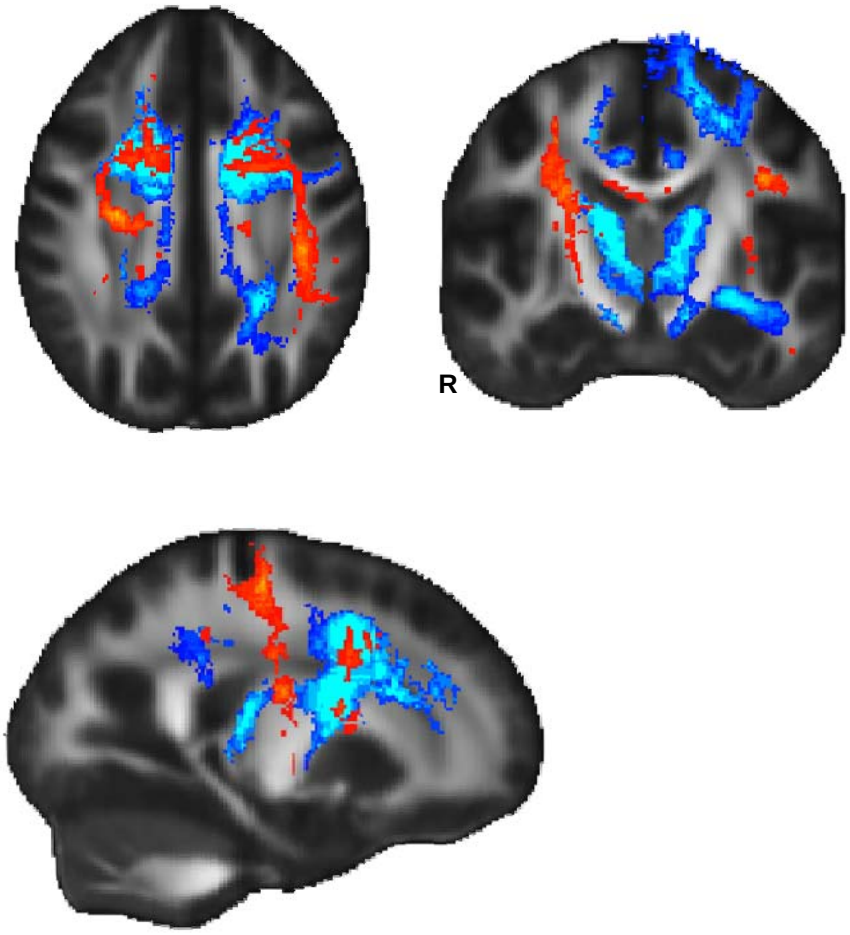


Figure 56: Comparison of two composite connectivity networks derived from the pre-SMA experiment at different IPIs (Figure 52 = red, and Figure 53 = blue). Composite connectivity networks of summed tracts generated by PDT from the FA clusters correlated with the TMS effects in the pre-SMA experiment at 6 ms (red) and 12 ms (blue) IPI.

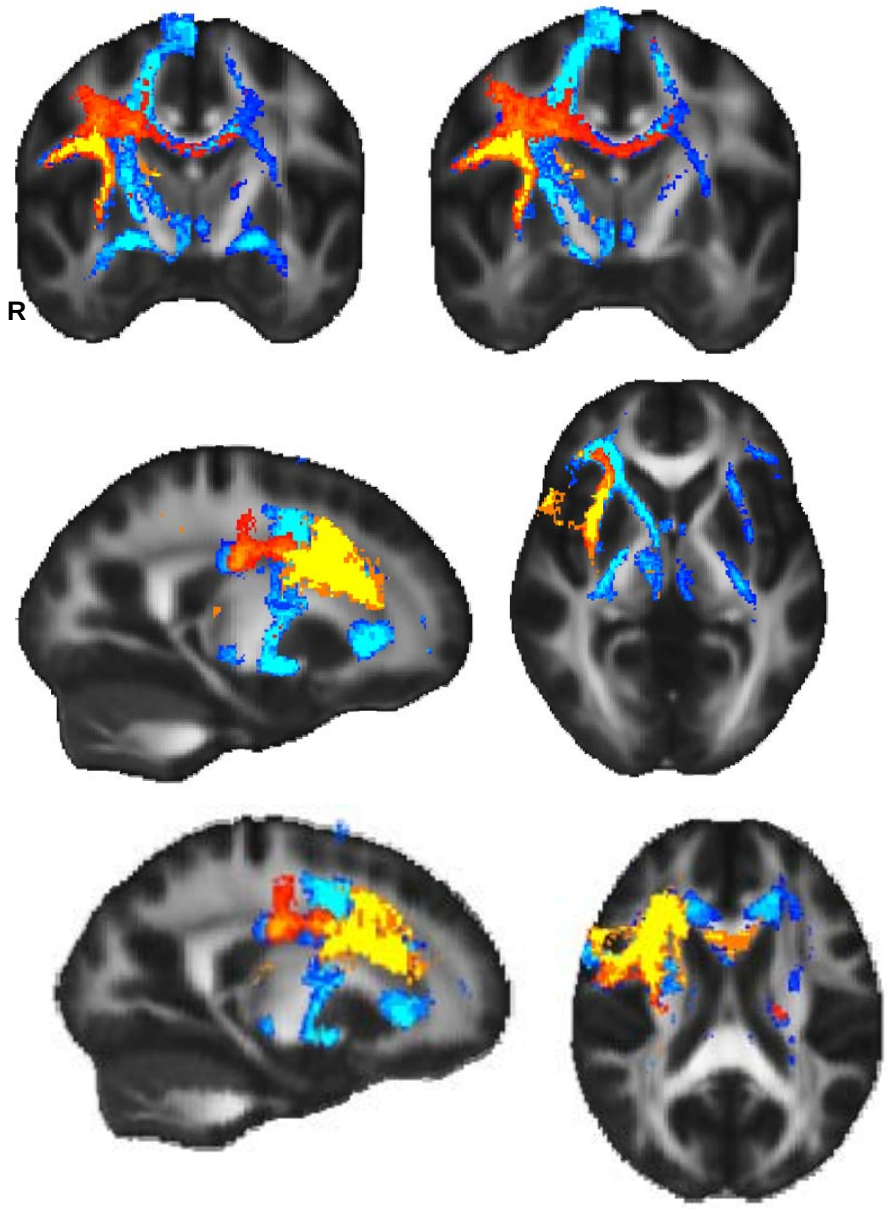


Figure 57: Comparison of two composite connectivity networks derived from the rIFC experiment at different IPIs (Figure 54 = orange, and Figure 55 = blue). Composite connectivity networks of summed tracts generated by PDT from the FA clusters correlated with the TMS effects in the rIFC experiment at 6 ms (orange) and 12 ms (blue) IPI.

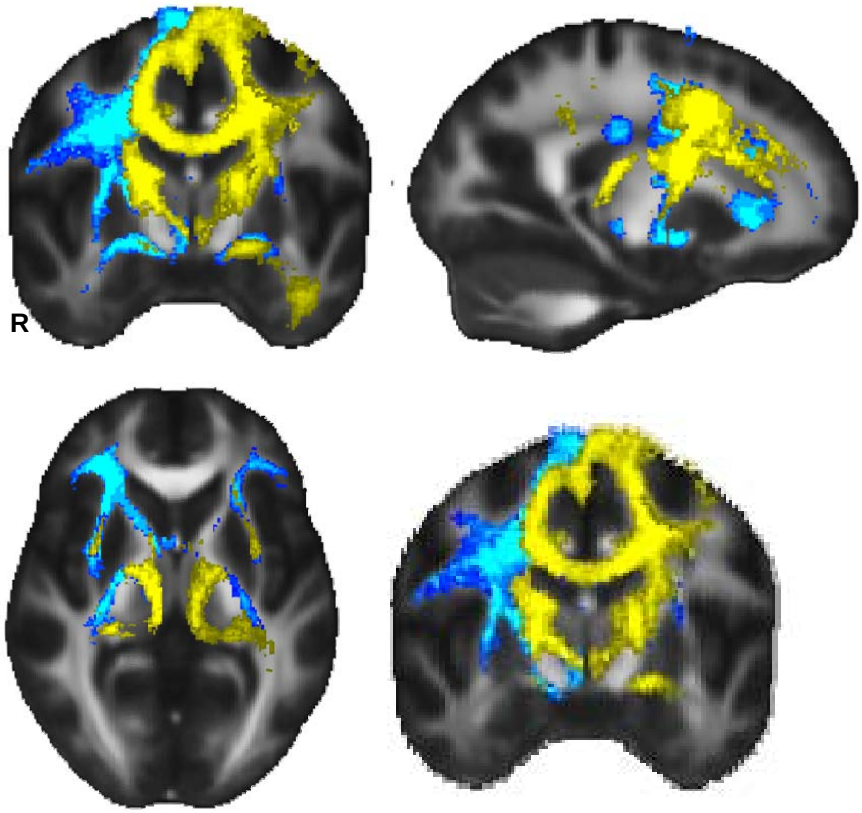


Figure 58: Comparison of two composite connectivity networks derived from the pre-SMA (yellow) and the rIFC (blue) experiment at the same IPI of 12 ms (Figure 53 = yellow, and Figure 55 = blue). Composite connectivity networks of summed tracts generated by PDT from the FA clusters correlated with the TMS effects in the two experiments at 12 ms IPI.

Discussion

We investigated different pathways of functional connectivity during action reprogramming and tried to relate these to anatomical white matter tracts. Action reprogramming and response inhibition is necessary if external or internal stimuli indicate that an expected and prepared action is no longer appropriate and therefore has to be inhibited and an alternative response needs to be selected and initiated. Pre-SMA and rIFC have been suggested to play an important role in these situations. However it is still a matter of debate (1) which distinct influence pre-SMA and rIFC exert on the motor system during action reprogramming, (2) when exactly in the time-course of action reprogramming these areas influence M1 and (3) by which anatomical routes pre-SMA and rIFC influence M1.

We tried to address some of these questions using a paired-pulse TMS paradigm and a very simple decision making task in combination with DTI. The task required the participants to inhibit a movement they had already prepared on the basis of their previous experience and their expectations about future events and to select an alternative response. We already knew the exact time points of maximum pre-SMA-M1 interaction and rIFC interaction, respectively. This knowledge was derived from a study conducted by (Mars et al., 2009) and the previously reported rIFC-M1 switch experiment (see Chapter 2.1.2). These experiments investigated the time-course of functional connectivity between pre-SMA and M1 (Mars et al., 2009) and between rIFC and M1 after the onset of a cue telling the subjects to reprogramme a prepared response. We applied single-pulse and paired-pulse TMS only at these time points of maximum pre-SMA-M1 and rIFC-M1 influence but with variable IPIs. Therefore we

could study the impact of a conditioning pulse over pre-SMA or rIFC on the MEP elicited by a M1 test pulse depending on the interval between conditioning and test pulses. Different IPIs allowed us to study functional interactions mediated by different anatomical tracts.

We replicated the effects reported earlier and observed similar effects of connectivity at longer IPIs. We found some paired-pulse TMS effects at certain IPIs to be correlated with effects at other IPIs. This could suggest that highly correlated paired-pulse TMS MEP effects at different IPIs might be mediated via similar pathways. Therefore pre-SMA or rIFC could exert its influence on the motor system through more than one pathway. The influences exerted at different IPIs appeared relatively similar for each area. As in a previous experiment by (Mars et al., 2009) we found that pre-SMA facilitated M1 excitability at 6, 9 and 12 ms IPI. Additionally we showed that rIFC inhibits M1 at 6, 9 and 12 ms IPI. It might be surprising that an area exerts similar influences on the motor system via different neuronal pathways. It seems likely that different functional pre-SMA-M1 pathways convey different information during the complex process of action reprogramming. These different influences exerted by pre-SMA (or rIFC) on M1 via different white matter tracts and intermediate grey matter areas might however be difficult to disentangle with such a paired-pulse TMS paradigm.

We related cross-subject variability in paired-pulse TMS MEP effects at different IPIs to cross-subject variability in white matter micro-architecture. We showed correlations between physiological measures of functional connectivity during action reprogramming and the micro-structural integrity of specific white matter networks presumed to mediate these physiological effects. Such structural-physiological relationships might explain some of the reported relations between brain structure and

behaviour (Gaser and Schlaug, 2003; Bengtsson et al., 2005; Johansen-Berg and Behrens, 2009). Structural connectivity could determine functional connectivity and functional connectivity could then influence behaviour. We used probability diffusion tractography to elucidate white matter tracts in which local regions of FA correlation were found. The correlated clusters and the tracts derived from these clusters were anatomically specific to certain IPIs. The clusters correlated with the pre-SMA-M1 6 ms IPI TMS effects pointed to tracts in the dorsal white matter, especially in the first branch of the SLF, whereas the clusters correlated with the pre-SMA-M1 functional connectivity at 12 ms IPI seemed to indicate pre-SMA-M1 pathways via the basal ganglia. A similar anatomical specificity of the location of the correlated clusters was apparent for the rIFC-M1 TMS effects. This suggests that a combined paired-pulse TMS-DTI approach could be used to elucidate anatomical pathways of functional connectivity. Moreover we found similar white matter clusters being correlated with the Pre-SMA-M1 and the rIFC-M1 paired-pulse TMS MEP effects at 12 ms IPI. This might suggest a functional as well as an anatomical network of action reprogramming and response inhibition. It has been suggested that experience influences brain anatomy and white matter architecture (Bengtsson et al., 2005; Johansen-Berg and Behrens, 2009). In many real life situations not only movement inhibition but a complex process of response inhibition and action reprogramming is necessary to act appropriately. Thus environmental demands could shape the structure of white matter connectivity networks. Individual patterns of several white matter tracts could determine functional connectivity of this network and thus individual cognitive function and behaviour.

2.3. Pre-SMA activity influences rIFC-M1 functional connectivity

Introduction

The neural basis of response inhibition and action reprogramming has been under debate and subject of extensive research in the recent years. The rIFC and the pre-SMA have been suggested by numerous studies as the cortical areas mediating response inhibition. However a functional dissociation between these two areas during response inhibition and action reprogramming has proven difficult to obtain.

Studies in patients showed that both lesions to pre-SMA and rIFC impair stopping performance (Aron et al., 2003a; Nachev et al., 2007). Neuroimaging studies have shown rIFC to be more active during response inhibition than during normal action execution (Garavan et al., 1999; Konishi et al., 1999; Aron and Poldrack, 2006; Li et al., 2006; Aron et al., 2007a; Mars et al., 2007b; Chikazoe et al., 2009). It was theorised that the rIFC as the cortical site of inhibitory control projects to the STN in a hyperdirect pathway (Aron and Poldrack, 2006; Aron et al., 2007a). Moreover rIFC activity has been found to be positively correlated with STN activity during response inhibition and negatively correlated with SSRT, whereas pre-SMA activity was neither correlated with STN activity nor with SSRTs (Aron et al., 2007a). However other imaging studies have also isolated the pre-SMA as a key locus of response inhibition (Nachev et al., 2005; Li et al., 2006; Mars et al., 2007b) and have shown that pre-SMA and not rIFC activity was correlated with SSRT (Li et al., 2006) and that pre-SMA is more likely to directly project to the STN than rIFC (Duann et al., 2009). The causal contribution of both rIFC and pre-SMA to response inhibition, task

switching and action reprogramming has been established by previous studies using neurophysiological recordings and electrical microstimulation (Sasaki et al., 1989; Stuphorn and Schall, 2006; Isoda and Hikosaka, 2007) as well as TMS (Rushworth et al., 2002b; Chambers et al., 2006; Mars et al., 2009).

