Transcranial magnetic stimulation (TMS) has become a mainstay of cognitive neuroscience, thus facing new challenges due to its widespread application on behaviorally silent areas. In this review we will summarize the main technical and methodological considerations that are necessary when using TMS in cognitive neuroscience, based on a corpus of studies and technical improvements that has become available in most recent years. Although TMS has been applied only relatively recently on a large scale to the study of higher functions, a range of protocols that elucidate how this technique can be used to investigate a variety of issues is already available, such as single pulse, paired pulse, dual-site, repetitive and theta burst TMS. Finally, we will touch on recent promising approaches that provide powerful new insights about causal interactions among brain regions (i.e., TMS with other neuroimaging techniques) and will enable researchers to enhance the functional resolution of TMS (i.e., state-dependent TMS). We will end by briefly summarizing and discussing the implications of the newest safety guidelines.

© 2010 Elsevier Ltd. All rights reserved.
1. Introduction

Cognitive neuroscience is concerned with the scientific study of the neural substrates of mental processes and their behavioral manifestations. Over several years, different neuroimaging techniques have provided correlational maps of cognitive processes in the adult human brain at different levels of temporal and spatial detail. These imaging techniques have in common that the signal and information that is gathered on brain function, such as changes in regional cerebral blood flow (rCBF), blood oxygenation level dependent (BOLD) signals or evoked potential changes (electroencephalography, EEG, and magnetoencephalography, MEG) covary with the mental process of interest (Walsh and Cowey, 2000). However, correlational approaches cannot differentiate between epiphenomenally and causally activated neuronal populations. Moving beyond a merely correlational description of the relationship between brain and behavior was the fresh approach offered by transcranial magnetic stimulation (TMS). A TMS-induced change in behavior (usually measured in reaction times, RTs, or accuracy) can be used to inform models of causal relations between specific brain regions and individual cognitive functions (Robertson et al., 2003). TMS has nowadays become a standard stimulation technique for the noninvasive investigation of cognitive function (Pascual-Leone et al., 2000), whereby neural tissue is stimulated by using the principles of electromagnetic induction to generate electrical currents in the brain (Barker et al., 1985). The key features of the technique are that the TMS stimulator delivers a large current in a short period of time and the current flowing in the TMS coil produces a magnetic field that lasts for only about a millisecond. Provided that appropriate stimulation parameters are selected, such rapidly changing magnetic field easily penetrates the scalp and skull and induces an electrical field sufficient to stimulate neuronal activity and change the pre-stimulus dynamics of neuronal firing in the stimulated region. Although its precise mechanisms of action are still far from clear, TMS is thought to activate neuronal axons in the cortex and subcortical white matter, rather than the cell bodies of cortical neurons (Ridding and Rothwell, 2007). TMS focality is currently expressed in square centimeters as a measure of cortical surface and can be optimized by combining two circular coils to form a figure-of-eight or butterfly coil. A standard 70-mm butterfly coil (56 mm inside turn diameter, 90 mm outside turn diameter, 73 mm mean diameter and nine turns of copper wire per winding) is thought to maximally stimulate about 1–2 cm² of cortex beneath its central junction. However, the behavioral spatial resolution of TMS effects ranges from 0.5 cm to 1.5 cm apart, depending on the specific tissue that is being stimulated (e.g., Beckers and Hötberg, 1992; Brasil-Neto et al., 1992a; O’Shea and Walsh, 2007a). The rapid decline in magnetic field strength with distance depending on coil size and stimulation intensity is a critical issue in cortical stimulation and limits its application to areas that are no deeper than 2–3 cm from the skull surface. For a standard butterfly coil with a maximum field strength of 2 T, the induced field is more or less hemispherical, peaking at about 2.5 cm from the surface of the skull. Considering skull thickness and the presence of interposed membranes, this implies that most brain tissue cannot be directly excited by TMS. It might be, however, indirectly excited by propagation of activity from the region underneath the coil via synaptic connections. TMS technology is not suitable at present to stimulate deep brain structures such as amygdala, hippocampus or the thalamus. It is possible that with future technical improvements, focality will be measured in units of volume. For example, the use of nonlinear coil materials (H-coils) for stimulation of deeper neural pathways has already been explored (Levkovitz et al., 2007; Roth et al., 2007), although the advantage of this coil with regard to depth of stimulation in comparison to the figure-of-eight coil has not been demonstrated yet (Fadini et al., 2009).

In this review we touch on some practical considerations that are relevant for researchers wishing to utilize this technique to study cognitive processes and their neural basis. We will also alert the reader to important technical and methodological considerations for the use of TMS in cognitive neuroscience. In doing this, we will mainly refer to evidence from studies on number processing (see e.g., Rusconi and Umiltà, 2008; Sandrini and Rusconi, 2009), our main field of expertise. All these studies will exemplify nicely how TMS can be used to investigate a variety of issues in the cognitive neurosciences in general, including location, timing, lateralization and functional relevance of the neural correlates underlying number processing. Finally, new research directions will be pointed out, that might guide the investigation of functional cortical interactions between two connected brain areas, the influence of TMS in remote brain areas effectively connected with stimulation site, the dynamics of cortical connectivity and how to enhance the functional resolution of the technique. Since methodological issues and technological advances cannot be entirely separated from safety and ethical considerations, the current synthesis will take into account the new safety guidelines (Rossi et al., 2009), which are meant to update the ones (Wassermann, 1998) that regulated TMS application during its first exciting decade of experimentation in laboratories all over the world.

2. Neural mechanisms of TMS-induced effects

Despite the widespread usage of TMS in basic research, its exact mechanisms of action and interactions with ongoing neural activity remain unclear.

Data from animal studies using TMS in combination with direct electrophysiological recording (e.g., Allen et al., 2007; de Labra et al., 2007; Moliadze et al., 2003; Pasley et al., 2009), metabolic (Valero-Cabré et al., 2005) or with optical imaging techniques (Allen et al., 2007) have provided insight into TMS effects, for both primary motor cortex (M1) and other neural structures, including primary visual cortex (V1). TMS has been shown to elicit distinct and complex episodes of enhanced and suppressed activity at the cortical level (Moliadze et al., 2003; Allen et al., 2007) also depending on the state of the stimulated area (Pasley et al., 2009). Allen et al. (2007) investigated effects induced by short TMS pulse trains at various frequencies (1–8 Hz) on neural and hemodynamic responses of spontaneous and visually evoked activity. For spontaneous activity their main result was a TMS-induced immediate increment lasting up to 60 s. In a task-free context, TMS appears thus to enhance activity in the area underneath the coil. Conversely, the same TMS stimulus suppressed for more than 5 min the neural activity evoked by a visual stimulus (Allen et al., 2007).

The reason why this happens is not yet completely clear. One possibility is that TMS induces intracortical inhibition. It has been indeed found that an induced electric field can increase GABA levels, which in turn suppresses activity (Mantovani et al., 2006). An alternative hypothesis is that TMS amplifies background activity, instead of reducing signal strength. In other terms, TMS might introduce random neural noise (Moliadze et al., 2003). It is also possible that TMS acts by disrupting the temporal relation between neurons belonging to a more extended circuit activated by the task (Pasley et al., 2009). Finally, it is important to point out that these effects were also dose-dependent; scaling with stimulation frequency (1–8 Hz) and duration (1–4 s), as they became more pronounced when longer TMS trains and higher stimulation frequencies were used.

In humans studies, TMS is generally applied with the aim to disrupt the neural activity that subserves cognitive processing (i.e., as a ‘virtual lesion’ method). However, the ‘virtual lesion’ hypothesis
does not provide insight about the precise mechanisms of action of TMS (Miniussi et al., 2010). In fact, it is unclear whether TMS suppresses neural signals (Harris et al., 2008), adds random neural activity to the ongoing processing (Ruggiero et al., 2010; Walsh and Cowey, 2000) or is a combination of each, with the exact balance depending on stimulation intensity but also on the anatomofunctional characteristics of the stimulated tissue (see Miniussi et al., 2010; Siebner et al., 2009a). For instance, Harris et al. (2008) investigated TMS-induced effects on V1 during a visual discrimination task. The subjects were asked to discriminate the orientation of visual gratings while the level of image noise in the visual stimulus was concurrently manipulated. The effect of interaction between TMS and stimulus noise on the visual discrimination threshold was interpreted as showing that TMS decreased signal strength without affecting neural noise. Ruggiero et al. (2010) investigated the effect of TMS (brief pulses) applied to V5/MT on the shape of the psychometric function in a visual motion-direction-discrimination task in which the visual stimulus featured two elements: a visual signal (dots that moved coherently in one direction) and visual noise (dots that moved randomly in many directions). The results showed that a TMS train induced a decrement in the slope of the psychometric curve and therefore seemed to add neural noise to the perceptual process.