The notion of rIFC being the centre of inhibitory motor control has been questioned by a recent study investigating functional connectivity based on fMRI data (Duann et al., 2009). According to this view rIFC is part of a ventral attention system, which is activated in response to the detection of a salient target stimulus, particularly when the stimulus is behaviourally relevant. This study argued that in a go/no-go task rIFC responds specifically to no-go trials, because they are salient and behaviourally relevant and presented less frequently than go-trials. Hence rIFC only serves to detect no-go signals and subsequently "energises" pre-SMA, which then exerts inhibitory motor control via STN (Duann et al., 2009). Regrettably (Duann et al., 2009) did not try to distinguish BOLD activity related to saliency and BOLD activity associated with movement inhibition. A similar but completely converse theory on pre-SMA-"posterior lateral PFC" interaction has been suggested for rule-guided decision making and action selection by (Kouneiher et al., 2009) based on fMRI data and functional connectivity analysis of this fMRI data. According to this notion pre-SMA detects motivationally salient stimuli and subsequently energises "posterior lateral PFC" (the MNI coordinates of this area are almost exactly the same as our rIFC coordinates, see Figure 18 and Figure 19) to increase executive control.

A recent fMRI study by (Chikazoe et al., 2009) tried to clarify whether activity in rIFC during go/no-go tasks is associated with response inhibition or processing of infrequent stimuli. They therefore introduced infrequent go-trials during a go/no-go paradigm. Interestingly both seemed to be the case: They showed greater activity in

posterior inferior frontal gyrus (pIFG) during no-go compared to infrequent go trials and greater activity in the inferior frontal junction area (IFJ) during infrequent go-trials compared to frequent go-trials. This suggests that pIFG is specifically related to response suppression whereas IFJ responds to salient stimuli. Both areas were very close (approximately only 2 cm apart) but showed different activation patterns. In a normal go/no-go task both areas would have just formed a large single cluster (Chikazoe et al., 2009). This pIFG region is very close to what has been termed "PMv" supporting the notion that PMv as the posterior part of IFC might be the centre of a network exerting inhibitory control on the motor system.

The following experiment attempted to investigate whether pre-SMA activity causally influences functional connectivity between rIFC and M1.The aim of this experiment was to establish whether 15 minutes of 1 Hz rTMS over the Pre-SMA would affect rIFC-M1 functional connectivity during action reprogramming and action execution. In a previous experiment we observed an inhibitory influence of rIFC on M1 excitability during "response switching" (i.e. action reprogramming). Additionally we showed a facilitatory influence of rIFC on M1 excitability during normal action execution trials. Both influences occurred 175 ms after centre cue colour onset in action reprogramming and action execution trials, respectively. An experiment conducted by (Mars et al., 2009) showed that Pre-SMA exerts an influence on M1 excitability during action reprogramming at 125ms after cue onset. Thus pre-SMA-M1 functional connectivity during action reprogramming occurs earlier in the time course of action reprogramming than the rIFC-M1 functional connectivity, suggesting that pre-SMA might be necessary to establish rIFC-M1 connectivity. Changes in activity of pre-SMA could therefore affect rIFC-M1 connectivity.

Methods

Participants. Eight healthy volunteers (age range between 24 and 41 years, mean age = 28.1 +/- 1.93SD, three females) with no personal or familial history of neurological or psychiatric disease participated in a combined paired-pulse TMS – rTMS experiment. The aim of this experiment was to establish whether 15 minutes of 1 Hz rTMS over the pre-SMA would affect rIFC-M1 functional connectivity during action reprogramming and action execution. Pre-SMA-M1 functional connectivity during action reprogramming occurs earlier in the time course of action reprogramming than the rIFC-M1 functional connectivity suggesting that pre-SMA might be necessary to establish rIFC-M1 connectivity. To infer whether a mild and transient disruption of pre-SMA activity by 1 Hz rTMS would change rIFC-M1 connectivity we (1) used paired-pulse TMS to investigate baseline functional connectivity between rIFC and M1, (2) then applied 15 minutes of 1Hz rTMS (900 pulses) over pre-SMA and (3) immediately afterwards examined rIFC-M1 functional connectivity again using the same paired-pulse TMS protocol (see Figure 59). The experiment was approved by the Oxfordshire Research Ethics Committee and conducted in accordance with the declaration of Helsinki. The same behavioural task as in the pervious two experiments was used in four experimental blocks, two experimental blocks before 1 Hz rTMS application to pre-SMA and two blocks immediately after rTMS. All participants were right-handed and gave written informed consent. They were all screened for adverse reactions to TMS and risk factors by means of a safety questionnaire.

Experimental setup. Participants were seated in a darkened room and wore a tight-fitting EEG cap, on which TMS sites were marked and earplugs to protect against TMS noise. A chin rest was used to minimise head movements.

Behavioural Task. The behavioural task was exactly the same as in the previous three experiments and as in the experiment conducted by (Mars et al., 2009)(see Figure 59). It was modelled on the paradigm developed by (Isoda and Hikosaka, 2007) and required participants to respond with the left or right index finger in response to visual stimuli presented on a computer screen. Custom software written in Presentation (version 0.53) controlled the experiment. As in the previous three experiments participants were familiarised with the task before the actual experiment. The experiment consisted of 4 experimental blocks and 15 minutes of 1 Hz rTMS. Two experimental blocks preceded the 15 minutes period of rTMS. The other two experimental blocks followed the rTMS immediately. Each experimental block contained 30 switch and 150 stay trials. Reaction times were recorded.

Paired-pulse TMS. As in the previous experiment we aimed to investigate the influence exerted by rIFC on M1 during action reprogramming and action execution. We therefore decided to use a paired-pulse TMS paradigm with one TMS test coil over the M1 and another TMS coil over rIFC. Pulses were delivered on 30 out of 180 trials per block. As in the previous experiment there were two types of TMS trials. On *"single-pulse" trials*, a single TMS test pulse was delivered over the left M1-representation of the right FDI. Intensity of this pulse was such that an MEP of 1 – 1.5 mV was evoked in the relaxed, contralateral FDI. This intensity was 39.5% (SEM +/- 2.13) of the maximum stimulator output. On *"paired-pulse" trials*, the M1 test pulse was preceded by a conditioning pulse over the rIFC. The intensity of the conditioning pulse was set at 110% RMT as in the previous study and as in (Buch et al.,

submitted). Average RMT was 35.9 (+/- 1.83 SEM) of maximum stimulator output. The inter-pulse interval was 8 ms. We applied pulses only at 175 ms after centre cue colour presentation as this was the time-point the rIFC-M1 switch experiment had found the strongest rIFC-M1 effects. We hypothesised that the preceding rIFC conditioning pulse would (1) again inhibit M1 excitability in action reprogramming trials, (2) again facilitate M1 excitability in action execution trials and (3) that these influences of rIFC on M1 during action reprogramming and execution might change after transient and mild disruption of the Pre-SMA. Pulses were delivered through two 55 mm diameter figure-of-eight coils directly connected to two high-power Magstim 200 MonoPulse machines (The Magstim Company ®). TMS coil applying test pulses to the left M1 was placed tangentially to the scalp, inducing posterior-to-anterior current flow perpendicular to the central sulcus. TMS coil applying conditioning pulses to the rIFC was placed tangential to the scalp with the handle of the coil pointing forwards and upwards (see Figure 60). MRI scans were acquired for every subject. MRI-aligned frameless stereotaxic neuronavigation system (Brainsight, Rogue Research Inc.®) was used to determine rIFC location. Areas of TMS (left M1 and rIFC) were evaluated with respect to the Montreal Neurological Institute (MNI) standard space using subject's individual MRI scans. The mean MNI location across subjects for rIFC conditioning coil location was x= 59.2 (+/-0.93), y= 15 (+/- 0.5) and z= 26.3 (+/-2.29) (see figure). The mean M1 test coil MNI location was x= -45.6 (+/-2.2), y= -13.1 (+/-2.88) and z= 60.9 (+/-1.61) (see Figure 61). TMS (single or paired) was delivered on switch and stay trials (15 TMS trials per block delivered on switch trials, 15 per block on stay trials). A total of 15 TMS trials per condition (single and paired pulse TMS trials, switch and stay, pre-rTMS and post-rTMS) were obtained

and used for the analysis. TMS trials were presented at least 7 seconds apart, to ensure that pulses on adjacent trials did not influence each other.

RTMS. Two blocks of paired-pulse TMS data were acquired before 1 Hz rTMS application, and two blocks were acquired afterwards. A biphasic Magstim Super Rapid machine (The Magstim Company ®) was used to deliver 15 minutes of 1 Hz rTMS (900 pulses) through a 70mmfigure-of-eight coil. The coil was held tangential to the skull with the handle pointing in the anterior direction, 4cm anterior to the electrode position Cz, previously shown to be an appropriate location for the stimulation of pre-SMA (Rushworth et al., 2002b; Mars et al., 2009)(see Figure 60). The mean MNI location across subjects for pre-SMA rTMS coil location was x= -1.101 (+/-1.91), y= 15.1 (+/-2.83) and z= 71.3 (+/- 0.65) (see Figure 61). Intensity of the rTMS stimulation was set at 90% of the individual RMT (as in (O'Shea et al., 2007a)). The coil was replaced after 7.5 min to avoid overheating with another 70mm figure-of-eight coil of exactly the same type. Coil changeover took approximately 30 s (O'Shea et al., 2007a). During rTMS delivery subjects were asked to fixate on a white fixation cross presented on a black computer screen and completely relax their hands. In the post-rTMS session, the time required for setting up the two paired-pulse TMS coils (rIFC and M1) and the experimental task meant that about 1 min had elapsed between the end of the 1 Hz rTMS and the onset of the paired-pulse TMS experiment. During the interval, the subjects were asked to sit still and relax their hands.

Electrophysiological recordings. Finger muscle EMG activity was only recorded in the pre-rTMS and post-rTMS paired-pulse TMS blocks. As in the previous experiment MEPs were recorded from two muscles, the FDI and the ADM, in the right hand using two surface Ag-AgCl electrodes in tendon-belly montage. An earth

electrode was placed on the right elbow. EMG responses were band-pass filtered between 10-1000 Hz, with an additional 50 Hz notch filter, sampled at 5000 Hz, and recorded using a CED 1902 amplifier, a CED micro 1401 Mk.II A/D converter, and a PC running Spike2 (Cambridge Electronic Design, Cambridge, UK).

Analysis. For the analysis of rIFC-M1 functional connectivity during action reprogramming and action execution before and after transient disruption of pre-SMA activity, we analysed MEP data from the switch and stay trials. As in previous experiments analysis of electrophysiological data concentrated on peak-to-peak amplitudes of the MEPs measured on "switch trials" and "stay trials", before and after rTMS delivery. Trials with incorrect responses, trials with premature (RT<150) responses, trials in which the test pulse failed to elicit a reliable MEP (amplitude<0.1 mV), and trials in which participants pre-contracted the FDI muscle prior to application of the TMS pulse (EMG amplitude>0.1 mV in the 80 ms before the pulse) were discarded from the analysis. Following this pre-processing, on average 2 (SEM± 0.4076) trials per condition (of a total of 15 trials per condition) had to be excluded. To account for differences in coil placement between experimental blocks, MEP sizes were median- normalised within each block. Analyses of MEPs were carried out on the ratio [paired-pulse TMS MEP / single-pulse TMS MEP]. Analyses of both behavioural and electrophysiological data were conducted using ANOVA tests, using repeated measures. Significant effects were identified based on Huynh-Feldt corrected ANOVA values, using SPSS 16.0. Post-hoc two-sided t-tests were used to further investigate significant effects in the ANOVAs.

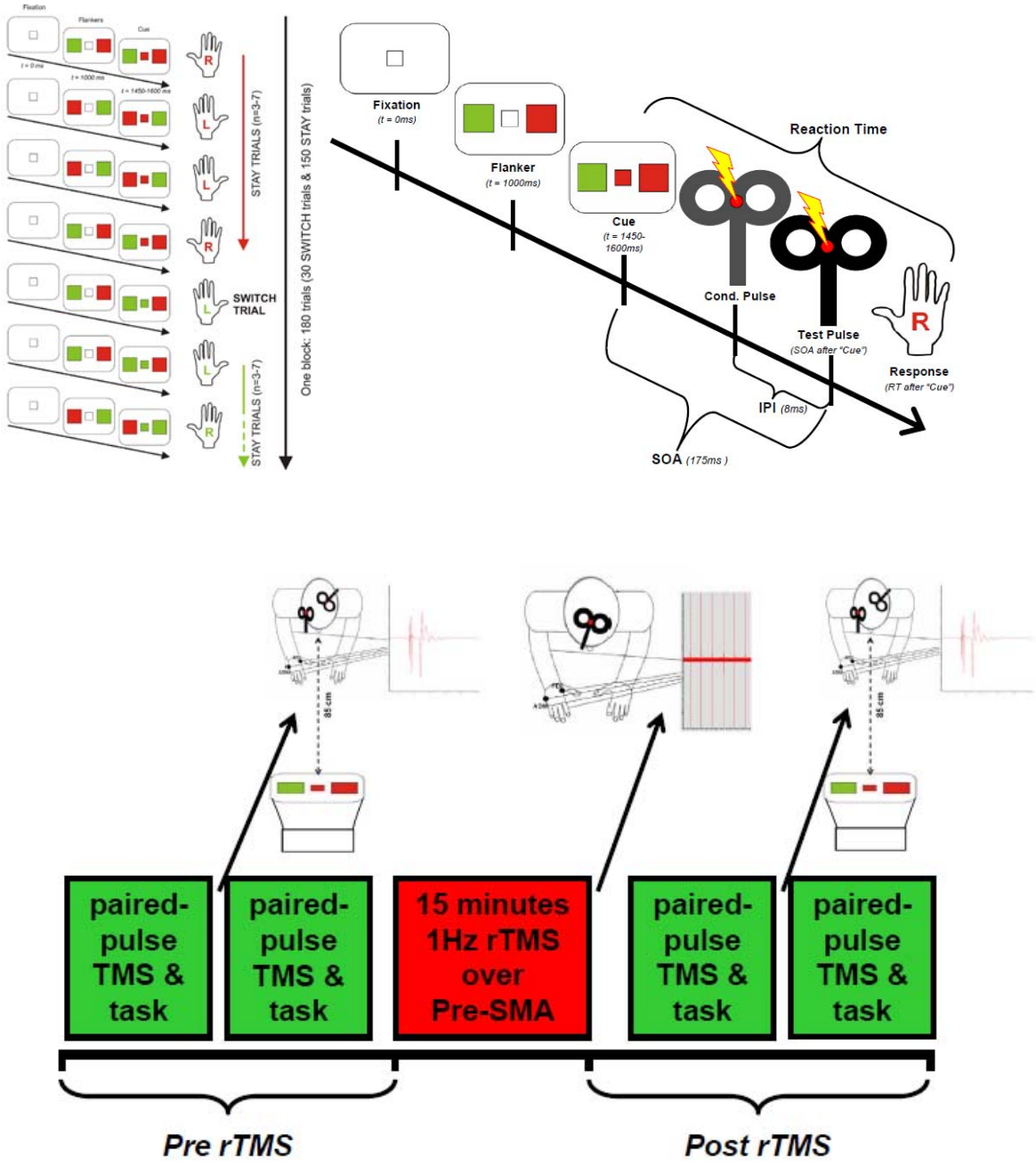


Figure 59: Experimental task. Pulses in the paired-pulse TMS blocks were delivered through two 55mm diameter figure-of-eight coils. The conditioning coil was placed over rIFC, the test coil was placed over left M1. One experimental block contained 180 trials, 30 switch trials and 150 stay trials. Single and paired-pulse TMS was delivered at 175 ms SOA with 8 ms IPI (top). Two experimental blocks preceded the 15 minutes period of rTMS. The other two experimental blocks followed the rTMS immediately (bottom).