Although the results of these two studies, which were based on different rationales, and employed different paradigms, sites and TMS parameters, are not in agreement, they suggest that through a psychophysical approach it is possible to highlight the functional activation state of the target area and link in a specific way the effects of TMS on behavioral performance and its putative effects at the neural level. The interpretation of TMS effects and the establishment of causal relations between activity in the targeted brain area and a given behavioral effect is thus much more complex than suggested by the virtual lesion metaphor. It is true that such metaphor has guided most of the published cognitive studies and provides a shortcut to predicting, describing and interpreting TMS effects on behavior, however, at least basic awareness of the main issues in TMS physiology is necessary to devise a correct experiment design and draw reliable conclusions (see e.g., Di Lazzaro et al., 2008; Siebner et al., 2009a; Ziemann, 2010). Studies of the molecular effects of noninvasive stimulation in the human brain will become increasingly relevant from the cognitive neuroscience perspective as the techniques of cortical stimulation, will also vary. Nowadays, a simple method for adjusting MT to account for variations in cortical distance is available (Stokes et al., 2005, 2007), providing a more accurate calibration than unadjusted MT for the effective application of TMS in cognitive neuroscience. It shows that for every millimeter from the stimulating coil, an additional 2.5–3% of TMS output is required to induce an equivalent level of brain stimulation to the motor cortex. Such gradient also depends strongly on the motor threshold itself (lower MT = lower gradient).

### 3. How to choose the most appropriate TMS protocol?

The selection of stimulation parameters (intensity, frequency and duration) and paradigm relies on a difficult and often arbitrary decision. Here below we will provide an essential overview of the most common issues that the cognitive neuroscientist is likely to encounter in the process of planning a TMS experiment.

#### 3.1. Stimulation intensity

Resting motor threshold (rMT) has been classically defined as the amount of TMS machine output (intensity) necessary to produce a motor evoked potential (MEP) exceeding a defined peak-to-peak amplitude (usually 50 μV) 50% of the time in a finite number of trials (usually 10). Accurate estimation of rMT is of utmost importance in both research and clinical studies as it is the unit most commonly used for TMS dosing (Wassermann, 2002). The original guidelines for assessment of rMT by the International Federation of Clinical Neurophysiology suggest that the TMS operator starts with a subthreshold TMS intensity and increases in steps of 2% or 5% of machine output until a stimulus strength is reached with “approximately” 50% successful MEPs in 10–20 consecutive stimuli (Rossini et al., 1994). In a revised proposal, Rothwell et al. (1999) stated that the procedure should start with a suprathreshold value from which TMS intensity is decreased in steps of 2% or 5% until a level is reached below which reliable responses disappear (where the definition of reliable response is that at least 50% successful MEPs are observed in 10–20 stimuli). However, neither procedure describes in detail how the sub- or suprathreshold strength value to initialize the procedure should be obtained.

Conventionally, hand muscles are chosen as a reference for the determination of the individual MT. Most researchers use the first dorsal interosseous (FDI) as target muscle (Kansaku et al., 2007; Oliveri et al., 2004b). MT can be measured also in a tense muscle (active motor threshold, aMT). In fact, it is the minimal intensity of stimulation required to produce an MEP having amplitude of about 150–200 μV on more than 5 out of 10 trials while the subject is maintaining a voluntary contraction of about 10–20% of maximum contraction using visual and/or auditory feedback. Studies have demonstrated high concordance between MT estimations using electromyogram (EMG) and visual inspection (Pridmore et al., 1998; Stokes et al., 2005). Therefore, in some studies applying TMS over non-motor areas, the MT is established as the lowest stimulation intensity that would result in a visible movement in a relaxed muscle (Rusconi et al., 2005, Exp. 1; Sandrini et al., 2004) or in a tense muscle (Göbel et al., 2001, 2006).

TMS protocols expressed as a percentage of MT allow stimulator output to be calibrated individually to an overt physiological response, even when applied to non-motor cortical regions (e.g., Andres et al., 2005, 130% of rMT; Göbel et al., 2006, 110% of aMT; Sandrini et al., 2004, 110% of rMT; Oliveri et al., 2004b, 90% of rMT; Rusconi et al., in press, 110% of aMT).

Individual differences in MT are closely related to variations in the cortical depth of M1. It is therefore reasonable to assume that, when the intensity of TMS applied to non-motor regions is based on MT, variations in depth from the overlying scalp surface will result in different levels of effective stimulation. Indeed, if targeted cortical sites vary in depth from the stimulating coil, the strength of the magnetic field necessary to reach them, and thus the amount of cortical stimulation, will also vary. Nowadays, a simple method for adjusting MT to account for variations in cortical distance is available (Stokes et al., 2005, 2007), providing a more accurate calibration than unadjusted MT for the effective application of TMS in cognitive neuroscience. It shows that for every millimeter from the stimulating coil, an additional 2.5–3% of TMS output is required to induce an equivalent level of brain stimulation to the motor cortex. Such gradient also depends strongly on the motor threshold itself (lower MT = lower gradient).

It is important to mention that MEP threshold varies widely in the healthy populations. Wassermann (2002) estimated that experimental error and other unstable determinants of threshold may account for about 36% of the across subjects variability at rest and about 50% during voluntary activation. The remaining between-subjects variability in MEP threshold presumably results from relatively stable biological differences between individuals. Most TMS studies did not find any differences in excitability threshold of the primary motor cortex (M1) depending on age (Rossi et al., 2004; Oliverio et al., 2006). Other studies suggest, however, a possible association between healthy aging and an increase in motor threshold (Rossini et al., 1992; Peinemann et al., 2001). Such discrepancies likely originate in technical and experimental differences.

Stimulation over non-primary motor areas often does not produce as readily an objective, quantifiable response (but see speech arrest on left inferior frontal gyrus (IFG)/Broca’s area and slowing of contraversive saccades during frontal eye fields (FEF) stimulation).
One important exception is, in most individuals, the visual cortex (e.g., V1, V4 and V5), whose stimulation can elicit phosphenes (bright spots of light in the visual field) that can be reported by subjects but cannot be directly quantified by observers (Boroojerdi et al., 2000). While phosphenes elicited by V1-TMS are stationary, those evoked by TMS on V5 are often moving. Their characteristics therefore reflect the perceptual specialization of a given area.

Analogous to MT, phosphenes threshold (PT) is the lowest stimulation intensity at which phosphenes (stable or moving) are perceived in at least 5 of 10 stimulations.

For example, Salillas Pérez et al. (2009) delivered TMS (110% of PT) over the ventral intraparietal sulcus (vIPS) to determine effects on performance in motion detection and number comparison tasks. The rationale of this study is based on data supporting a link between representations of number and space (for reviews, see Fias and Fischer, 2005; Hubbard et al., 2005; Umiltà et al., 2009). More precisely, because motion perception can influence visuospatial imagery processes (Logie, 1995), it was predicted that it could influence numerical representations as well. The finding that vIPS-TMS results in impaired efficiency in both motion perception and number comparison may suggest that these processes share a common neural substrate, although, given the limited spatial resolution of TMS when used as a virtual lesion, this should be interpreted with caution.

Traditional methods of determining stimulation threshold have to take also into consideration two parameters that have a significant impact on it: stimulus waveform (monophasic vs. biphasic) and current direction. As for waveform, in the past most TMS studies on M1 were performed with monophasic waveforms, in particular those examining MT (Mills and Nithi, 1997). The new generation of stimulators is however capable of producing biphasic pulses, and studies on M1 suggest that, for a given amplitude of initial current, biphasic stimulation is more effective than monophasic stimulation (Sommer et al., 2006). As for current direction, in order to obtain responses by lower stimulus intensities in M1, induced currents from monophasic pulses should flow in a posterior-to-anterior direction, and from biphasic pulses in the posterior-to-anterior direction during the second phase, and thus in the opposite direction from the first phase (Kammer et al., 2001a). This suggests that the coil should be positioned in opposite directions depending on whether a monophasic or a biphasic pulse is used for stimulation. In the visual cortex, PT is lower if the direction of the induced current is oriented form lateral to medial in the occipital lobe rather than vice versa (Kammer et al., 2001b). The seemingly reasonable assumption that a relevant proportion of TMS threshold measures from different neocortical regions could reflect a shared component of within-individual responsiveness to TMS has been criticized by studies that did not find any correlation between MT and PT. It should, however, be noted that such studies did not take intra-individual variations in scalp–cortex distance between M1 and V1 in account (Antal et al., 2003; Boroojerdi et al., 2002; Gerwig et al., 2003; Stewart et al., 2001; but see Deblieck et al., 2008; see also Oliver et al., 2009, for the finding of a correlation between MT and the intensity level at which TMS is effective on a parietal site). As a consequence, it has become common practice to use a fixed intensity defined as a percentage of the stimulator output (see Cappelletti et al., 2007; Cattaneo et al., 2009b; Cohen Kadosh et al., 2007a; Dormal et al., 2008; Knops et al., 2006; Rusconi et al., 2005, Exp. 2; 2007). This approach reduces experiment duration and limits the number of TMS pulses, by eliminating the preliminary phase of threshold hunting. Stimulation intensity is usually fixed in these studies at the lowest stimulation intensity that can successfully affect behavior when TMS is delivered over the area of interest, based on the literature and/or the researcher’s previous experience (see Fig. 1 for a schematic summary regarding stimulation intensity).

On a conclusive note, it is worth mentioning that a comprehensive and individually tailored approach (encompassing not only stimulation intensity but also brain-specific coil orientation, stimulus duration, etc.—see below) is not yet possible with current technical facilities; it may be implemented, however, in a not-so-far future, when fine-grained information regarding individual

---

**Fig. 1.** Schematic illustration of different approaches used to set the intensity of stimulation.
anatomy and function data will be made available to neuronavigation systems (Wagner et al., 2009). Ideally it will also be possible to model the current flow over different areas by taking into account not only scalp–cortex distance but also the specific tissues, with their conductivity characteristics, that fill such distance under different scalp regions.

3.2. TMS paradigms

TMS can be applied as single pulses (spTMS) or as trains of pulses and in the latter case it is named repetitive transcranial magnetic stimulation (rTMS; for review see Pascal-Leone et al., 2000). By applying spTMS at variable times during task execution (on-line paradigm, where both stimulation and task performance occur concurrently), it is possible to investigate with a temporal resolution of a few tens of milliseconds (ms) at what exact time point neural activity at the stimulation site is critical for successful task performance (chronometry of functional relevance). In spTMS studies, the interval between pulses has to be sufficiently long to prevent interactions between consecutive pulses. However, an interval of about 6–7 s between pulses may be sufficient.

Andres et al. (2005) used spTMS to investigate the involvement of a portion of the bilateral IPS in number comparison, which is directly related to the representation of number magnitude. TMS pulses were delivered over left and right IPS, either unilaterally or bilaterally (in which case two coils were made to discharge simultaneously), while participants were engaged in a single-digit number comparison task against a fixed standard (5). The timing of the TMS pulses was varied: stimulation could occur 150, 200, or 250 ms after digit presentation. When the distance between target and reference numbers was small, responses were significantly slower in unilateral left and bilateral stimulation conditions than in unilateral right stimulation and control conditions. When numerical distance was large, responses were significantly slower in the bilateral stimulation only. Left and right parietal cortices, therefore, showed a different resolution in single-digit comparison, the left being necessary for fine discrimination, and both right and left being able to support coarser comparisons. What remains unclear is whether, by having a larger range of TMS timings, effects could be modulated. Event-related potentials (ERPs) studies indicate that number semantic processing from Arabic input occurs between 170 and 240 ms (Dehaene, 1996). Introducing TMS timings beyond the +150/250 ms window might thus reveal a more complex picture of the interaction between left and right IPS.

A nice demonstration, of how TMS can be used to differentiate distinct time points of functional relevance within a brain region, has been provided by Chambers et al. (2004).

The authors applied spTMS to the parietal cortices, at 12 different time points (30-ms intervals) starting at 30–360 ms after the onset of the target, to reveal which part of the parietal cortex, and at what time, is functionally relevant for spatial attention shifts. They could reveal that activity in the right angular gyrus (AG) is crucial at two distinct time points during reorienting of spatial attention (between 90 ms and 120 ms; and between 210 ms and 240 ms after stimulus onset), probably reflecting receipt of information via two anatomically distinct visual pathways.

The possibility to cover a larger time window is offered by rTMS in which pulse trains with rates up to 50 Hz are delivered. Lengthening the duration of on-line rTMS is presumed to cause more disruption by virtue of temporal summation of the effects of the stimulation.

Sandrini et al. (2004) found that rTMS applied over left supra-marginal gyrus (SMG) slowed down RTs when subjects were asked to select the largest digit among a pair, whereas right-SMG rTMS had no effect. However, rTMS did not interact with numerical distance. This result could therefore be interpreted as evidence that the processing chain is interfered with before accessing magnitude (i.e., operations between input and semantics are slowed down). Another possible explanation is interference on motor response selection, a process in which the left SMG is thought to play a fundamental role (e.g., Rushworth et al., 2001). Rusconi et al. (2007) found an involvement of the anterior portion of posterior parietal cortex (PPC) in a typical stimulus–response (S–R) correspondence (Simon) effect, but not in the Spatial-Numerical Association of Response Codes (SNARC) effect, thought to be driven by number magnitude. In contrast, they found that the posterior portion of PPC disrupted the numerical S–R correspondence effect. Likewise, Göbel et al. (2001) found interference, mostly on the left posterior PPC, when participants were performing a magnitude task and rTMS was delivered on stimulus presentation.

The spTMS-induced change in neuronal activity can last for roughly 40–60 ms (Amanian et al., 1989; Brasil-Neto et al., 1992b). If short burst of rTMS are applied, the influence of TMS on neuronal activity can be prolonged (Pascal-Leone et al., 2000). This implies that temporal specificity of rTMS is much less than spTMS but with the advantage to be more suited for inducing measurable behavioral effect sizes in higher cognitive functions than spTMS. An interesting rTMS approach to get insights into the temporal characteristics of the involvement of a brain area during a cognitive process has recently been adopted by some studies (Rossi et al., in press; Schuhmann et al., 2009). For example, Schuhmann et al. (2009) investigated the temporal dynamics of Broca's area involvement in picture naming using triple-pulse TMS (inter-pulse interval of 25 ms) at various time points after picture presentation onset, namely at: (1) 150–175–200 ms, (2) 225–250–275 ms, (3) 300–325–350 ms, (4) 400–425–450 ms and (5) 525–550–575 ms. The results showed an increase in RTs only when applied in the 300-ms range after picture presentation. These data confirm the relevance of Broca's area during picture naming and provide new insights into the temporal characteristics of its specific contribution during speech production.

rTMS can be also applied in an off-line paradigm, in which magnetic stimulation and task performance are dissociated in time. In a common approach, performance is measured initially and then rTMS is usually applied over a site of interest for several minutes (from 10 to 25), generally at a low-frequency (<1 Hz), then performance is measured again. This paradigm is based on data showing reduction of visual (Boroojerdi et al., 2000) and motor cortex (Chen et al., 1997) excitability beyond the duration of the rTMS application itself.

Previous studies show that the duration of the after-effects is short-lasting, that is at least half the duration of the stimulation train (Robertson et al., 2003). However, these studies only report behavioral measures and provide no information about the duration of the neurophysiological effect of off-line low-frequency rTMS. Recently, Eisenegger et al. (2008) assessed the duration of this effect after stimulation of the right dorsolateral prefrontal cortex (DLPFC). Subjects underwent positron emission tomography (PET) scans after application of long-train low-frequency rTMS (1 Hz for 15 min). Immediately after the stimulation train, rCBF increases were present under the stimulation site as well as in other prefrontal cortical areas, including the ventrolateral prefrontal cortex (VLPFC) ipsilateral to the stimulation site. The mean increases in rCBF returned to baseline within 9 min, showing that the duration of rCBF changes in the prefrontal cortex is approximately 60% the duration of the stimulating train itself.