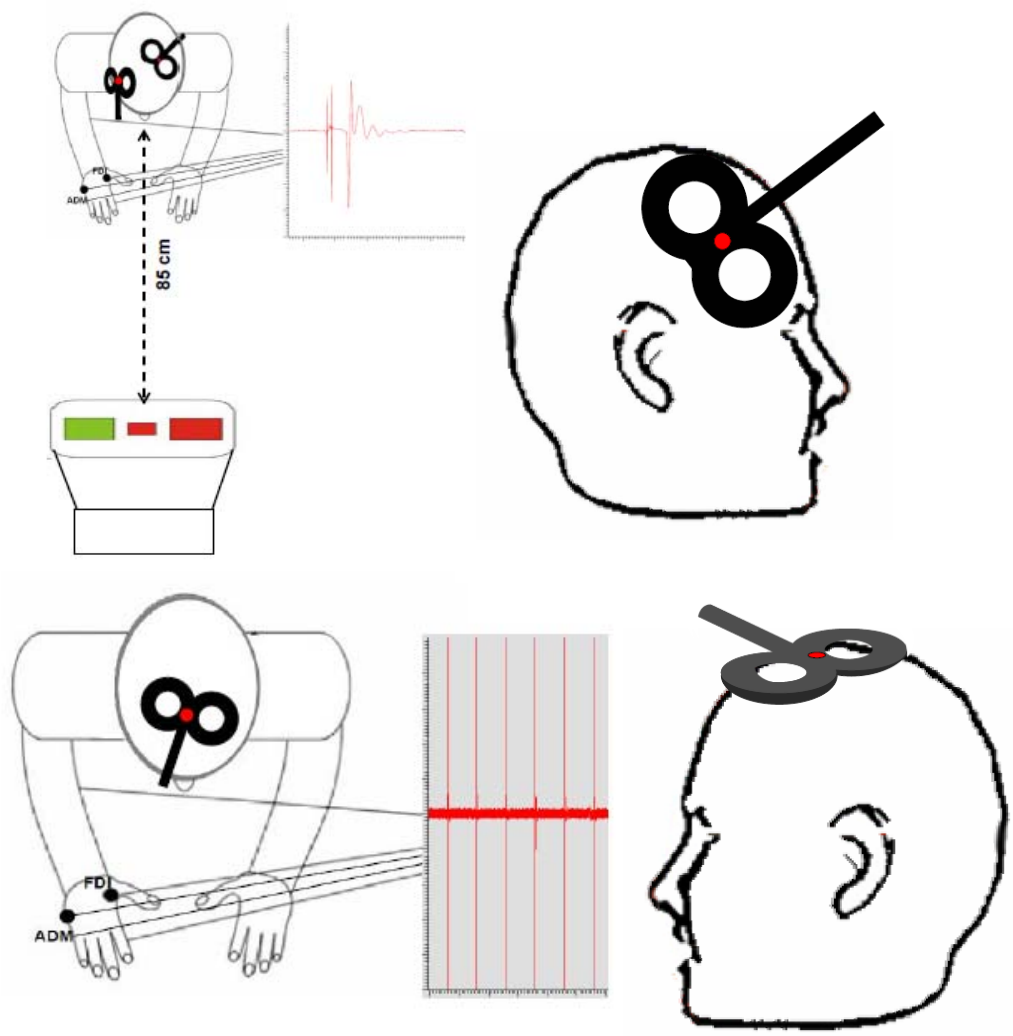


Figure 60: Experimental setup. TMS coil applying test pulses to left M1 was placed tangentially to the scalp, with the handle oriented posteriorly. TMS coil applying conditioning pulses to the rIFC was placed tangentially to the scalp with the handle of the coil pointing forwards and upwards (top). TMS coil applying 1 Hz rTMS to the pre-SMA (bottom) was placed tangentially to the scalp with the handle pointing in the anterior direction 4 cm anterior to the electrode position Cz.

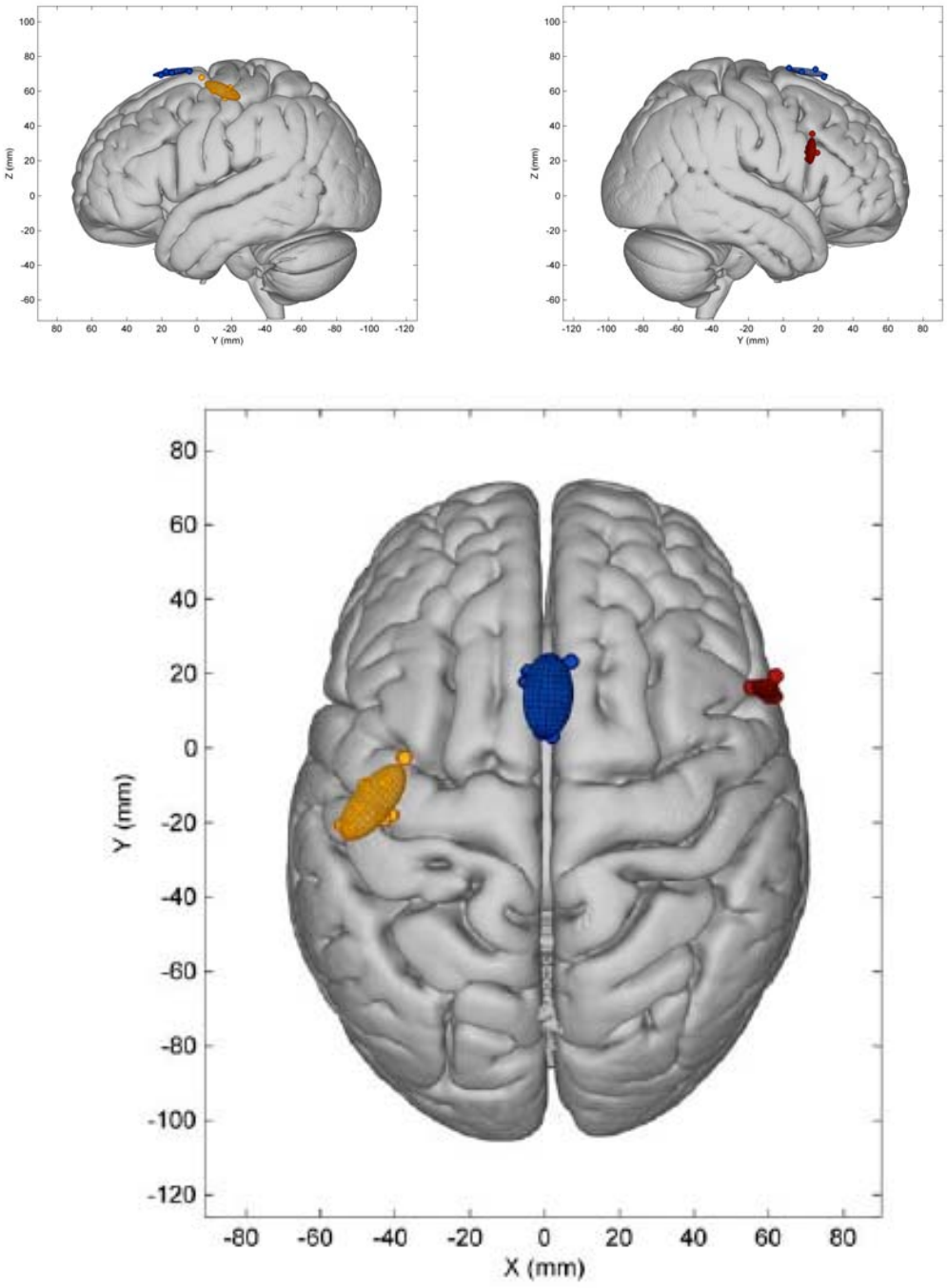


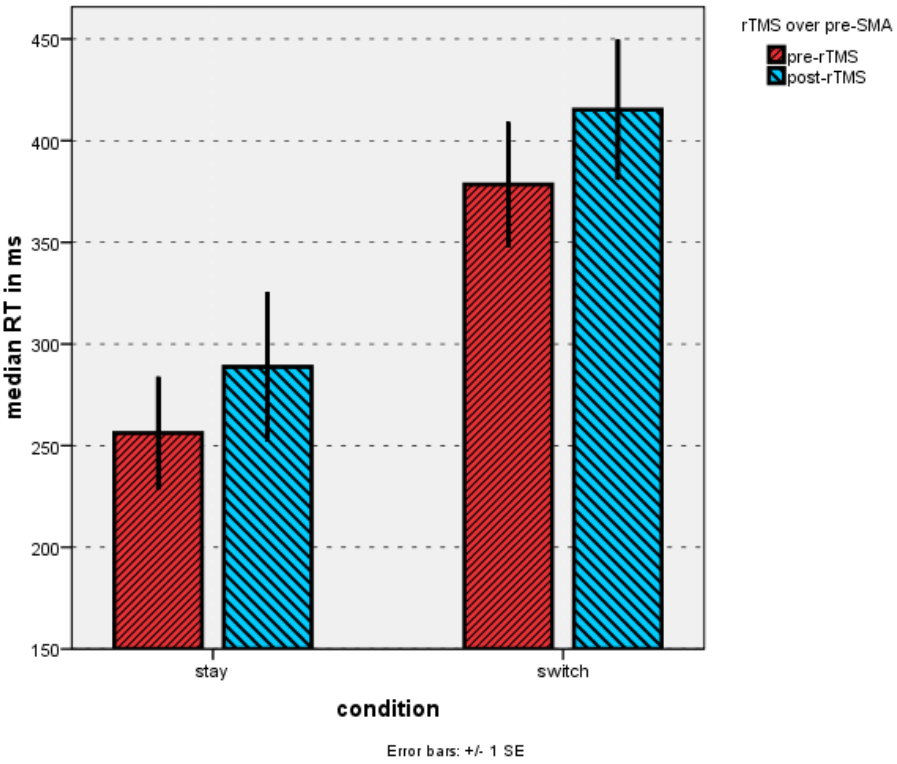
Figure 61: MNI coordinates for TMS targets. Circular symbols indicate individual subjects' stimulation locations in MNI152 space. Ellipsoids represent 95% confidence limits of the mean group stimulation location for each area. The mean MNI location across subjects for the pre-SMA rTMS application (blue) was x= -1, y= 15 and z= 71. The mean MNI location of the rIFC conditioning coil location (red) was x=59, y=15 and z=26.

Results

Behavioural results. ANOVAs of median RTs on correct trials and of error rates (incorrect responses / total number of trials) with *"trial type"* (switch vs. stay) and *"rTMS"* (before 1 Hz rTMS application to pre-SMA vs. after 1 Hz rTMS) as within-subject factors showed a main effect of *"trial type"* ($F_{1,7}$= 36.273, p<0.001 for RTs; $F_{1,7}$= 8.955, p=0.02 for error rates), but no main effect of *"rTMS"* and no interaction between *"rTMS"* and *"trial type"* (p>0.35). Post-hoc paired-samples t-tests on the behavioural data confirmed subjects were significantly slower on switch trials than on stay trials (RT 272 ms on stay vs. 405 ms on switch trials, t_7= -5.286, p=0.001) and made significantly more mistakes (error rate 30.7% on switch trials vs. 1.8% on stay trials, t_7= -2.499, p=0.041). This confirmed the effectiveness of the task manipulation. However paired samples t-tests testing differences between the pre-rTMS and post-rTMS blocks in RTs, RTs in switch trials, RTs in stay trials, error rates, error rates in switch trials and error rates in stay trials did not yield any differences in these behavioural measures. This indicates that performance was not changed by 15 minutes 1 Hz rTMS application to the pre-SMA. If anything median RTs in switch trials were slightly higher in the post-rTMS session compared to the pre-rTMS session (378.4 ms in the pre-rTMS session and 415.1 ms in the post-rTMS, t_7= -1.544, p= 0.166).

Pre-SMA rTMS influence on functional connectivity between rIFC and M1. The aim of this experiment was to investigate whether functional interactions between rIFC and M1 depend on activity in pre-SMA. Activity in cortical areas can be decreased by a 15 minutes period of 1 Hz rTMS (O'Shea et al., 2007a). We compared

functional connectivity between rIFC and M1 175 ms after cue onset before transient pre-SMA interruption with rIFC-M1 functional connectivity immediately after pre-SMA interruption. We analysed single and paired-pulse TMS MEPs and calculated [paired-pulse TMS MEP / single-pulse TMS MEP] ratios for switch and stay trials in the two pre-rTMS blocks and the two post-rTMS blocks separately. An ANOVA test on the paired-pulse TMS / single-pulse TMS MEP ratios with within-subjects contrasts of *"trial type"* (switch vs. stay), and *"rTMS"* (pre-rTMS vs. post-rTMS) revealed a significant *"trial type" × "rTMS" interaction* ($F_{1,7}$=11.918, p=0.011). Post-hoc two-tailed one-sample t-tests against baseline (ratio of 1.0 or 100%) revealed significant inhibition of M1 excitability due to rIFC conditioning pulses for switch trials (t= -2.709, p= 0.03) and a significant facilitation facilitation of M1 excitability due to rIFC conditioning pulse application on stay trials (t=2.635, p=0.034) before rTMS application. This effect was reversed for the rIFC influence on M1 during switch trials after rTMS application to pre-SMA with significant facilitation of M1 due to rIFC conditioning pulse stimulation during switch trials (t_7= 3.076, p= 0.018). Paired-samples t-tests between single-pulse / paired-pulse TMS MEP ratios revealed a significant difference rIFC-M1 interaction between switch and stay trials (t_7=-4.134, p= 0.004, stay > switch) only before rTMS application. This difference disappeared or if anything was even reversed after 15 minutes of 1 Hz rTMS over pre-SMA (switch > stay, p>0.15). These results are interesting, as they suggest that a mild and transient inactivation of pre-SMA activity does not have a big influence on behavioural measures of task switching and action reprogramming but does induce changes to rIFC-M1 functional connectivity.



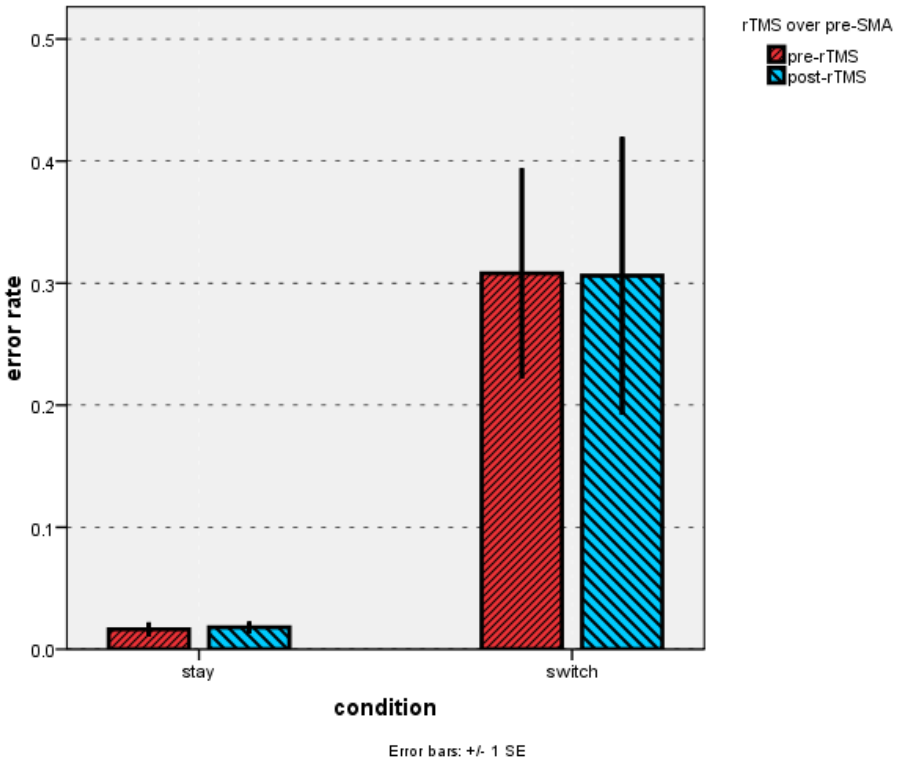


Figure 62: Behavioural results. Subjects were significantly slower on switch trials than on stay trials (top) and made significantly more mistakes (bottom). 15 minutes of 1 Hz rTMS over pre-SMA did not significantly change task performance.

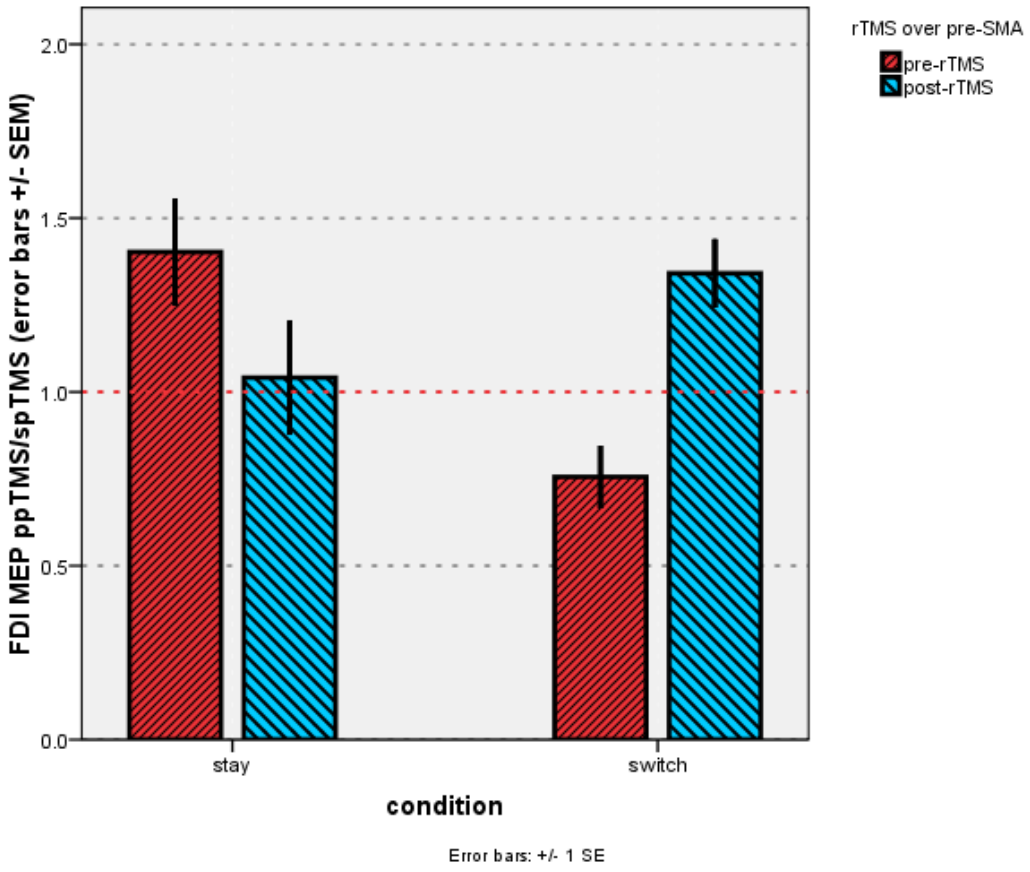


Figure 63: RIFC - M1 functional connectivity during action reprogramming (right) and action execution (left) at 175 ms SOA before (red) and after (blue) 15 minutes of 1 Hz rTMS application to pre-SMA. MEP data was recorded from the FDI and paired-pulse / single- pulse TMS MEP ratios were calculated. Pre-SMA inactivation induces changes to rIFC-M1 functional connectivity.

Discussion

Numerous studies on response inhibition, action reprogramming, task switching and conflict resolution suggested an important contribution of rIFC (Sasaki et al., 1989; Garavan et al., 1999; Konishi et al., 1999; Aron et al., 2003a; Aron et al., 2004a; Aron and Poldrack, 2006; Chambers et al., 2006; Li et al., 2006; Aron et al., 2007a; Mars et al., 2007b; Duann et al., 2009) and pre-SMA (Rushworth et al., 2002b; Nachev et al., 2005; Isoda and Hikosaka, 2007; Mars et al., 2007b; Nachev et al., 2007; Sumner et al., 2007; Taylor et al., 2007; Nachev et al., 2008) to these processes. However the different role of these areas in action reprogramming and response inhibition has proven difficult to disentangle. It has been suggested that rIFC exerts an inhibitory influence on the motor system via projections to the STN (a so called hyperdirect pathway). Hence rIFC could be the cortical site implementing inhibitory control. Especially a posterior region within rIFC is well placed to be the centre of a network of brain areas inhibiting M1 corticospinal activity (Baumer et al., 2009; Chikazoe et al., 2009; Buch et al., submitted). However pre-SMA could exert inhibitory control over M1 via the same cortico-STN-pallidal pathway (Isoda and Hikosaka, 2007, 2008). Some recent imaging studies have found that rIFC and STN activity was negatively correlated with SSRTs in go/no-go tasks but not pre-SMA activity (Aron and Poldrack, 2006; Aron et al., 2007a). Others found pre-SMA activity but not rIFC activity to be negatively correlated with SSRTs (Li et al., 2006). A recent study by Duann and colleagues suggested that rIFC activity related to no-go trials in go/no-go paradigms is just a response to a behaviourally relevant and salient target stimulus (Duann et al., 2009). As a part of the ventral attention system IFC detects the no-go signal and expedites response inhibition, which is actually carried out by a Pre-SMA

→ STN → globus pallidus internus loop. This could be an interesting reinterpretation of the studies investigating inhibitory control. However whereas the study by (Duann et al., 2009) did not even try to dissociate processes of attentional control and processes of inhibitory control in no-go trials in a go/no-go task, a study by (Chikazoe et al., 2009) was able to distinguish two functionally different areas within the IFC by introducing infrequent (and hence more salient) go-signals into their go/no-go task. An anterior area in IFC referred to as IFJ showed activity related to saliency in both infrequent go and infrequent no-go trials. A region in the posterior IFC showed greater activity related to no-go trails but not to infrequent go-trials. According to the location of this posterior IFC area (MNI: x=56, y=16, z=16; (Chikazoe et al., 2009)) this area could be identical the area that was often referred to as "PMv" and that has now been shown to exert inhibitory influence on M1 corticospinal excitability (Baumer et al., 2009; Buch et al., submitted).

Lesions to the pre-SMA have been reported to impair conflict resolution and movement inhibition (Nachev et al., 2005; Nachev et al., 2007). Rather than studying patients this experiment used a 1 Hz rTMS protocol to induce mild and transient disruption to pre-SMA. The experiment aimed to investigate whether this transient deactivation of pre-SMA activity would influence behavioural performance and functional connectivity between rIFC and M1 in a task requiring action selection and action reprogramming. We showed that although rTMS over pre-SMA did not significantly change behavioural performance it did change the pattern of functional connectivity between rIFC and M1. As in previous experiments we found that rIFC exerted an inhibitory influence on M1 excitability during switch trials. After inactivating pre-SMA activity by rTMS the inhibitory influence of rIFC on M1 disappeared. This could suggest that physiological measures of functional

connectivity could be more sensitive to activity changes in other areas critically involved in the task than behavioural measures such as RTs and error rates. The results suggest that pre-SMA plays a causal role for the inhibitory influence of rIFC on the motor system. It could explain why lesions of the pre-SMA impair conflict resolution and action reprogramming. According to a popular view of medial frontal and lateral frontal cortex interaction during executive control and decision making (Kerns et al., 2004; Ridderinkhof et al., 2004; Rushworth et al., 2004; Nachev et al., 2008; Kouneiher et al., 2009) it could be interpreted as the pre-SMA detecting prediction violation or conflict and subsequently energising inhibitory control implemented by an rIFC → STN → globus pallidus internus → thalamus → M1 loop.

3. Conclusion and Future Directions

Actions are selected in the context of environmental demands and internal goals. Both change constantly and dynamically and several studies have addressed the issue of how information about these is represented, updated and integrated to form appropriate decisions and action plans. According to a traditional view the brain constantly samples information about the environment to build up a "real-time representation" of the current state of the world. However other theories suggest the brain as being mainly driven by predictions about the environment and therefore mainly sampling violations of these predictions (Cisek, 2007; Mars et al., 2007b; Rushworth et al., 2009). Additionally this "picture" of the current state of the world and the prediction-error driven updating of this picture might specifically relate to our current goals and behavioural strategies. This is similar to the phenomenological notion of readiness-to-hand (Heidegger's "Zuhandenheit", "handiness") (Heidegger, 1927; Heidegger et al., 1962). We are constantly *involved* in the world. We are doing things with the aim to achieve something. Hence our picture of the world is formed by our desires, goals and action plans, i.e. the world is not represented as "the thing in itself" but as an environment "ready to hand". These considerations imply that actions are often planned, selected and initiated in the context of ongoing actions and already present action plans. In the previous chapters it was referred to this idea as "action reprogramming". Action reprogramming requires the inhibition of prepared or already initiated but inappropriate movements, resolution of response conflict and initiation of an alternative and appropriate action. The prevention of planned movements and the reprogramming of actions have been suggested to involve regions in the frontal cortex and the basal ganglia. The rIFC and the pre-SMA have been thought to play a major

role in response inhibition and action reprogramming (Aron et al., 2004a; Chambers et al., 2006; Li et al., 2006; Aron, 2007; Nachev et al., 2007; Taylor et al., 2007; Nachev et al., 2008; Chambers et al., 2009; Chikazoe et al., 2009; Mars et al., 2009). We aimed to investigate (1) general excitability of the motor system and M1 internal inhibitory processes during action execution and action reprogramming, (2) functional connectivity between rIFC and M1during action execution and action reprogramming, (3) different anatomical pathways mediating functional rIFC-M1 and pre-SMA-M1 connectivity and (4) rIFC-M1 functional connectivity after a virtual lesion to pre-SMA. The previous chapters already discussed many different aspects of these experiments and the results.

Paired-pulse TMS has proven to be a very efficient technique to investigate functional connectivity between neuronal networks within a certain cortical area or even between cortical areas (O'Shea et al., 2008; Reis et al., 2008; Wasserman et al., 2008). A single TMS test pulse over the M1 is used to examine M1 corticospinal excitability. A conditioning pulse preceding the test pulse activates a certain "group" of neurons depending on its intensity and the area of stimulation. These additionally activated neurons might influence M1 excitability depending on the time interval between the two pulses.

We used this technique to study general M1 excitability and M1 internal short-interval inhibitory mechanisms (so called SICI (Rothwell et al., 2009)). We hypothesised that excitability and internal inhibitory processes in the M1 would change during the time course of action reprogramming and would differ from situations when only action execution was required. We found that during action reprogramming the excitability of the motor system changes according to the necessity of inhibiting a prepared response and selecting an alternative action between 75 ms and 175 ms after the cue

onset. In switch trials 75 ms after cue onset excitability of the motor system of the prepared but incorrect response is still bigger than the excitability of the motor system of the unprepared but appropriate response. This effect is reversed 225 ms after cue onset suggesting that action reprogramming on the level of the motor system takes place between 75 ms and 225 ms after the onset of the instructive cue. This reversion could be achieved by GABA-ergic M1 internal inhibitory interneurons. We observed a release of SICI for the unprepared but correct hand in action reprogramming trials. This suggests that one mechanism achieving successful action reprogramming is an even stronger release of SICI to overcome the prepared but incorrect action plan.

To investigate the source of the inhibitory control of the motor system we studied functional connectivity between rIFC and M1 during normal action execution and action reprogramming. A paired-pulse TMS paradigm was used with a conditioning pulse over the posterior rIFC and the test pulse over the left M1. Our results indicate that rIFC influences M1 corticospinal excitability in both, action execution and action reprogramming situations at about 175 ms after instruction cue onset. Whereas rIFC facilitates the correct movement in action execution trials, it inhibits the wrong movement in action reprogramming trials. Estimates of the duration of the stopping processes in go/no-go paradigms based on SSRTs (150 to 200 ms) (Aron et al., 2007b) are very similar to the observed time-course of rIFC-M1 inhibitory effects during action reprogramming, suggesting that stopping could be mediated by these observed inhibitory physiological mechanisms. However we did not only find inhibition of M1 corticospinal output during response inhibition and action reprogramming trials, but also facilitation of the correct and expected response in normal action execution trials. These findings are similar to the results of paired-pulse TMS experiments investigating PMv-M1 connectivity during grasping movements

(Davare et al., 2008; Baumer et al., 2009; Davare et al., 2009; Buch et al., submitted). "Inhibition" is a widely used concept in the field of cognitive and molecular neuroscience, psychology and even psychotherapy (see Chapters 1.1.1 and 1.1.2.). However the degree to which inhibition of actions at a behavioural level can be related to physiological inhibition is unknown. It is very unlikely that the extremely diverse processes of synaptic inhibition, circuit inhibition, response inhibition or inhibition of emotions and memories are exerted by similar neural mechanisms. Our results suggest a complex relationship between inhibition on a behavioural level and inhibition on a neurophysiological level. We found inhibition on the level of general M1 corticospinal excitability and M1 internal inhibitory mechanisms but also on the level of rIFC – M1 functional connectivity. These different physiological inhibitory mechanisms might play different roles in action reprogramming. However these results indicate that inhibition on a neurophysiological level might at least to some extend correspond to behavioural processes of response inhibition. Some of these might even serve other types of inhibitory control in higher cognition.

In a third experiment we again used a paired-pulse TMS paradigm to investigate different pathways of functional pre-SMA-M1 connectivity and rIFC-M1 connectivity during action reprogramming and response inhibition. The aim of this experiment was to show different pathways of functional pre-SMA-M1 and rIFC-M1 connectivity during action reprogramming and to localise these pathways within the brain's white matter. To elucidate whether these pre-SMA-M1 and rIFC-M1 pathways belong to a bigger network involved in action reprogramming, conflict resolution and response inhibition we carried out probabilistic diffusion tractography based on the white matter clusters that showed FA values significantly correlated with the paired-pulse TMS measurements. In this experiment we replicated the effects of pre-SMA-M1 and

rIFC-M1 functional connectivity reported earlier (Mars et al., 2009) and observed similar effects of connectivity at longer IPIs. Additionally we found clusters that showed FA values significantly correlated with the TMS effects. These clusters of significant correlation and the tracts derived from these clusters were anatomically specific to certain IPIs. TMS effects occurring at shorter IPIs (6 ms) were correlated with FA clusters in the dorsal white matter (for example in the SLF) whereas TMS effects occurring at longer IPIs (12 ms) seemed to indicate pathways via the basal ganglia. We found similar white matter clusters being correlated with the pre-SMA-M1 and the rIFC-M1 paired-pulse TMS MEP effects. This might suggest an extended functional and anatomical network of action reprogramming and response inhibition.