However, it should be kept in mind that the after-effect can vary a lot in its duration, depending on the context. Off-line paradigms can avoid nonspecific disruption of performance due to discomfort, noise, muscle twitches and intersensory facilitation associated with rTMS during the task. Moreover, compared to on-line paradigms, they require the use of a task that lasts no longer than the duration
of the after-effect and they miss any kind of specificity in terms of temporal involvement of a cognitive process. Moreover, the total duration of an experiment is longer before proceeding to stimulate a new site one has to wait for effects of rTMS to wash out and sometimes the session has to be split in different days. As for the interpretation of results, in the presence of facilitation effects, it is important to rule out learning effects.

Off-line rTMS at 1 Hz has been used extensively in recent years (Chen et al., 1997). It was applied to investigate a variety of higher functions over different brain areas, such as parietal contributions to spatial attention (Hilgetag et al., 2001; Thut et al., 2005), spatial hearing (Lewald et al., 2002) and number processing (Cappelletti et al., 2007; Knops et al., 2006; Rusconi et al., 2005) or prefrontal contributions to working memory (Mottaghy et al., 2002), affective processing (d’Alfonso et al., 2000) decision making (Knoch et al., 2006) or supplementary motor area (SMA) to motor learning (Perez et al., 2007; Tanaka et al., in press). Moreover, it was recently applied to evaluate the role of anterior temporal lobe in semantic cognition (Pobric et al., 2009, 2010; Lambon Ralph et al., 2010) that has been shown to cause behavioral changes that last for at least 30 min. Silvanto et al. (2007) also used a customised protocol (8 pulses at 40 Hz, separated by 1800 ms for a duration of 50 s with a stimulation intensity of 60% of maximum stimulator output) over V1–V2 during a motion-direction–discrimination task (see Fig. 2c).

In studies seeking to compare the effects of different frequency or patterns of pulses, it is fundamental to dose the number of pulses in the two protocols. Thus, in protocol of continuous TMS (low-frequency or cTBS) pulses are applied in a continuous train, whereas in protocols of intermittent TMS (high-frequency and iTBS), short periods of rTMS is separated by periods of no stimulation (see Fig. 2 for a schematic summary regarding TBS protocols).

Relatively short-term effects, of the order of seconds to a few minutes, could be due to changes in neural excitation caused by shifts in ionic balance around populations of active neurons or even the electrical capacitative effects storing charge induced by the stimulus. On the other hand, longer-lasting effects presumably involve changes in the effectiveness of synapses between cortical neurons (long-term depression, LTD, and long-term potentiation, LTP, of synaptic connections; Ridding and Rothwell, 2007).

Conventional ‘off-line’ rTMS needs a longer application time (between 10 and 25 min) and produces shorter-lasting effects (i.e., in the range of 5–20 min) than patterned off-line rTMS. It therefore seems plausible that, for future studies, TBS techniques may be the elective option for ‘off-line’ rTMS studies on higher functions.

4. Dependent variables and effect directions

A TMS-induced change in behavior (in the form of RTs, accuracy or signal detection measures) can be used to inform models of causal relations between specific brain regions and individual cognitive functions. TMS is generally used in cognitive neuroscience with the aim to disrupt some specific neural activity that subserves cognitive processing (i.e., as a ‘virtual lesion’). However, in several studies, certain cognitive tasks have been in fact enhanced by TMS, thus revealing the potential of TMS-induced paradoxical functional facilitation (e.g., interhemispheric or intrahemispheric interactions; Sack and Linden, 2003; Theoret et al., 2003; Ward and Cohen, 2004; facilitation is defined paradoxical as it is generally attributed to disinhibition of connected distant areas rather than actual improvement of the computation of the targeted site). This interpretation is based on animal studies and a few human case reports which suggest that direct or indirect neural ‘damage’ to specific areas in the central nervous system may result in facilitation of behavioral functions (see Kapur, 1996). Causality, therefore, is always assumed when a significant effect occurs (whether facilitative or inhibitory). Most TMS studies predict changes in behavior, but are rarely explicit about their direction (if they are, it is probably a post hoc statement disguised as an a priori hypothesis). This is due to the fact that for cognitive functions, the way in which
TMS modulates behavior (i.e., if it will exert facilitatory or disruptive effects) depends upon a number of variables, such as intensity, duration, frequency, and pattern of pulses applied, as well as possible interactions between them. In addition, other factors may play an important role, such as brain region and the cognitive task being performed (Walsh et al., 1998). Regarding stimulation parameters it is important to mention that in some TMS studies different frequency of stimulation on the same site and task led to similar (Hilgetag et al., 2001; Jin and Hilgetag, 2008) or opposite (Knoch et al., 2005) effects, whereas in other studies identical stimulation parameters (frequency, duration and intensity) and site led to opposite results in different tasks (Cappa et al., 2002; Sandrini et al., 2003; Rossi et al., 2001). Moreover, studies show on the same task opposite effects between different areas (Dräger et al., 2004) or between homologous areas of the human cerebral cortex (Cappelletti et al., 2007). For example, Cappelletti et al. (2007) investigated the causal role of IPS in processing both two-digit numbers and dots: in either case, performance was slower following left-IPS rTMS, and faster following right IPS rTMS (attributed to disinhibition of the homologous region in the left hemisphere).

Such evidence suggests that different protocols may induce similar effects on the same area or same protocols may induce opposite effect, and that no simple inference may be drawn from one cortical area to another or from one cognitive function to a related one. Another important point that should be taken into consideration is that TMS can induce a range of behavioral effects depending on the initial activation state of the stimulated region ("state-dependency" see Siebner et al., 2009a; Silvanto et al., 2008). In on-line paradigms, these effects can be either facilitatory or disruptive depending on the time point of stimulation. For example, spTMS usually disrupts cognitive functions when applied during the time period in which the stimulated area contributes to the task, but a number of studies have reported facilitation effects when TMS is delivered early in the time course of a trial – before activation in the region was expected (Grosbras and Paus, 2003; Pulvermüller et al., 2005; Stoeckel et al., 2009; Töpper et al., 1998). When TMS is delivered before the onset of a cognitive process, neural populations are less influenced by central factors than when TMS is applied during a cognitive process. This difference in the initial neural activation state could explain why the effects of TMS are opposite in the two circumstances. However, further studies are necessary to evaluate the validity of this interesting hypothesis. Moreover, although the neural mechanisms of long-lasting effects of off-line rTMS are different from those induced by spTMS or short bursts of rTMS, they have also been shown to be state-dependent (Andoh et al., 2008; Brighina et al., 2002; Lang et al., 2004; Siebner et al., 2004). Understanding state-dependency is thus crucial for an accurate interpretation of TMS studies (Pasley et al., 2009). In conclusion, the available evidence shows a very complex picture in relation with the application of TMS in cognitive studies. Factors such as experimental design, TMS parameters, type of task, stimulated brain area and its neural activation state are all important, as well as their possible interactions, which makes sometimes difficult to generate clear predictions about the direction of the TMS effect (facilitation vs. interference).

Moreover, the precise neurological mechanisms underlying facilitatory effects of rTMS on behavior are far from understood and even more obscure than the mechanisms of interference (Rossi and Rossini, 2004; Sack and Linden, 2003, but see Miniussi et al., 2010 for a fresh interpretative approach).

Perhaps less ambiguously, TMS can be used to measure functional connections between cognitive processing and activity in the visual cortex as well as the motor cortex. This can be done by measuring the change in the PT or in the amplitude of MEPs under various TMS experimental conditions and cognitive tasks. For example, it is possible to use PT to investigate whether a given task modulates visual cortical excitability. A recent study by Cattaneo et al. (2009d) showed that number priming modulates excitability of the visual cortex in a topographic fashion: small numbers, associated with left side space, increase the excitability of the right
early visual cortex and decrease the excitability of the left early visual cortex. The opposite pattern was found for large numbers. These data suggest that the attentional shifts induced by the mental number line can be traced down to the earliest cortical stages of visual processing. Regarding the motor cortex, when a task modulates MEPs in a region of motor cortex, this provides evidence of a functional link between the task and the motor cortex. MEPs can be recorded in the motor cortex that represents the hand, the arm, the leg, the foot and even the tongue (Fadiga et al., 2002).

Two studies (Andres et al., 2007; Sato et al., 2007) investigated the relation between numbers and hand/finger motor representations. Andres et al. (2007) employed TMS over M1 and found right FDI MEPs increase during serial counting, regardless of the use of numbers or letters to keep track of items, but no increase in arm and foot MEPs. They suggested that hand motor circuits are active above baseline whenever a correspondence is to be drawn between items and ordered series. Sato et al. (2007) measured abductor pollicis brevis (APB) and abductor digiti minimi (ADM) MEPs from both right and left hands in a parity judgment task, and found an increase in MEPs for the right hand only during small number processing, a modulation they relate to their participants’ counting habits. It is important to know that a modulation of the motor system can also consist of an amplitude decrease of the recorded MEPs (Buccino et al., 2005). Note that TMS is used in this fashion to investigate the causal role of a given cognitive task on the activation state of the motor cortex in that task. Change in motor cortex excitability during counting shows that this cognitive process can recruit motor cortex – it does not tell whether motor cortex plays a necessary role in counting.

Since changes in resting MEPs, as measured with single pulse TMS are not an unequivocal measure of activity in cortical areas (Di Lazzaro et al., 2001), the use of paired pulse TMS (Kujirai et al., 1993) should be preferred in future studies. In paired-pulse TMS, a conditioning stimulus (CS) below the threshold intensity needed to elicit an MEP is followed at short interstimulus intervals (ISIs) by a suprathreshold test stimulus (TS). The effects of the CS on the size of a test MEP depend on stimulus intensity and ISIs. Maximum inhibitory effects are found at short ISIs of 1–4 ms and CS of 60–80% of the rMT (Kujirai et al., 1993; Schäfer et al., 1997). Facilitatory effects of the CS on the test MEP can be observed at intervals 7–20 ms (Kujirai et al., 1993; Ziemann et al., 1996). The magnitude of the intracortical inhibition and facilitation vary depending on the amplitude of the test MEPs and the degree of contraction of the target muscle, a critical variable to control for in paired-pulse TMS studies. This modulation of MEP size takes place at the cortical level and is thought to reflect the activation of separate populations of inhibitory and excitatory cortical interneurons without affecting spinal circuits (see Di Lazzaro et al., 2008; Reis et al., 2008, for reviews about physiological mechanisms of this technique). Hence paired-pulse TMS provides a reliable index of motor cortical activation (see Oliveri et al., 2004a). Such method was first introduced for the study of motor cortex but can also be applied to non-motor areas (Oliveri et al., 2000). This technique has also been used to study functional interactions between two connected brain areas. In a creative protocol, in which one pulse is applied over each of two brain areas, it is possible to investigate functional cortical interactions on a subsecond timescale (dual-site paired-pulse TMS). In the motor system, one can test the causal influence of a connected area on the activation state of M1 by first stimulating that area, and measuring consequent changes in the amplitude of MEPs. A CS is first used to activate putative pathways to the motor cortex from the site of stimulation, whereas a second TS delivered over M1 a few ms later probes any changes in excitability that are produced by the input. Some studies have been conducted (either at rest or during tasks) with the CS delivered over contralateral M1 (Ferbert et al., 1992), cerebellum (Ugawa et al., 1995), pre-SMA (Mars et al., 2009), pre-motor cortex (Baumer et al., 2006; Boorman et al., 2007; Buch et al., 2010; Davare et al., 2008, 2009, 2010; Koch et al., 2006, 2007a; O’Shea et al., 2007c) and parietal cortex (Koch et al., 2007b, 2008, 2009), confirming the existence of these pathways in humans. Analogous studies have been conducted in the visual system, where PT evoked by TMS over V1 or V5 has been used to index cortical excitability. These experiments have tested functional connectivity in the visual system by measuring how a prior TMS pulse over another area changes PT over V1/V5 (Pascual-Leone and Walsh, 2001; Silvanto et al., 2006, 2009). The particular advantage of this protocol is that it can reveal with subsecond resolution how activity changes in one brain area causally impact on activity in connected areas (see for reviews Koch and Rothwell, 2009; O’Shea et al., 2008). Finally, interaction studies need not be limited to primary motor and sensory areas. Performance modulations after dual-site compared to single-site TMS, can also provide a marker of causal interaction (Ellison et al., 2007) (see Fig. 3 for a schematic summary regarding TMS protocols).

Finally, we will conclude this section with a mention that gender and aging may modulate TMS-induced effects. As for gender effects in TMS studies, only few studies reported different effects between male and female participants (De Gennaro et al., 2004; Knops et al., 2006). Evidence is instead more robust for age effects. For instance, traces of elementary motor-related memories, reflecting synaptic adaptation properties, can be revealed by TMS. Some studies showed that the ability to encode such motor-related memories seems to be age-dependent, as it progressively diminishes with age (Celnik et al., 2006; Sawaki et al., 2003). Furthermore, neuroimaging studies suggest that lateralization of prefrontal cortex activation associated with episodic memory declines with aging. The effects of rTMS applied to the left or right DLPFC on a visuospatial recognition memory task were compared in a population of healthy subjects divided into two classes of age (<45 and >50 years). In younger subjects, rTMS of the right DLPFC interfered with retrieval more than left DLPFC stimulation. The asymmetry of this effect was reduced with aging, and bilateral interference effects were found on memory performance. These data suggest that the bilateral engagement of the DLPFC may have a compensatory role on episodic memory across the life span (Rossi et al., 2004).

5. Control conditions

Different control conditions can be used to try and ensure that changes in performance are ascribed to TMS effects upon a specific brain area. One of the most common strategies is the use of sham stimulation. TMS is indeed associated with a number of sensory perceptions that can nonspecifically interfere with task performance. For instance, the discharging coil produces a click sound that may induce arousal, thereby modulating task performance, irrespective of the experimental demands (i.e., via intersensory facilitation; see Herschenson, 1962; Marzi et al., 1998). Sham rTMS stimulation is generally carried out by tilting the coil away from the scalp (Sandrini et al., 2004), so that both sound and scalp contact are roughly similar to those experienced during active stimulation, whereas the magnetic field does not reach cortical neurons or cutaneous receptors or superficial muscles. Nowadays, also sham-coils are commercially available. They still produce the same sound as during stimulation, but no magnetic field is generated, so that they can rest tangential to the scalp surface exactly as they are during active stimulation (see Cappelletti et al., 2007; Cohen Kadosh et al., 2007a). However, neither of the above sham conditions is fully satisfactory because none of them produces exactly the same sensations as a real TMS application. The introduction in the mar-
Fig. 3. Schematic illustrations of TMS protocols showing how TMS or rTMS can be applied.
over the right AG abolished the effect of number priming, whereas application of TMS over the left AG had no significant effect. In contrast, both left and right AG TMS modulated the effect of priming by large numbers (which presumably shift attention to the right side of visual space). These findings show that the AG plays a critical role in the allocation of visuospatial attention modulated by the mental number line. Another possibility is to stimulate different brain sites. Rusconi et al. (2007) reduced the SNARC effect by applying rTMS over left and right posterior, but not anterior portion, of PPL. The effect was attributed to disruption of the link between numbers and visuospatial attention rather than to interference with core number representations.

Relatively small changes in position can cause substantial changes in the sensory effects of stimulation. Moreover, even taking spatial specificity for granted, interference with a single task does not contain enough information to allow testing specific hypotheses about the cognitive operation that is being interfered with. As a consequence, observing behavioral dissociations induced by TMS on a given area across distinct tasks (Cappelletti et al., 2007; Dormal et al., 2008; Kansaku et al., 2007) or even in the same task but for different markers (e.g., Rusconi et al., 2007) may be as important as observing behavioral dissociations between stimulated sites. In other words, the inclusion of appropriate control tasks and/or conditions within tasks in the experimental design can enhance the cognitive resolution of TMS studies. A recent study (Dormal et al., 2008) tested a possible dissociation between numerosity and duration processing in the parietal cortex using a task in which participants had to compare the numerosity of flashed dot sequences or the duration of single dot displays. RTMS was applied over the left or right IPS or the vertex, chosen as a control site. Compared to the control site, performance was slowed down only for the numerosity comparison task after left-IPS stimulation, whereas it was not affected in the duration comparison task for either parietal sites. These data, together with Cappelletti et al.’s study, confirm recent imaging studies in humans and cell-recording in primates indicating that, besides comparison of symbolic stimuli, the parietal regions are also involved in quantity processing of non-symbolic numerosities (e.g., Castelli et al., 2006; Piazza et al., 2007; Nieder and Miller, 2004). Moreover, they show that at least one of the parietal areas that are critically involved in numerosity processing is not crucial for duration processing.