A fourth experiment attempted to investigate whether pre-SMA activity causally influences functional connectivity between rIFC and M1. Lesions to the pre-SMA have been reported to impair conflict resolution and movement inhibition (Nachev et al., 2005; Nachev et al., 2007). Rather than studying patients this experiment used a 1 Hz rTMS protocol to induce mild and transient disruption to pre-SMA. The experiment aimed to investigate whether a transient deactivation of pre-SMA activity would influence behavioural performance and functional connectivity between rIFC and M1 in a task requiring action selection and action reprogramming. We showed that rTMS induced deactivation of pre-SMA activity reduces the inhibitory influence of rIFC on M1 corticospinal excitability. In the Introductory Chapter theories on hierarchical organisation of the frontal lobes were discussed (see Chapter 1.1.2). Our experiments did not aim to explicitly test these hypotheses. However the results suggest that regions in the lateral PFC, such as the rIFC, and in the medial PFC, such as the pre-SMA directly influence motor output and that these influences depend on the cognitive state, i.e. whether participants needed to execute a prepared response or

whether they had to reprogramme their prepared actions. A combined rTMS / paired-pulse TMS paradigm was used to investigate interactions between medial PFC and lateral PFC regions. These findings suggest that the inhibitory influence of rIFC over M1 might depend on pre-SMA activity. This could indicate an interaction between distinct hierarchies of attentional and executive control in the medial and lateral PFC, similar to the theory proposed by (Kouneiher et al., 2009)

Using paired-pulse TMS we were able to actually show that activity in the rIFC reduces M1 excitability, i.e. induces "inhibition of M1" in the context of action reprogramming and response inhibition. Whether rIFC really is the cortical site implementing inhibitory control was a matter of extensive debate and research. However fMRI might not be the appropriate neuroscientific method to quantify mechanisms of (physiological) inhibition (Waldvogel et al., 2000). However a paired-pulse TMS paradigm helped us to shed some light on this issue. It still needs to be explored whether different regions in the (r)IFC play functionally distinct roles during action reprogramming and response inhibition (as suggested by (Chikazoe et al., 2009)). It has been proposed that a region in the anterior IFC, the IFJ area is active when infrequent and salient stimuli are presented and attentional control needs to be adjusted, whereas a region in the posterior (r)IFC is active, when responses need to be inhibited (Chikazoe et al., 2009). This hypothesis might be tested with a paired-pulse TMS paradigm with conditioning pulses over IFJ and posterior rIFC aiming to investigate whether functional connectivity between IFJ and M1 is related to attentional control and connectivity between posterior rIFC and M1 is related to inhibitory control. This so-called "posterior IFC" area is likely to be the same as PMv (Buch et al., submitted). But further research is needed to clarify this issue. Additionally it might be interesting to investigate "surprise" or "uncertainty" related

fluctuations in rIFC-M1 or IFJ-M1 connectivity as those fluctuations have been found for M1 excitability previously (Bestmann et al., 2008b). We tried to investigate uncertainty-related fluctuations in PMd-M1 connectivity using paired pulse TMS combined with a rule-guided decision making task based on the notions by (Koechlin and Summerfield, 2007; Bestmann et al., 2008b) (see Appendix 4.1).

We suggested a new methodological approach of combining paired-pulse TMS using various IPIs to elucidate different pathways of functional connectivity with diffusion MRI data in order to uncover anatomical white matter tracts mediating certain effects of functional connectivity. This approach should be used to investigate interactions of other areas such as PMd, IFJ or posterior parietal cortex with M1 in different cognitive tasks.

Moreover the combination of paired-pulse TMS and rTMS allowed us to investigate the impact of a transient disruption of an area on the functional connectivity between two other areas. This allows us to investigate whether virtual lesions causally affect interactions between brain areas and whether one area is necessary to establish a certain pattern of connectivity between other areas. This methodological approach could be used together with offline rTMS, such as 1 Hz rTMS or theta burst TMS (Huang et al., 2005) or with online TMS with single pulses or trains of pulses over the area that shall be deactivated as for example in (Rushworth et al., 2002b; Kennerley et al., 2004).

We used a relatively simple task requiring the participants to respond to coloured stimuli on a computer screen. Sometimes they had to inhibit an already prepared movement and reprogramme their action. We found pre-SMA and rIFC exerting a causal influence on M1 during action reprogramming and response inhibition. Both areas were already suggested to be part of a bigger executive control network.

However it is likely that multiple other functions are implemented in these areas. Moreover similar processes of inhibition and reprogramming might employ the same areas, pathways and networks in different situations. Future research needs to clarify whether these principles of inhibition and reprogramming apply over and above the immediate control of motor output and into the control of emotion, attention, memory, and task sets and whether the same regions (rIFC, pre-SMA, STN) are recruited across this wide range of control mechanisms.

4. Appendix

4.1. Changes in functional connectivity between PMd and M1 during action selection related to uncertainty

Introduction

Cognitive control allows flexible behaviour in a constantly changing world by selecting actions that are consistent with our own goals and appropriate to environmental demands. The PFC has an established role in cognitive control and research on these processes tried to elucidate the functional organisation of the PFC (Koechlin and Summerfield, 2007; Badre, 2008; Badre and D'Esposito, 2009). A recent approach based on information theoretical quantifications of (rule-guided) decision making processes suggested an anterior-to-posterior executive control hierarchy in the lateral PFC (Koechlin and Summerfield, 2007; Haggard et al., 2008). Data from several fMRI experiments supported this idea (Koechlin et al., 2003; Koechlin and Hyafil, 2007; Kouneiher et al., 2009). According to this theory even complex executive control processes can be decomposed into simple mechanisms of action selection. The demand of cognitive control is proportional to the amount of information needed to select a certain action among alternative actions. The "quality" of information (or type of information) corresponds to the source of the information, i.e. whether the information to select a certain response is conveyed by a stimulus (sensorimotor control), by the immediate context of the stimulus (contextual control) or by a stimulus presented previously to instruct a longer episode of decisions (episodic control). These different "types" or "levels" of information are processed alongside the anterior-to-posterior executive control hierarchy in the lateral PFC. The

"quantity" of information is proportional to the likelihood that a certain action might be required. If an action (or context, or episode) is the only possible action (or context, or episode) in a certain task, no sensorimotor (or contextual, or episodic) information processing is necessary to select this action. Conversely if an action (or context, or episode) is very rare (likelihood is close to zero), more sensorimotor (or contextual, or episodic) information processing is necessary to select this action. It has been suggested that BOLD activity in different lateral PFC areas along the proposed executive control hierarchy corresponds to the different levels of information, whereas the magnitude of the BOLD response in one of these areas corresponds to the quantity (in bits) of information that needs to be processed on each level (Koechlin and Summerfield, 2007). In this model the premotor cortex, especially PMd is responsible for processing sensorimotor information, i.e. relate simple stimuli to their appropriate responses. PMd-M1 functional connectivity has already been studied using a paired-pulse TMS paradigm (Koch et al., 2006; Boorman et al., 2007; O'Shea et al., 2007b). These studies suggested that functional connectivity between PMd and M1 changes during the time course of action selection and these temporal patterns of functional connectivity depend on whether sensorimotor information needs to be processed by PMd and conveyed to M1 or whether no information is necessary to "select" an action. (O'Shea et al., 2007b) referred to the former condition as the "select" condition, where a button press needed to be selected based on a previously learned stimulus-response rule, and referred to the latter condition as the "execute" condition, because subjects were asked to execute the same response in every trial regardless of which cue was presented. (O'Shea et al., 2007b) found differences in PMd-M1 connectivity between the two conditions. However in this experiment the amount of information only varied between 0 and 1 bit. In another single-pulse TMS

study (Bestmann et al., 2008b) showed that during the preparation for action period corticospinal excitability varied proportional to the uncertainty (entropy in terms of information theory) and the surprise (the stimulus-bound information conveyed by a visual cue) on a trial-by-trial basis. The experiment presented below aimed to find changes in functional connectivity between PMd and M1 related to the amount of information that needs to be processed by PMd and conveyed to M1 to select an appropriate action. However we did not observe clear information-related changes in PMd-M1 connectivity. Therefore the experiment is presented and discussed only briefly in the appendix of the thesis.

Methods

Participants. Eight healthy volunteers (age range 21 – 32 years, mean age = 28 +/- 1.52, 7 females) with no personal or familial history of neurological or psychiatric disease participated in one experiment investigating functional connectivity between PMd and M1 during rule-guided action selection. The experiment was approved by the Oxfordshire Research Ethics Committee and conducted in accordance with the declaration of Helsinki. All participants were right-handed and gave written informed consent. They were all screened for adverse reactions to TMS and risk factors by means of a safety questionnaire.

Experimental setup. Participants were seated in a darkened room and wore a tight-fitting EEG cap, on which TMS sites were marked and earplugs to protect against TMS noise. A chin rest was used to minimise head movements.

Behavioural Task. The task (see Figure 64 and Figure 65) was inspired by the "response experiment" in the fMRI study conducted by (Badre and D'Esposito, 2007) and required participants to respond to different visual stimuli with the fingers of their left hand. White stimuli were presented on a black 17 inch computer screen ~85 cm in front of them. Only one stimulus was presented at a time (~6º width) and subjects were asked to respond to the stimulus as fast and as accurate as possible. This experiment manipulated the response competition by varying the number of possible responses on a given experimental block. In the "one choice" block four different stimuli mapped to one single response: a left index finger button press. This block was similar to the "execute" condition in the experiment conducted by (O'Shea et al., 2007b). In the "two choices" block four different stimuli mapped to two eligible

responses, i.e. two stimuli mapped to an index finger button press and the other two stimuli mapped to a little finger button press. This condition was similar to the "select" condition in (O'Shea et al., 2007b). In the "four choices" block four different stimuli mapped to four eligible responses, i.e. each stimulus mapped to different finger responses (index, middle, ring and little finger). The stimulus-response associations were learned before each experimental block in a training block. Training stopped after 18 correct responses in a train of 20 trials ensuring that stimulus-response mappings were well known. Stimulus-response mappings were randomised between subjects. During the training blocks no TMS was delivered. One experimental session contained one 1-choice block (80 trials, 80 TMS trials), two 2-choices blocks (60 trials per block, 60 TMS trials per block) and four 4-choices blocks (70 trials per block, 35 TMS trials per block). Custom software written in Presentation (version 0.53) controlled the experiment. Reaction times were recorded, defined as the duration between the onset of the stimulus and the respective finger response.

TMS. The aim of this experiment was to investigate functional connectivity between left PMd and right M1 during action selections of different difficulty. This can be achieved using a paired-pulse TMS paradigm with one TMS coil over the right M1 (test pulse) and another TMS coil over left PMd (conditioning pulse) (see Figure 65). As in the previous experiment intensity of the right M1 TMS test pulse was such that an MEP of 1 – 1.5 mV was evoked in the relaxed, contralateral FDI. This intensity was 45.13% (SEM+/- 1.14) of the maximum stimulator output. The intensity of conditioning pulse was set at 110% of the resting motor threshold (RMT) of the right FDI muscle (left M1). These values were based on studies about functional connectivity between PMd and M1 during action selection (Koch et al., 2006; O'Shea

et al., 2007b). This intensity was 38.5% (SEM+/- 1.81) of the maximum stimulator output. The inter-pulse-interval was 8 ms. We hypothesised that the preceding conditioning pulse would change the MEP amplitude elicited by the test pulse, depending on the block type, i.e. the amount of information that needs to be processed by PMd to reduce uncertainty about the correct response and to guide action selection. In the 1-choice block the stimuli do not convey any information guiding action selection (0 bit). In the 2-choice condition the information guiding response-selection conveyed by each stimulus is 1 bit. In the 4-choice condition the amount of information conveyed by each stimulus is even bigger (2 bit). Temporal pattern and size of functional connectivity between PMd and M1 might change with the amount of information that has to be processed in PMd and conveyed to M1 to guide the selection of the appropriate movement. TMS pulses were delivered 50 ms, 75 ms, and 100 ms after stimulus onset. 20 single and paired-pulse TMS trials were delivered at rest to obtain a baseline value of functional connectivity. Pulses were delivered through two 55 mm diameter figure-of-eight coils directly connected to two high-power Magstim 200 MonoPulse machines (The Magstim Company®). A total of 10 TMS trials per SOA (50 ms, 75 ms, 100 ms), condition (1-choice, 2-choice, 4-choice), finger response and pulse type (single vs. paired) were obtained and analysed. In each block, TMS trials were distributed evenly over eligible responses, SOAs and single- or paired-pulse TMS.

Electrophysiological recordings. MEPs were recorded from two muscles, the FDI and the ADM, in the left hand using two surface Ag-AgCl electrodes in tendon-belly montage. An earth electrode was placed on the left elbow. EMG responses were band-pass filtered between 10-1000 Hz, with an additional 50 Hz notch filter, sampled at

5000 Hz, and recorded using a CED 1902 amplifier, a CED micro 1401 Mk.II A/D converter, and a PC running Spike2 (Cambridge Electronic Design, Cambridge, UK).

Analysis. For the analysis of functional connectivity between PMd and M1 during action selection we concentrated on peak-to-peak amplitudes of the MEPs measured on TMS trials. Trials with incorrect responses, trials with premature (RT<100) responses, trials in which the test pulse failed to elicit a reliable MEP (amplitude < 0.1 mV), and trials in which participants pre-contracted the FDI muscle prior to application of the TMS pulse (EMG amplitude>0.1 mV in the 80 ms before the pulse) were discarded from the analysis. To account for differences in coil placement between experimental blocks, MEP sizes were median- normalized within each block. Analyses of MEPs were carried out on the mean of the normalized MEP amplitudes in each condition. Analyses of both behavioural and electrophysiological data were conducted using ANOVA tests, using repeated measures where possible. Significant effects were identified based on Huynh-Feldt corrected ANOVA values, using SPSS 16.0.