Following up on their parietal stimulation study (Rusconi et al., 2007), Rusconi et al. (2008, in press) moved the exploration of the substrate of number–space interaction forwards, in the frontal lobe. They found that suppression of the SNARC effect is dependent on task and number magnitude with right FEF–rTMS and is dependent on task but generalized to all magnitudes with right IFG–rTMS. In general, when simple dissociations between tasks are found (i.e., modulation of performance in one task but not in the other when stimulating the same site), it is important to evaluate whether the differential effect of TMS may be attributed to global task characteristics such as relative difficulty rather than specific mental operations. It is indeed possible that the manipulation is powerful enough to modulate performance in the more difficult task but not in the easier one, which can still be performed at baseline levels even under modified neural conditions. This caveat is most popular in the neuropsychological literature. One other issue that may be often overlooked is that a reversed type of argument needs also to be taken into account in TMS studies. Rusconi et al. (2008, in press), for example, found a selective suppression of the SNARC effect during the easier task, that is magnitude comparison, which was on average 50 ms faster than parity judgment. In that case, therefore, based on behavioral performance the task difficulty objection could not be applied. Moreover, in Rusconi et al.’s study, the difference in terms of RTs between the two tasks was relatively small and the TMS effectiveness window could be assumed to cover all the time from stimulus to response selection in either task (Wassermann et al., 2008). In theory, cases where a similar dissociation is present, which escapes the difficulty objection since an index in the easier and not in the more difficult task is modulated by TMS, might however be detecting spurious effects. If the critical process that is affected by TMS occurs quite some time after stimulus presentation, it is possible that only the faster task reveals an effect because the TMS protocol covers all of the important processing stages, whereas it fails to cover all processes in the slower task. It is therefore very important, before deciding on a specific TMS protocol, to carry out a task analysis and specify what processing stages are expected to be involved and show sensitivity to the TMS manipulation.

In conclusion, experimenters have to choose carefully the appropriate experimental design to use in order to test their hypothesis and their theoretical framework. The use of more than one control condition is crucial for determining the contribution made by a cortical area to a specific behavior (see Fig. 4 for a schematic summary regarding control conditions).

6. Site localization

The spatial resolution of TMS depends on the geometry of the coil, stimulus intensity, stimulus configuration and the electrical properties of the cortex under the coil. Its temporal resolution depends on the duration of TMS and the duration of an area’s involvement in the task. In studies on cognitive functions, the information about the when and where of TMS application is derived from previous studies using complementary brain mapping techniques (ERP, MEG, PET, functional magnetic resonance imaging (fMRI)) and neuropsychological data with the task of interest. Even when exact temporal and spatial information are available, the precise and reliable positioning of the TMS coil is not a trivial issue.

Some areas of interest in cognitive TMS studies, when stimulated, have a signature output (for instance, M1 and V1). However, many others are behaviorally silent. A possible approach consists of locating brain areas relative to those that have a reasonably certain position. For example, DLPFC can be defined as 5 cm anterior of the region from which the most prominent motor response of the muscle APB can be recorded (Pascual-Leone et al., 1996) or the lateral cerebellum can be defined 1 cm under and 3 cm to the left or to the right of the inion (Torriero et al., 2004; Théoret et al., 2001).

A different approach of localizing the TMS target is the application of TMS in relation to specific individual scalp landmarks, for example, based on the standard 10–20 electrode scalp positioning system (Jasper, 1958). Locations (such as P3, P4, sites of the 10–20 EEG positions for left and right posterior parietal cortex) can be determined in relation to landmarks on the scalp such as the vertex (e.g., van Donkelaar and Müri, 2002), which is itself found by finding the crossing point between the nasion–inion line and the line connecting the pre-auricular points. Oliveri et al. (2004b) showed that when comparing numerical intervals, normal subjects overestimate the difference between the middle number and the outer number positioned at its left side. The right parietal cortex plays a major role in such mental space “deformation,” as its transient disruption by means of rTMS over P4 abolishes the leftward bias. Similar results were obtained by Göbel et al. (2006) in a mental number bisection task when the stimulation site was localized both functionally and with reference to individual anatomical scans.

With the above methods, coil positioning can be related to certain cortical areas with an estimated resolution in the range of centimeters. Although it may be appropriate for some purposes, in general this approach accounts for neither interindividual differences in the correspondence between scalp landmarks and...
underlying brain anatomy nor for interindividual differences in the functional organization of the brain (Herwig et al., 2003; Okamoto et al., 2004).

Coil position relative to individual anatomy can be precisely defined post hoc by marking the stimulation sites with a locally attached MR contrast marker (e.g., vitamin E capsules) and performing a structural magnetic resonance scan (Terao et al., 1998). The limit of this method is that localization has to be done only afterwards and does not allow the selection of the stimulated site on neuroanatomical basis in advance.

Instead of using anatomical information, it is also possible to position the TMS coil by using a function-guided approach: if the stimulation effect on the performance of a given behavioral task is known, this task can serve as a “functional” probe to position the coil for the subsequent investigation of another task. For instance, Göbel et al. (2001) applied the “hunting procedure” used by Ashbridge et al. (1997) for determining coil position on a visual search task. Thus, to select the region of parietal cortex that would be stimulated in the main task, the experimenter asked subjects to carry out a visual conjunction search task first, while receiving rTMS in the area of the P4 electrode site (right posterior parietal cortex). Sites on and around this location were probed for blocks of 20 trials until one region was found that consistently and markedly increased mean RTs on the search task. The site at which rTMS disrupted visual search was first established for each subject. The number task was then carried out while rTMS was delivered on that same site. The location of the AG/adjacent posterior part of the IPS was later confirmed with contrast marker in an MRI scan. The authors found increased RTs when rTMS was applied over this site during comparison of two-digit numbers to 65. RTs increase following left hemisphere stimulation was specific to numbers close to and larger than 65, whereas a trend for a more generalized effect was observed after right hemisphere stimulation. No effect was found when participants were stimulated on either left- or right-SMG and it was suggested that the AG/adjacent posterior part of the IPS might play a fundamental role in the spatial representation of number magnitude, with the left hemisphere containing a spatiotopic representation (i.e., the mental number line).

Although influential, the hunting procedure of Ashbridge et al. (1997) is time-consuming and relies on RT differences that do not undergo any statistical tests. An alternative or supplementary localizer has been developed by Oliver et al. (2009). The authors introduced a procedure for right parietal TMS effects upon visuospatial performance, in a task where signal detection theory could be applied and sensitivity measures, such as d', computed. Assessment of the TMS intensity required to produce the phenomena of interest found this was linearly related to individuals’ rMT over hand M1. In general, the hunting method has limited applications because it enables the localization of only very specific regions for which a TMS localizer is available. In addition, different laboratories tend to develop their own localizer that better matches their theoretical assumptions, empirical interests, and previously employed paradigms (see Ro et al., 1999; Ryan et al., 2006).

With the introduction of frameless stereotaxic systems, the TMS coil can be navigated to target specific anatomical sites based on individual subjects’ structural brain images. These systems provide “on-line” information about the location of the coil (Paus, 1999; Sparring et al., 2010). They achieve a virtual linkage between MRI images and real anatomy, and allow three-dimensional (3D) orientation by interactive visual navigation. Stereotaxic neuronavigation can be based on subject’s structural MRI (e.g., see Cappelletti et al., 2007; Rusconi et al., 2005). For instance, in Cappelletti et al.’s paper, the horizontal segment of the IPS was defined as middle part of the sulcus in the dorsal parietal lobe that intersects the postcentral sulcus whereas the AG was defined as the gyrus on the lateral surface of the parietal lobe curving around the posterior end of the superior temporal sulcus. However, although this method accounts for interindividual differences, reliable anatomical landmarks exist for very few target sites and individual differences of gyral folding and cortical layering have to be taken into account. Another approach exploits functional neuroimaging data (“probabilistic approach”, Paus et al., 1997). Talairach coordinates could be generated from: (1) a previous fMRI study with the same task paradigm on different subjects (Cappelletti et al., 2009; Kansaku et al., 2007); (2) a study investigating similar cognitive processes (Cattaneo et al., 2009b; Dormal et al., 2008; Rusconi et al., 2007); (3) a meta-analysis of sev-
eral studies (Rusconi et al., 2008, in press). For example, Kansaku et al. (2007) used the same counting task in two experiments—one with fMRI and the other with TMS. fMRI was utilized to localize an area (or areas) additionally recruited for large vs. small number counting, and TMS would be applied to disrupt the function of target area(s) to determine whether they play an indispensable role in the exact large number counting process. It turned out that the upper part of the left ventral premotor cortex (PMv) was preferentially activated and its subsequent stimulation confirmed its causal role in the process as it selectively disrupted exact large number enumeration.