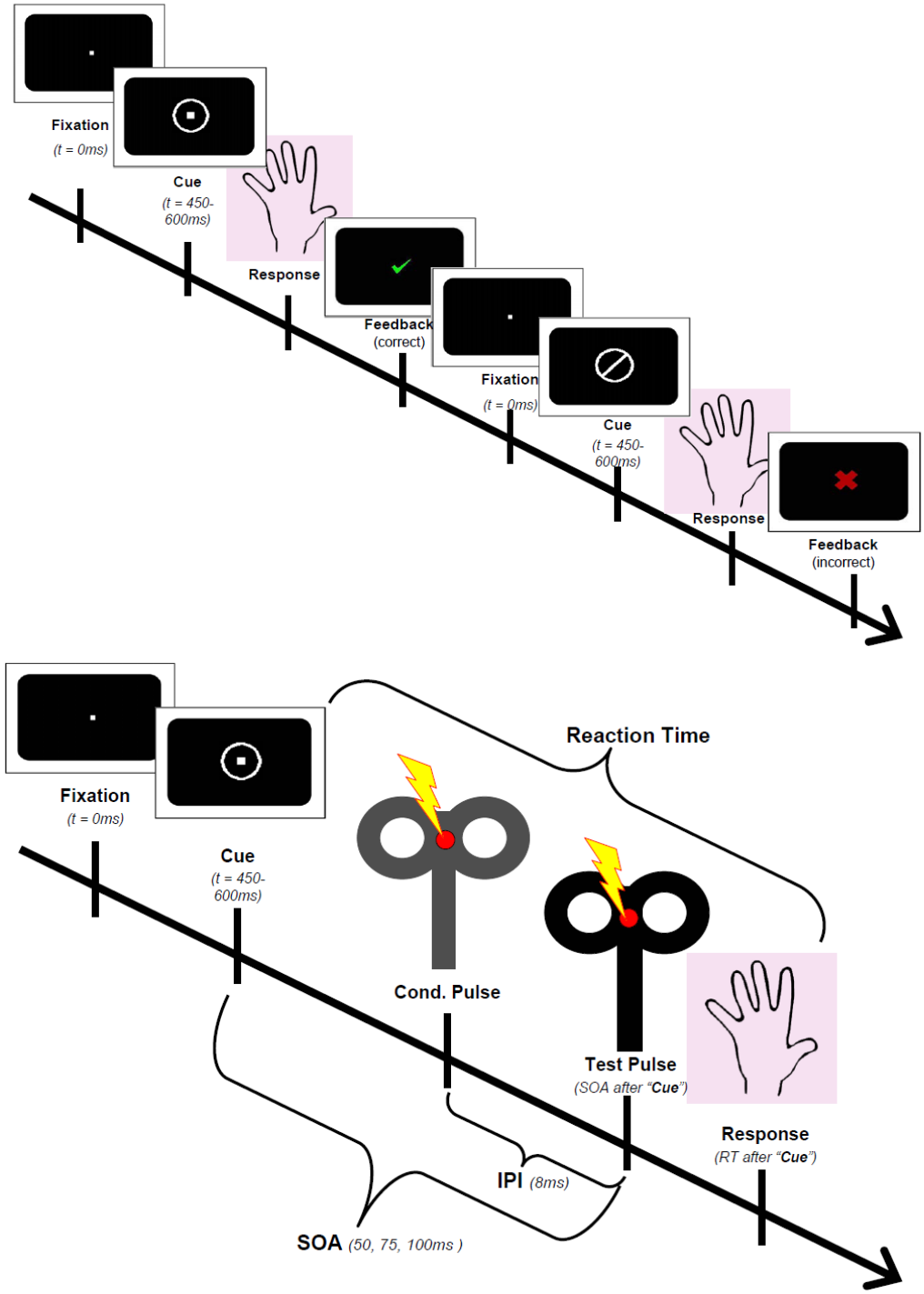


Figure 64: Behavioural task. Previous to the actual experimental TMS block subjects learned the stimulus response mappings in a training block (without TMS, top: two trials in a training block). During the actual experimental block pulses were delivered through two 55mm diameter figure-of-eight coils. Single and paired-pulse TMS was delivered at 50, 75 and 100 ms SOA with an IPI of 8 ms in the paired-pulse TMS trials (bottom).

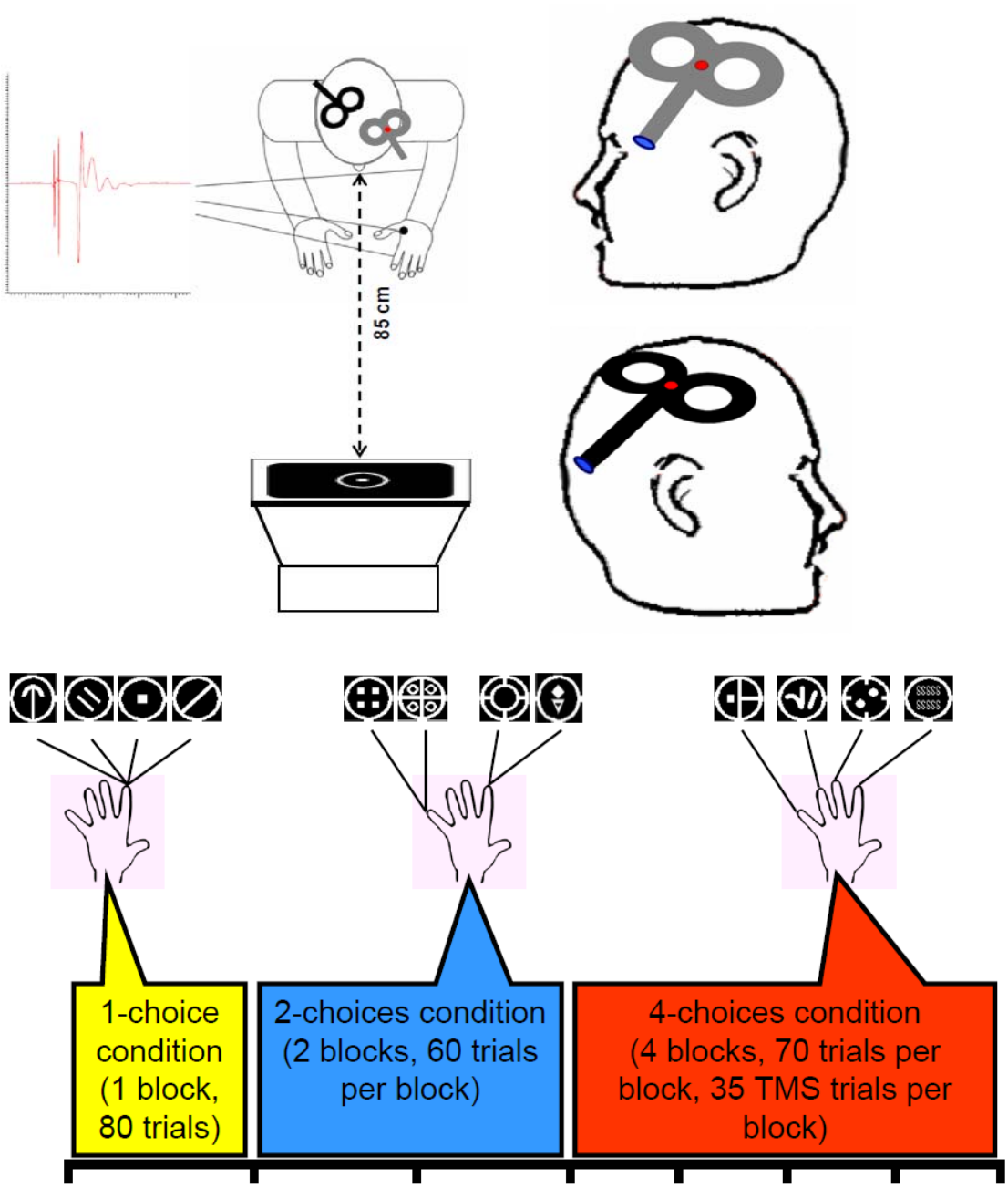


Figure 65: Experimental setup. Pulses were delivered through two 55 mm diameter figure-of-eight coils, one coil placed over the right M1 (test pulse) and another TMS coil over left PMd (conditioning pulse) (top). The experiment manipulated the response competition by varying the number of possible responses on a given experimental block. In the "one choice" block four different stimuli mapped to one single response: a left index finger button press. In the "two choices" blocks four different stimuli mapped to two eligible responses, i.e. two stimuli mapped to an index finger button press and the other two stimuli mapped to a little finger button press. In the "four choices" blocks four different stimuli mapped to four eligible responses, i.e. each stimulus mapped to different finger responses (index, middle, ring and little finger). The order of the blocks was randomised. The stimulus-response associations were learned before each experimental block in a training block.

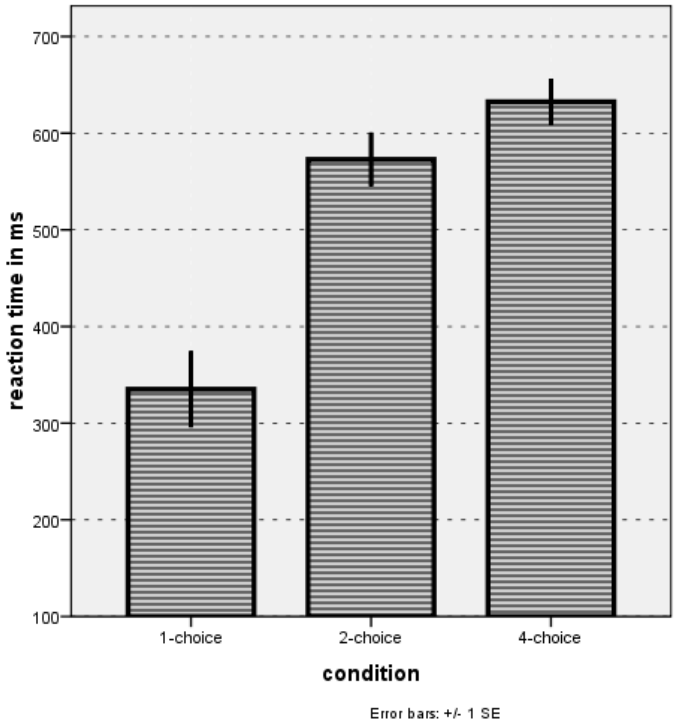
Results and Discussion

Behavioural results. Increasing amounts of information conveyed by stimulus were associated with a parametric increase in median RTs. An ANOVA on the median RTs with the within subject factor *"choice"* (1choice, 2-choices, 4-choices) revealed a significant effect of *"choice"* ($F_{2,14}$=61.421, p<0.001). Post-hoc paired samples t-tests between the median RTs showed a significant difference between RTs in the 1-choice and 2-choices condition (t_7=-7.620, p<0.001, 2-choices>1choice), between RTs in the 1-choice and 4-choices condition (t_7=-9.523, p<0.001, 4-choices>1choice), and between RTs in the 4-choices and 2-choices condition (t_7=-2.752, p=0.028, 4-choices>2choice). This replicates the findings by (Koechlin et al., 2003; Badre and D'Esposito, 2007). These differences could be interpreted in line with several different theories about lateral frontal cortex hierarchies of executive control (Koechlin and Summerfield, 2007; Badre, 2008; Badre and D'Esposito, 2009). The increase in RTs parallel to the increase of eligible choices in different blocks of the experiment could reflect an increase in competition, working memory load or information processing.

Paired-pulse TMS results. An ANOVA on the paired-pulse / single-pulse TMS MEP ratios with the within subjects factors *"choices"* (1, 2, 4 choices) and *"SOA"* (50 ms, 75 ms and 100 ms) revealed no significant effects (all p>0.15). However one-sample t-tests of the MEP ratios against baseline (=1 or 100%) showed significant inhibition for PMd-M1 resting state connectivity (t_7=-2.506, p=0.041) and significant inhibition 50 ms after stimulus onset in the 4-choice condition (t_7= -2.805, p=0.026). This suggests that PMd inhibits M1 excitability at rest and replicates the findings by (Mochizuki et al., 2004; Koch et al., 2006). Paired-samples t-tests of MEP ratios during action selection against the resting state MEP revealed significant facilitation

100 ms after stimulus onset in the four choice condition (t_7=3.328, p=0.013, 4-choice task 100 ms SOA>resting state). These results suggest that we could not find uncertainty or information-related changes in PMd-M1 connectivity during the time course of action selection. Imaging studies found a correlation between the amount of information conveyed by stimulus guiding a decision and the BOLD activity in a cortical area processing this type of information (Koechlin et al., 2003; Koechlin and Summerfield, 2007; Kouneiher et al., 2009). PMd has been suggested to be involved in the selection of movements according to learned arbitrary stimulus-response associations. We therefore hypothesised that functional PMd-M1 connectivity would be correlated with the amount of information necessary to select a response. This might suggest that (1) the magnitude of PMd-M1 connectivity increases with increasing information or (2) the latency of maximum PMd-M1 connectivity switches to later SOAs with increasing amounts of information (as information processing in PMd takes longer). We could not reliably observe any of the two effects. However this does not necessarily indicate that PMd-M1 functional connectivity does not vary with the amount of information conveyed by a stimulus. Negative results of paired-pulse TMS experiments are very difficult to interpret. It could be that we were not able to reliably sample the exact time points of behaviourally relevant PMd-M1 connectivity, because we (a) did not choose the correct SOA for TMS delivery or (b) big trial by trial variability of the time courses of functional PMd-M1 connectivity made it difficult to obtain clear-cut facilitatory or inhibitory effects at fixed time points after stimulus onset. Additionally conditioning pulse intensity, IPI, coil placement and coil orientation although chosen with care and on the basis of previous paired-pulse TMS studies investigating PMd-M1 connectivity during action selection (Koch et al., 2006; O'Shea et al., 2007b) might have been inappropriate. Although the

behavioural effects suggest that our manipulation of the amounts of information conveyed by simple stimuli worked, it might be worth to test a different experimental paradigm. Moreover it could be interesting to test uncertainty or conflict-related changes in pre-SMA-M1 connectivity using a paired-pulse TMS paradigm similar to (Mars et al., 2009). Surprise-related changes in functional connectivity between rIFC and M1 or between IFJ and M1 might be obtained with a similar paired-pulse TMS setup. It could be worth following up the question whether functional connectivity changes with uncertainty, surprise or information. Information-related changes in functional connectivity have already been shown using effective connectivity analysis on the basis of BOLD fMRI data (Koechlin et al., 2003; Koechlin and Summerfield, 2007; Kouneiher et al., 2009). Changes in M1 excitability related to uncertainty and surprise were observed using single-pulse TMS (Bestmann et al., 2008b).



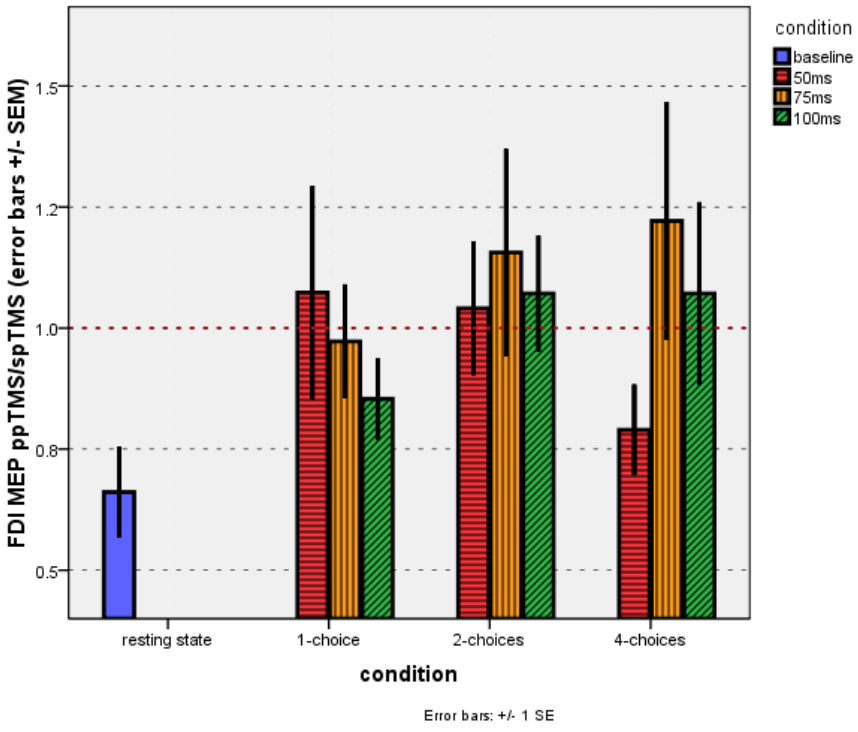


Figure 66: Median RTs showed a significant difference between RTs in the 1-choice, 2-choices and 4-choices condition. Median RTs increased with the amount of information that needed to be processed to guide an action (top). Paired-pulse / single-pulse TMS MEP ratios plotted for every condition (three SOAs: 50, 75 and 100 ms; 1-choice, 2-choices, 4-choices condition; and a resting state PMd-M1 baseline condition).