This method considers both structural and functional imaging data relevant to the cognitive task to be used, but it does not take into account individual structure–function differences. Finally, a very efficient method consists of identifying target areas based on individual functional activation maps. For example, to clarify whether there is a portion of the IPS that is specifically selective for number or whether the IPS supports all analog magnitude judgments (e.g., size, weight, density, brightness), Cohen Kadosh et al. (2007a) combined fMRI and neuronavigated TMS in a size congruity paradigm, a Stroop-like task in which numerical values and physical sizes were varied independently. In line with previous studies (Cohen Kadosh et al., 2007b; Pinel et al., 2004), the size congruity effect activated the bilateral IPS. In order to accurately stimulate the functionally defined region of interest along the IPS for each participant in the TMS experiment, the authors analyzed the IPS size congruity effect activation for each individual separately. The results revealed considerable interindividual differences in the IPS activation both in extent and in layout and, therefore, in the stimulated coordinates. The results showed that TMS to the right (but not to the left) IPS is sufficient to produce a size congruity pattern (i.e., lack of facilitation), independent of whether subjects compared the numerical value (and ignored the physical size), or compared the physical size (and ignored the numerical value). Importantly, an analogous behavioral pattern was also obtained when participants with developmental dyscalculia performed the same task (albeit without TMS) (Rubinste and Henik, 2005, 2006). Therefore, the automatic processing of numerical value or physical size seems to be equally impaired due to structural abnormality, or stimulation, of the right IPS. This approach thus accounts for individual differences both in brain anatomy and in the functional architecture of the brain (see for a comparison of these methods, Sack et al., 2009; Sparing et al., 2008; see Fig. 4 for a flow chart on site localization).

The same authors (Sack et al., 2009) investigated whether the effects of right parietal TMS on size congruity revealed in their previous study (Cohen Kadosh et al., 2007a) could be obtained when using other approaches to determine the target stimulation site. They repeated their original study testing the same number of participants (n = 5) while basing TMS localization either on: (1) individual fMRI-guided TMS neuronavigation; (2) individual fMRI-guided TMS neuronavigation; (3) group functional Talairach coordinates; or (4) 10–20 EEG position P4. They quantified the behavioral effects induced by TMS using each approach, calculated their standardized effect sizes, and conducted a statistical power analysis in order to calculate the optimal sample size required to reveal statistical significance. Their results indicate that when the right IPS is stimulated in the location indicated by the individual fMRI results, the size congruity effect (SCE) decreases significantly. This effect was found to be significant with a sample size of n = 5 when using fMRI-guided TMS neuronavigation to approach the TMS target site. Although all TMS approaches showed the same trend as the fMRI-guided TMS neuronavigation, the effect size was not large enough to reach statistical significance. Power analyses revealed the number of necessary participants increased to n = 9 when employing MRI-guided neuronavigation, to n = 13 in case of a site localization based on group Talairach coordinates, and to n = 47 when applying TMS over P4.

It may be that for other brain regions or other cognitive tasks, the interindividual differences in brain anatomy and structure–function relationships are less pronounced, thus reducing the difference between fMRI-guided, MRI-guided, and probabilistic approaches, making these latter two approaches acceptable compromise between stimulation accuracy and pre-experimental effort. However, there are also data showing a qualitative difference among different approaches. A paper by Feredoes et al. (2007) used fMRI to localize TMS sites to disrupt the short-term storage of verbal information during a delayed letter-recognition task. Results indicated that rTMS targeting regions identified by single-subject analysis (SS, based on fMRI) in left perisylvian and sensorimotor cortex impaired performance, whereas rTMS targeting regions identified by the spatially normalized group-averaged (SNGa, based on structural MRI) in left caudal PFC had no effect on performance. This seems to suggest that the brain basis of some cognitive functions, in which there is considerable interindividual variability, may be better detected by SS than by SNGa approaches to fMRI data analysis (see Postle and Feredoes, 2010).

In conclusion, it seems incontestable that individual fMRI-guided TMS offers the highest experimental power. It however limits the range of possible targets to those that can be identified by fMRI (see Fig. 5 for a schematic summary regarding site localization).

7. Combining TMS with other techniques

7.1. TMS and neuroimaging—off-line and on-line approaches

It is worth mentioning that progress in cognitive neuroscience often depends on the convergence of evidence from multiple methods. Since every single technique has its own limitations, there is a clear theoretical advantage in combining different approaches. The timing of TMS relative to neuroimaging (PET, fMRI, EEG) defines which questions can be tackled using a combined TMS-neuroimaging approach. TMS can be applied “off-line” before or after neuroimaging. We have already discussed the importance of available temporal information (e.g., EEG, MEG) to define the precise time window during which TMS should be applied and the spatial information (e.g., PET or fMRI) that can be used to guide the positioning of the TMS coil over the cortical target area. Neuroimaging techniques can also map the spatio-temporal pattern of functional reorganization induced in the brain by TMS (De Gennaro et al., 2007; Huber et al., 2007; Siebner and Rothwell, 2003) or the lasting functional impact of TMS on task-related neural activity at the systems level (O’Shea et al., 2007b; Sole-Padullés et al., 2006).

As a rule, neuroimaging should start as quickly as possible after rTMS to ensure that short-lasting after-effects are captured. One excellent way to detect the effects of rTMS on neural activity is to compare fMRI task-related activation before and after stimulation. It does pose technical challenges, however.

In TMS-fMRI off-line studies safety procedures and technical issues are the main constraints on timing. Necessary actions, which cause a delay between TMS administration and fMRI recording, can be summarized as follows:

(1) Move subject into the MRI room.
(2) Position subject inside the scanner. With a high field scanner (e.g., 4T) positioning takes about 50 s, as vertigo induced when the gradient of the field, the gradient-field product, or the gradient-velocity product experienced by the subject’s head are
high. In lower field (up to 3T) the bed speed can be increased thus reducing the positioning time to 10–15 s.

(3) Let personnel out (about 10 s).

(4) Localizer (about 50 s).

(5) Pre-EPI acquisition (distortion correction before each echo-planar image takes about 45 s, when there is not enough time it can be done off-line).

Therefore, total time before the fMRI experiment can take place is around 4 min. This is an important aspect to keep in mind when deciding on the duration and type of rTMS protocol.

For instance, a mechanism of rTMS effect on brain activity has been shown supporting the hypothesis that some brain functions operate in a state of interhemispheric compensation after a virtual brain lesion, that is, a form of adaptive plasticity in the nondominant hemisphere for functional recovery (O'Shea et al., 2007b). These plastic-adapting changes of the intact hemisphere could occur rapidly (>4 min after the end of TMS) and be specific to functions that are normally mediated by the stimulated area. O'Shea et al. (2007b) directly demonstrated the interhemispheric compensation by testing the hypothesis that, if the pattern of reorganization causes recovered behavior, then recovery performance should breakdown when activity is disrupted in the compensatory hemisphere. Subject underwent two fMRI sessions, one of which was preceded by 15 min of 1 Hz TMS to dorsal premotor (PMd) area. The results showed a compensatory increased in fMRI response in the right PMd and the connected medial premotor areas. Subsequent TMS of the “reorganized” right PMd disrupted performance, confirming that the pattern of functional reorganization of the right PMd made a causal contribution in preserving behavior after neuronal interference.

In the TMS–EEG off-line study, the only point to consider is whether TMS should be applied with electrodes being attached to the scalp. The decision might depend on the experimental design as well as number and montage of electrodes. Standard electrodes increase the distance between the TMS coil and the cortex requiring a higher intensity of stimulation to induce a stimulation effect. However, flat electrodes have become available to reduce this problem.