4.2. Short-Latency Influence of Medial Frontal Cortex on Primary Motor Cortex during Action Selection under Conflict[†]

Introduction

Medial frontal cortex, more specifically the presupplementary motor area (pre-SMA), is important in situations involving the direct competition (Ullsperger and von Cramon, 2001), inhibition (Nachev et al., 2007), updating (Shima et al., 1996), or reprogramming (Isoda and Hikosaka, 2007) of actions. Previous work has shown that repetitive transcranial magnetic stimulation (TMS) over pre-SMA during response conflict results in a greater activation in the motor cortex controlling the competing response, as indexed by the lateralized readiness potential (LRP) (Taylor et al., 2007), indicating that the pre-SMA is in a position to influence the motor cortex. However, the questions of how and when this influence is exerted remain. Importantly, previous studies could not investigate whether the effect of pre-SMA was predominantly to inhibit the incorrect motor response or to facilitate the correct motor response. Moreover, the limited temporal resolution of repetitive TMS means that the timing of this influence could not be determined. Finally, there has been speculation about the anatomical route by which medial frontal cortex (MFC) influences action selection (Kerns et al., 2004; Isoda and Hikosaka, 2008) and a better understanding of the timing of the influence of pre-SMA might clarify this issue. In the present study, we address these issues by looking at the functional connectivity of pre-SMA with

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primary motor cortex (M1) using the paired-pulse TMS technique. We asked healthy human participants to perform a task modelled on the paradigm developed by Isoda and Hikosaka (Isoda and Hikosaka, 2007). The task (Figure 67) required participants to either execute a prepared response or switch to another response. A test TMS pulse was delivered over left M1. On some trials it was preceded by a conditioning pulse over pre-SMA. The conditioning pulse can modulate the amplitude of the motor-evoked potential (MEP) elicited in the hand muscle by the M1 test pulse (Figure 68) providing a quantification of the influence of pre-SMA on corticospinal excitability. We then probed the differential influence of pre-SMA on M1 depending on the cognitive state of the participants, i.e., whether participants executed the prepared response or switched to another response. Previous studies of functional connectivity between dorsal premotor cortex (PMd) and M1have reported changes time-locked to task events (Koch et al., 2006; O'Shea et al., 2007b), suggesting the technique may be suitable for probing the influence of pre-SMA on M1 during cognitive control of action selection.

Materials and Methods

Participants and experimental setup. Forty healthy volunteers (age 19–40 years) with no personal or familial history of neurological or psychiatric disease participated in one or more of the experiments (approved by the Oxfordshire Research Ethics Committee and conducted in accordance with the declaration of Helsinki): 11 participants (7 females) in the switch experiment, 10 (6) in the stay experiment, 6 (3) in the M1-control experiment, 7 (5) in the PMd-control experiment, and 6 (3) in the interpulse interval (IPI) experiment. All participants were right-handed and gave written informed consent. Participants wore tight-fitting bathing caps, on which TMS

sites were marked, and earplugs to protect against TMS noise. A chin rest was used to minimize head movements.

Behavioral task. The task (Figure 67) required participants to respond with the left or right index finger in response to visual stimuli presented on a screen ~85 cm in front of them. Each trial began with the presentation of a central white square (4.7° width) followed by the presentation of flanker stimuli on each side of the central square. The flankers were always one square (width 6°) on each side, one of them red, one of them green, with trial-wise random assignment of color to side of display. After a variable delay (450–600 ms, uniform distribution), the central square became either green or red, instructing the participant to respond with the index finger on the side corresponding to the flanker of the same color. The critical manipulation imbedded in the task was that the central cue took the same color for trains of 3–7 consecutive trials (uniform distribution). This afforded participants the opportunity to prepare, in the period between the onset of the flankers and the onset of the central color cue, the response that was most likely to be required. The manipulation meant that there were two types of trials: stay trials, on which the fixation color was identical to that of the previous trial, thus allowing participants to execute the prepared response, and switch trials, on which the fixation color was different from the previous trial, thus requiring participants to inhibit the prepared response and reprogrammea response with the other hand. Stimuli were pseudo-randomly generated and a different stimulus order was used for each block and for each participant. Custom software written in Turbo Pascal controlled the experiment. Before the actual experimental session, participants were familiarized with the task for 30 trials. The main experiment consisted of 7 (switch and stay experiments) or 5 (PMd and M1-control experiments) blocks of 180

trials each. Each block contained 30 switch and 150 stay trials. The IPI experiment consisted of 5 blocks of 220 trials, each containing 36 switch and 184 stay trials.

Switch and stay experiments. During the switch and stay experiments, TMS was delivered through two figure-of-eight shaped coils, connected to monophasic Magstim 200 stimulators (Magstim Company). Test coil intensity was such that an MEP of 1–1.5 mV was elicited in the contralateral first dorsal interosseous (FDI) muscle. Conditioning coil intensity was set at 120% of the resting motor threshold (RMT), which in turn was defined as the minimum intensity, when the coil was over the M1 hotspot, needed to elicit an MEP of ~50 μV in the relaxed FDI muscle on 5/10 trials. The IPI between conditioning and test pulses was 6 ms, which has been shown to be effective in a previous study of medial premotor areas (Civardi et al., 2001). The relative frequency of switch nd stay trials remained the same in both experiments and, in both cases, TMS was delivered either 75, 125, or 175 ms after the onset of the central color cue [stimulus– onset asynchrony (SOA)] (Figure 67). These times were chosen to cover the period during which pre-SMA neuronal activity changes occurred in the experiment performed by Isoda and Hikosaka (2007) and the times when other premotor areas have been shown to exert an influence over M1 (O'Shea et al., 2007b). The test coil was placed over the position which allowed elicitation of the largest MEP for a given intensity in the FDI muscle of the righthand, with the coil held tangentially to the skull with the handle oriented posteriorly at ~45° from the mid-sagittal axis. The conditioning coil was placed with the handle pointing in the anterior direction, as close as possible to a position 4 cmanterior to electrode position Cz, previously shown to be an appropriate location for stimulation of pre-SMA (Rushworth et al., 2002b) (Figure 68). Coil positions were assessed in nine participants using Brainsight frameless stereotaxy (Rogue Research) (Figure 68).

Average Montreal Neurological Institute coordinates for the conditioning coil were [4 18 65] and therefore clearly within pre-SMA (Picard and Strick, 1996). Average coordinates for the test coil were [-40, -10, 60], just anterior to the central sulcus, consistent with previous reports of the hand area of M1. In addition, diffusion-weighted imaging (DWI) was used to further assess the anatomical pathways mediating the observed effects (supplemental Material II, available at www.jneurosci.org as supplemental material). Wefirst collected pre-SMA/M1 interaction data in two separate experiments involving different participants. During these switch and stay experiments, pulses were delivered almost exclusively on switch and stay trials, respectively. For these two experiments, a total of 14 pulse trials per hand, SOA, and pulse type (single or dual pulses) were delivered and used for the analyses on switch and stay trials, respectively. The presence or absence of TMS could not serve as precue indicating trial identity, because the pulses were only applied after the switch or stay cue had already occurred. However, six instances of pulse application on the opposite trial prevented participants from detecting, as assessed by subsequent report, any relationship between trial type and TMS delivery. For the same reason, each trial type was also presented in the absence of TMS on at least 20% of instances of that trial type in each block. TMS trials were presented at least 7 (mean 10.5) seconds apart, to ensure that pulses on adjacent trials did not influence each other. In each block, TMS trials were distributed evenly over response hand, SOA, and single- or dualpulse TMS. For the analysis of the effect of pre-SMA on M1, we thus concentrated on a between-session design: we analyzed TMS data from switch trials in one session (referred to as the switch experiment) and stay trials in a separate session (the stay experiment). This was necessary because probing both an adequate number of switch and stay trials at three different SOAs with both single and paired

pulses would have resulted (1) in the participants receiving a very large number of TMS pulses and (2) in an exceedingly long experiment.

M1 and PMd-control experiments. We tested the anatomical specificity of pre-SMA/M1 interactions in two control experiments. In the M1-control experiment, we applied TMS on both switch and stay trials at an SOA of 125 ms through a single figure-or-eight coil, connected to the stimulators via a BiStim module, placed over the hand area of M1. This is a critical control because it tests whether any observed changes in MEPs are caused by the mediating influence of pre-SMA or merely the result of processes internal to M1 (O'Shea et al., 2007b). A second control experiment tested whether pre-SMA TMS effects were caused by spreading of activation from the pre-SMA coil into the adjacent PMd. During this PMd-control experiment, the conditioning coil was placed over the right hemisphere, because the average pre-SMA location was also just within the right hemisphere, at a location 2 cm anterior and 1 cm medial to the location in right hemisphere which resulted in the largest MEP in the contralateral FDI for a given TMS intensity ("hotspot"), which has previously been shown to be a reliable landmark for PMd and dorsal precentral sulcus (O'Shea et al., 2007b). TMS intensities were the same as in the switch and stay experiments. In both of these control experiments the IPI was 6 ms, as in the switch and stay experiments. These control experiments are thus not comparable with previous paired-pulse TMS experiments probing PMd/M1 functional connectivity, which used an IPI of 8ms (Koch et al., 2006; O'Shea et al., 2007b).

IPI experiment. In a final control experiment, we tested for effects of different IPIs between conditioning pulses over pre-SMA and test pulses over M1. Single pulses and dual pulses at IPIs of 3, 6, 9, 12, and 18 ms were delivered on switch trials at an SOA of 125 ms. A total of 15 trials were presented for each pulse and hand

combination, distributed equally across five experimental blocks. Stimulation parameters were the same as in the other experiments.

Electrophysiological recording and data analysis. MEPs were recorded from the right-hand FDI muscle using Ag-AgCl electrodes in a tendonbelly montage. EMG responses were bandpass filtered between 10 and 1000 Hz, with an additional 50 Hz notch filter, sampled at 5000 Hz, and recorded using a CED 1902 amplifier, a CED micro 1401 Mk.II A/D converter, and a PC running Spike2 (Cambridge Electronic Design). Analysis of electrophysiological data concentrated on peak-to-peak amplitudes of the MEPs measured on TMS trials. Trials with incorrect or premature [reaction time (RT)<150 ms] responses, those in which the test pulse failed to elicit a reliable MEP (amplitude >0.2 mV), and those in which participants precontracted the FDI muscle before application of the conditioning pulse (EMG amplitude<0.1mV in the 80 ms before the pulse) were discarded from the analysis. Grubb's test was used to detect outliers in the obtained values of one block and these were excluded from the analysis. After this preprocessing, on average 11.45 (SEM=0.62) and 11.65 (+/-0.67) trials were included per condition in the switch and stay experiments, respectively. To account for differences in coil placement between blocks, MEP sizes were normalized within each block. Analyses of MEPs were performed on the median of the normalized MEP amplitudes in each condition. Analyses of both behavioral and electrophysiological data were conducted using ANOVA tests, using repeated measures where possible. Significant effects were identified based on Huynh-Feldt corrected ANOVA values, using SPSS 15.0. Post hoc paired-samples two-sided t tests were used to further investigate significant effects in the ANOVAs. Post hoc tests on electrophysiological data were performed on the dualpulse MEP amplitudes expressed as percentage of the respective singletrial MEP amplitude ("MEP changes").

Results

Behavioral data. ANOVAs of RTs on correct trials and of error rates, with within-subjects factor TRIAL_TYPE (switch or stay) and between-subjects factor EXPERIMENT (switch or stay experiment), showed that participants responded more slowly (420 vs 290 ms, main effect of TRIAL_TYPE: $F_{(1,19)} = 145.894$, $p < 0.001$) and made more errors (21.85% vs 1.40%, main effect of TRIAL_TYPE: $F_{(1,19)}=94.614$, $p=0.001$) in switch compared with stay trials, confirming the effectiveness of the task manipulation. Behavioral effects did not differ between the two experiments, as indicated by the absence of any other significant effects. In all three control experiments (M1-control, PMd-control, and IPI), these effects were replicated. Participants responded more slowly (M1: $t_{(5)}=15.790$, $p=0.002$; PMd: $t_{(6)}= 3.882$, $p= 0.008$; IPI: $t_{(5)}=3.733$, $p=0.014$) and made more errors (M1: $t_{(5)} =5.716$, $p=0.001$; PMd: $t_{(6)}=6.252$, $p=0.001$; IPI: $t_{(5)}=3.191$, $p = 0.025$) on switch compared with stay trials.

Pre-*SMA/M1 paired-pulse TMS during switch and stay trials.* The main question addressed in the present study was whether pre-SMA exerts a context-dependent influence on M1. An ANOVA on the MEP data gathered in the switch and stay experiments, with within-subjects factors PULSE (single vs dual) and SOA(75, 125, 175 ms) and between-subjects factor TRIAL_TYPE (switch vs stay, based on the stimulated trials in the switch and stay experiments, respectively), revealed a significant interaction between TRIAL_TYPE, PULSE, and SOA ($F_{(2,18)} = 3.533$, $p = 0.039$) (Figure 69) and between TRIAL_TYPE and SOA ($F_{(2,18)} = 4.734$, $p=0.021$). *Post hoc* tests revealed that for the switch trials, the dual-pulse MEP differed from the single-pulse baseline only at the 125 ms SOA (mean %MEP = 117.82, $t_{(10)} = 6.373$, p

= 0.001). Furthermore, within the switch experiment, MEP changes at SOA 125 ms were significantly greater than MEP changes at SOA 75 ms ($t_{(10)}$=3.361, p=0.007) and SOA 175 ms ($t_{(10)}$ = 2.591, p = 0.027). None of these effects reached significance in the stay trials. It might be argued that switches after a larger number of stay trials were anticipated by the participants. However, the modulatory effect of pre- SMA TMS on M1 was still present even on such "late" switch trials (supplemental Material IV, available at www.jneurosci.org as supplemental material).

Anatomical specificity. In the M1-control experiment, the influence of the conditioning pulse over M1 led to an increase in MEP amplitude (%MEP = 176%, main effect of PULSE ($F_{(1,5)}$ = 7.731, p = 0.039). However, this influence was no longer context specific; there was no main effect of TRIAL_TYPE ($F_{(1,5)}$ = 1.336, p= 0.300) and, crucially, no interaction between TRIAL_TYPE and PULSE ($F_{(1,5)}$ = 0.317, p = 0.598). In the PMd-control experiment, there were no significant effects (main effect of PULSE: $F_{(1,6)}$ = 1.611, p = 0.251; main effect of TRIAL_TYPE: $F_{(1,6)}$ = 2.041, p = 0.203; TRIAL_TYPE = PULSE: $F_{(1,6)}$ = 4.033, p=0.091). Although the TRIAL_TYPE_ PULSE interaction approached significance, the effect is in the opposite direction of the effect of pre-SMA, with PMd influence during switch trials trending toward an inhibitory effect on M1. Thus, the facilitatory effect on M1 activity on switch trials is mediated via a pre-SMA/M1 pathway and does not reflect processes internal to M1 or spreading of activation to PMd (Figure 69). To further establish the anatomical specificity of the present effects, we correlated theMEPchanges in the switch experiment with individual differences in the white matter integrity of anatomical tracts (Fig. 4*c*; supplemental Material II, available at www.jneurosci.org as supplemental material). This analysis suggested that the effects were mediated solely by pathways underlying premotor and primary motor areas.

Selectivity of pre-SMA/M1 interactions. We further investigated the specificity of the pre-SMA influence on M1 on switch trials by analyzing data from the switch experiment for trials in which participants switched toward and ultimately selected the hand contralateral to the stimulated M1 (here, right hand), and trials in which participants had prepared, but ultimately inhibited, the hand contralateral to the stimulated M1 and finished the trial by switching to the hand ipsilateral to the stimulated M1. Although there was unambiguous evidence that pre-SMA facilitated the M1 corresponding to the unprepared hand to which subjects were about to switch, evidence for a significant influence of pre-SMA on the M1 corresponding to the preprepared hand that was not to respond was less clear-cut (supplemental Material III, available at www.jneuroci.org as supplemental material). The absence of any inhibitory influence on such trials may be considered surprising. A final experiment was run to test whether this effect might be present at other IPIs. This experiment (Fig. 4*a*) replicated the significant facilitation of MEP size at SOA 125 using a 6 ms IPI when switching toward the stimulated hand compared with baseline ($t_{(5)} = 3.193$, $p = 0.024$). There was no significant modulation, including no inhibitory modulation, of MEPs when switching away from the stimulated hand at any IPI (all $p = 0.45$). There was also a significant facilitation when switching toward the stimulated hand at a 12ms IPI ($t_{(5)} = 3.726$, $p = 0.014$). Additionally, there was a significant difference in MEP facilitation in the IPI experiment when switching toward the stimulated compared with the nonstimulated hand at a 6 ms IPI ($t_{(5)} = 2.932$, $p = 0.033$). The presence of a hand-selective facilitation effect here but not in the switch experiment might be explained by individual differences in RTs. If the pre-SMA facilitates the correct response, as suggested by the hand difference in the IPI experiment, then the difference between influences on the motor areas might be greater later in the

response selection process. Hence, we assessed whether, in the combined data from the switch and IPI experiments, there was evidence that shorter switch RTs were associated with a greater relative facilitation of the contralateral, compared with the ipsilateral hand. After rejection of two outliers using Grubb's test, we indeed found such a negative correlation (Pearson= 0.510, $p = 0.026$) (Figure 70).

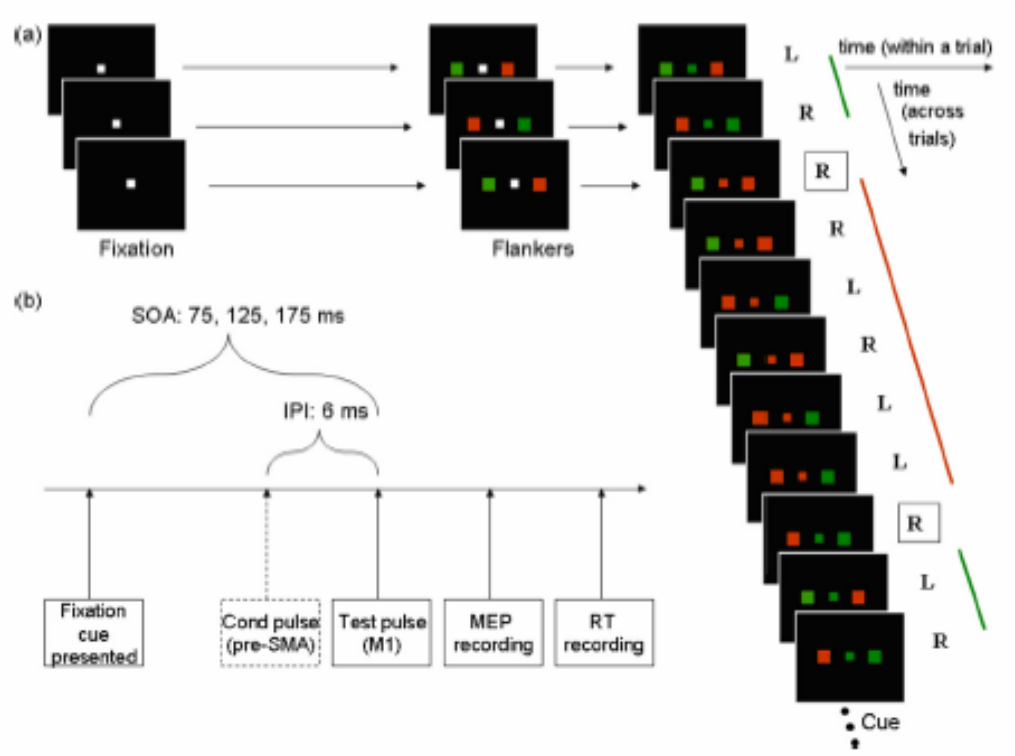

Figure 67: *a*, On each trial of the action reprogramming task participants were presented with a centrally displayed white square. Subsequently, two colored flankers (red and green, sides random) appeared on either side of fixation. Four hundred and fifty to six hundred milliseconds after flanker onset, a central colored cue appeared, to which participants responded with the index finger of the hand on the side with the congruent color. Trials were blocked into groups with the same cue color, so that as soon as flankers were presented, participants could anticipate and thus prepare an action based on the cue color presented in the previous trial. The prepared response would, however, be incorrect when the central cue color changed from one trial to the next (switch trials, boxed letters). Correct actions are indicated by "R" (right) and "L" (left). *b*, The M1 test pulse was applied 75, 125, or 175 ms after the central color cue onset. A pre-SMA conditioning pulse preceded the M1 test pulse by 6 ms on half of the TMS trials.

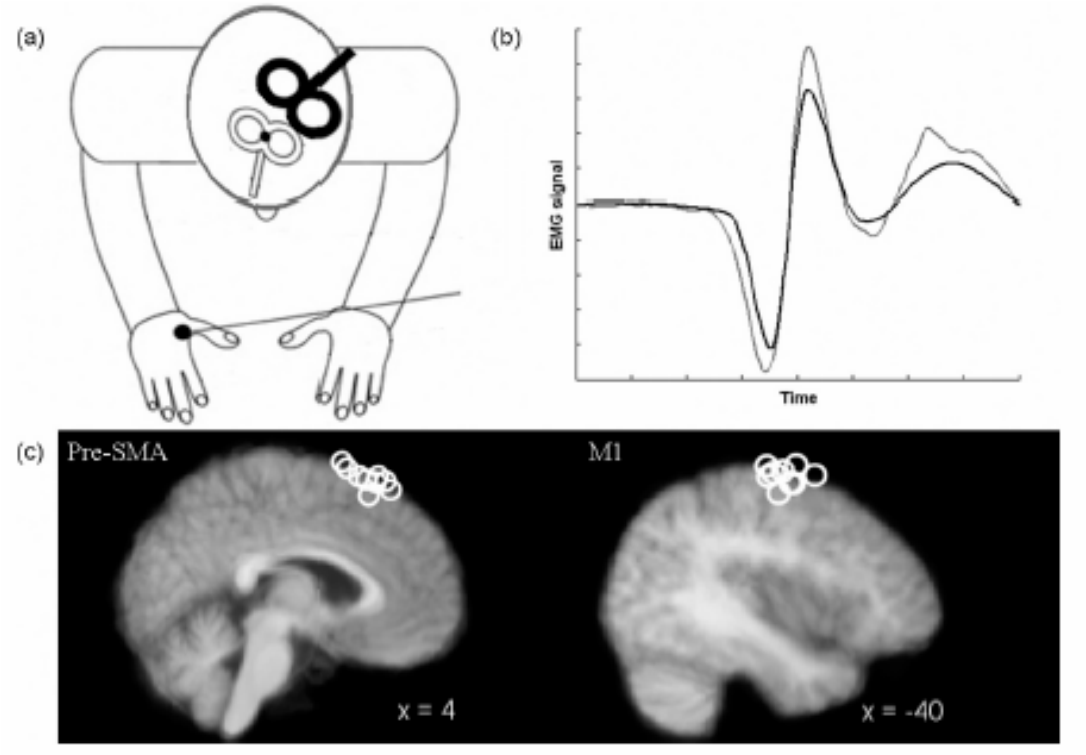

Figure 68: *a*, In the switch and stay experiments, the test coil (black) was placed over left M1, whereas the conditioning coil (white) was placed over pre-SMA. *b*, Example of MEPs recorded on a single pulse (black) and a dual pulse (gray) trial. The conditioning pulse can modulate peak-to-peak MEP amplitude. *c*, Sagittal views of the mean anatomical image indicating pre-SMA (left) and M1 (right) TMS sites. Each circle represents the coil location in one participant.

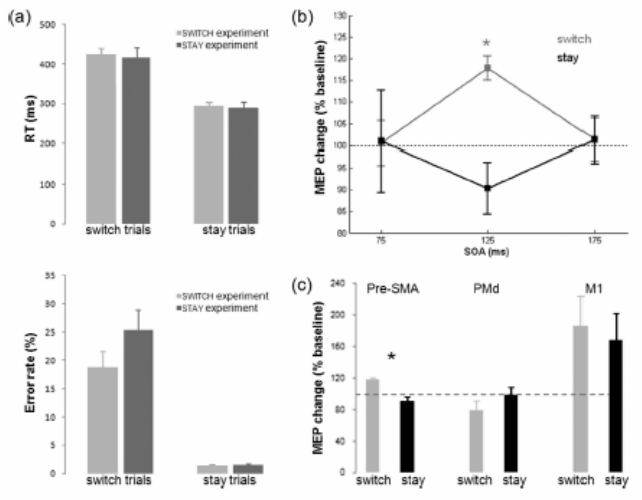

Figure 69: *a*, Behavioral data in the main switch and stay experiments indicate participants reported faster (top) and more accurate (bottom) on switch compared with stay trials. *b*, The effect of pre-SMA conditioning pulses onM1test pulse-elicited MEP amplitudes was specific to behavioral context and SOA. Pre-SMA/M1 functional connectivity significantly increased on switch trials. * indicates significant modulation of MEP amplitudes in dual-pulse compared with respective single-pulse trials. *c*, Contextspecific facilitations of MEP amplitude at a SOA of 125 ms were only present when the conditioning coil was place over pre-SMA and were absent when the conditioning coil was over PMd or M1.

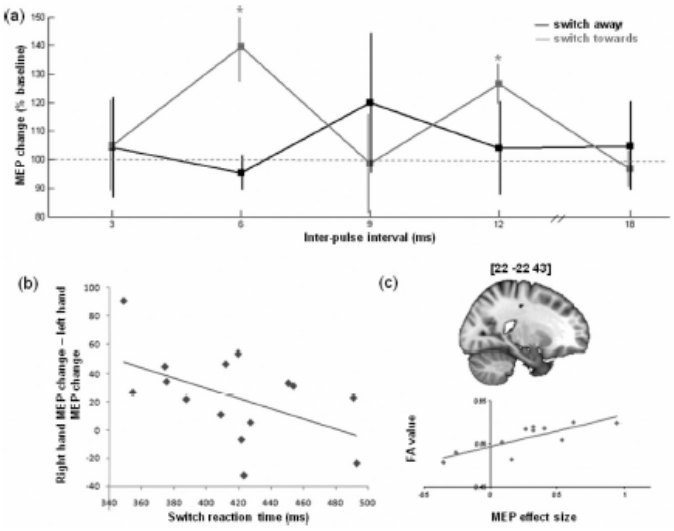

Figure 70: Additional results. *a*, Significant MEP facilitation was seen when switching toward the contralateral hand at 6 and 12 ms interpulse intervals, but no significant effect was seen when switching to the ipsilateral hand. *b*, A negative correlation was present between the relative facilitation of thecontralateral, compared with the ipsilateral, hand and the RT on switch trials. *c*,Example location of significant correlation between MEP effect size and white matter intensity and scatter plot of individual data within this cluster.

Discussion

Our results show that pre-SMA influences corticospinal excitability at a short latency of 6 ms. The effect is temporally specific to a period 125 ms after movement instruction, anatomically specific to pre-SMA, and occurs only during action reprogramming. Previous studies have used paired-pulse TMS to demonstrate increased functional connectivity between premotor and motor regions during the initiation of a sensorimotor transformation (Koch et al., 2006). The current study is the first to employ this technique to study action selection under conditions of response conflict, specifically in the case of the inhibition of a prepared response and the selection of an alternative, a process we refer to as action reprogramming. Action reprogramming recruits a number of neural structures in addition to those involved in normal action selection (Ullsperger and Von Cramon, 2001; Mars et al., 2007), including pre-SMA. Pre-SMA modulation of M1 did not reflect the initiation of a sensorimotor transformation, which was similar on both stay and switch trials; instead, the effects reported reflect a change in expectation, built up over the course of several trials, about which of two sensorimotor transformations should be enacted. The fact that earlier paired-pulse TMS studies (Koch et al., 2006; O'Shea et al., 2007) focused on the initial action selection, whereas the current study focuses on the more complex process of reprogramming an action, might explain why the timing of the effects in the current study were at a later SOA, 125 ms, than in the earlier studies. Furthermore, at the critical SOA the latency (IPI) of influence of pre-SMA over M1 was shorter (6 ms) than the latency studied in previous PMd/M1 experiments (8 ms). Our IPI control experiment showed no effects at the latency normally used in PMd/M1 experiments. The pattern of influence of pre-SMA on M1 reflected whether

the stimulated M1 needed to be inhibited or facilitated. Pre-SMA facilitated M1 activity associated with the correct, but unprepared, action. The M1 associated with the incorrect, but prepared, action was not, however, inhibited at any pre-SMA/M1 stimulation latency. It was relatively less facilitated by pre-SMA stimulation than the correct hand M1, and this relative difference was most prominent in the fastest switchers. This may indicate that pre-SMA normally brings about action reprogramming through a complex combination of facilitatory and inhibitory influences that are not mimicked by the artificial activation patterns induced by TMS, or that pre-SMA is concerned with a higher level of control. That pre-SMA/M1 effects are reliably seen at latencies as short as 6ms in both switch and IPI experiments is important, because it constrains hypotheses about the routes by which pre-SMA might influence M1. Although a basal ganglia/subthalamic route might mediate important aspects of action inhibition (Isoda and Hikosaka, 2008), even the most direct path through this route is associated with neuronal latencies in excess of the 6 ms pre- SMA/M1 latency (Nambu et al., 2000). Similar considerations also rule out that a pathway via lateral prefrontal cortex mediated these short-latency effects, even if that pathway is important for regulating longer term changes in cognitive control (Kerns et al., 2004). These observations are consistent with the fact that correlations between white-matter integrity and changes in pre- SMA/M1 functional connectivity were restricted to dorsomedial frontal cortex. Previous work has suggested that corticospinal excitability is modulated by recent trial history (Bestmann et al., 2008) and it might be argued that differential switch expectancies, based on the number of preceding stay trials, are what actually influence pre-SMA/M1 functional connectivity. Although the present study was not designed to test such a hypothesis, a preliminary investigation showed that switch-related pre-

SMA/M1 effects were apparent even when the switches were very predictable, because many stay trials had elapsed. In conclusion, the pre-SMA influencesM1at a short latency of 6ms during action reprogramming, but not during simple action selection. The effect is specific to the cognitive context, anatomically specific to pre-SMA, and temporally specific, being strongest 125 ms after the onset of a cue, indicating the need for action reprogramming.

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