This off-line approach, in which TMS and neuroimaging are separated in time, is technically easier to implement than the “on-line” approach, in which TMS and neuroimaging overlap in time with TMS having the possibility to adversely affect data acquisition during neuroimaging (for a review about the combination of TMS with neuroimaging see Siebner et al., 2009b). The potential of this on-line approach is the possibility of using TMS to test how focal cortex stimulation acutely modifies the activity and connectivity in the stimulated neural circuits (see for a review Driver et al., 2009). If the goal of the experiment is to investigate the spatial pattern of TMS-induced changes in the brain activity, an on-line combination of TMS with PET or fMRI allows one to visualize the causal impact of target neural interference on connected brain structures. Conversely, if temporal aspects of neuronal processing are the main focus, the combination of TMS with EEG provides a methodology to investigate causal and dynamic relations between brain areas. Finally, it is important to remember that a complementary approach to on-line TMS–neuroimaging combinations (TMS–PET, TMS–fMRI and TMS–EEG) is feasible just using a TMS protocol (dual-site paired pulse TMS), in which behavioral effects of TMS applied to one cortical area (e.g., hand twitches for M1-TMS, or phosphenes for V1-TMS) can be modulated by preceding ‘conditioning’ TMS pulses to a second, potentially interconnected cortical areas.

Fig. 5. Schematic illustration of approaches used for the localization of brain sites.
region. The advantage of this protocol is the possibility to collect information about neural processing on a milliseconds timescale, while its limit is due to its restriction to pairs of cortical sites without offering the possibility to record neural activity changes in the whole-brain.

An elegant advancement in the application of this protocol has been demonstrated recently by Davare et al. (2010). The authors used a triple coil TMS approach, and investigated the consequences of interfering with cTBS over anterior intraparietal area (AIP) on physiological interactions between PMv and M1 at rest or during preparation to grasp objects with either a precision grip or a whole-hand grasp. They found that task-related interactions, but not those at rest, were disrupted following AIP cTBS. Behaviorally, disruption of AIP was also associated with a relative loss of grasp-specific pattern of digit muscle activity. These data suggest that AIP is critical in processing context- and grasp-dependent information, which enables PMv to bias excitability levels of M1 hand representation during preparation for an upcoming grasp.

7.2. TMS with concurrent PET or fMRI

The successful combination of TMS with PET was reported in the late 1990s (Paus et al., 1997; Siebner et al., 1998; Fox et al., 1997). At around the same time Bohning et al. (1997) reported the first combined TMS–FMRl study.

The earliest TMS–PET (see for a review Paus, 2005) and TMS–fMRI studies (see for reviews Bestmann et al., 2008a; Ruff et al., 2009a) measured the effects of focal brain stimulation while subjects were at rest, that is, in the absence of any cognitive task. These data show that the neural consequences of focal TMS were not restricted to the site of stimulation but also modulated the activity of distal brain areas. Another important observation was that the impact of stimulation was dose-dependent, as stronger stimulation intensities evoked larger activity changes in those regions.

Related to this distal connective spread, some studies used the combination of TMS and ligand PET to study neurotransmitter system function, showing that cortical stimulation (prefrontal and motor cortices) led to an activation of specific cortico-striatal glutamatergic pathways, which in turn, induced local release of dopamine at their terminations in the striatum (Strafella et al., 2001, 2003). Moreover, a recent study (Cho and Strafella, 2009) provided the first evidence of extrastriatal brain dopamine modulation following acute rTMS of the left (but not right) DLPFC with its effect limited to the medial part of prefrontal cortex, including pregenual, subgenual anterior cingulate cortex and medial orbitofrontal cortex. These studies of neurochemical functions with rTMS may be useful for exploring hemispheric differences and the effects of these differences on striatal and extrastriatal dopaminergic systems.

Moreover, a recent series of studies (Ruff et al., 2006, 2008, 2009b; Sack et al., 2007) has moved towards another domain, showing the potential of this approach to investigate interregional interactions and their possible functional consequences for perception and cognition. The data from these studies seem to converge on the following conclusions: (1) focal TMS applied to a particular cortical area has both local and remote neural effects in the brain; (2) cortical interactions depend on the functional state of connections (state-dependency). For example, Ruff et al. (2008) investigated functional interactions between fronto-parietal and visual cortical areas. The stimulation of right IPS, but not right FEF, elicits a pattern of activity changes in visual cortices that depends on current visual context (i.e., affecting the BOLD signal in V1–V4 during the absence of visual input whereas affecting the BOLD signal in V5/MT only when moving stimuli were present). These state-dependent TMS effects on cortico-cortical interactions have been also demonstrated in the motor system (Bestmann et al., 2008b); (3) the state-dependent remote neural effects of TMS may be functionally relevant for behavior. For instance, Ruff et al. (2006) reported that TMS to the right FEF leads to BOLD increases for peripheral visual field presentation whereas BOLD decreases for the central visual field. Based on these data, the authors predicted that FEF stimulation should enhance peripheral vision, relative to central, in both visual hemifields. This new prediction was confirmed in a psychophysical experiment testing the effects of FEF-TMS upon contrasts judgment for Gabor gratings presented in the central vs. peripheral visual field. The results showed that perceived contrast was enhanced for peripheral relative to central stimuli whereas vertex stimulation (control site) was ineffective. Remote neural consequences of TMS can thus be used to generate new predictions for behavioral TMS effects outside the MR scanner.

If this is true, however, can behavioral TMS studies still be considered crucial to verify the functional necessity of a stimulated cortical brain region?

Although it seems reasonable to interpret significant remote neural effect of TMS as also functionally relevant, the causal involvement of such remote effects in behavioral changes needs to be demonstrated. In the absence of empirical proofs it is sensible to keep attributing the primary cause to changes at the site where stimulation is maximal (Sack, 2010).

Physical modulation of a targeted cortical region, as with TMS, is not the only approach to test for interplay between human brain regions. In recent years, functional neuroimaging has moved beyond conventional brain mapping and is now challenging the monopoly on causality analysis ascribed to functional interference techniques in humans (i.e., transcranial direct current stimulation and TMS). For fMRI especially, new techniques are increasingly applied providing mathematical models that can be used to assess possible changes in “effective connectivity (EC)” between brain regions under different conditions (Friston et al., 2003; Roebroeck et al., 2005). For example, Granger causality mapping (GCM) is a data-driven EC method with the aim to identify, with reference to a seed region Y, which other brain regions (e.g., X) engage in directed interactions with region Y (Roebroeck et al., 2005). Therefore, using spTMS neuronavigation to stimulate fMRI EC-identified clusters at single-subject level, it is possible to test behavioral relevance of an fMRI EC functional network underlying a cognitive process (see de Graaf et al., 2009). Moreover, the combination of different methods for studying EC can allow new hypotheses to be tested that otherwise would be difficult to address. Finally, since several technical problems make such combination very challenging, until now only few groups worldwide have been able to realize simultaneous TMS and fMRI. All these studies used 1.5 or 3T scanner, however some technical and safety aspects has been already tested in 4T scanner (Ferrari et al., 2010).

7.3. TMS with concurrent EEG

At around the same time of the first combination of TMS with PET and fMRI, Ilmoniemi et al. (1997) also showed that the combination of TMS with EEG was feasible, thus offering an approach to investigate whole-brain causal dynamics with excellent temporal resolution. As other TMS-neuroimaging approaches, the earliest TMS–EEG studies (see for reviews Ilmoniemi and Kičič, 2010; Komssi and Kähkönen, 2006; Minussi and Thut, 2010; Taylor et al., 2008) measured the effects of focal brain stimulation while subjects were at rest. With this approach some studies investigated the electrical responses evoked by TMS not only in the stimulated area (reactivity) but also in the connected sites (connectivity). It has been shown that the reactivity of the cortex is related to TMS intensity (Komssi et al., 2004; Kähkönen et al., 2005) and coil orientation (Bonato et al., 2006) and the measures of cortical activity and connectivity are state-dependent. For example, Massimini et al. (2005)
applied spTMS over PMd during EEG. The ERPs data showed remote TMS effects in awake subjects that became more restricted during sleep, suggesting that cortical functional connectivity changes with subjects’ level of arousal.

In recent studies, however, the efforts to combine TMS with simultaneous ERP recording have been successful in studying the temporal and functional impact of TMS interference on cognitive processes. The value of this combination lies in the fact that the fine temporal resolution of EEG allows one to make an on-line measure of the effects of TMS at different stages of processing (e.g., sensory and post-perceptual), within brain regions which are anatomically remote from the area impaired by TMS.

Therefore, by using this approach, it is possible to investigate where, when and how TMS interacts with task performance on a whole-brain scale (Fuggetta et al., 2006, 2009; Taylor et al., 2007). For instance, Taylor et al. (2007) tested whether the stimulation of FEFs had a causal impact on distal visual cortex during the endogenous orienting of visuospatial attention. When TMS was delivered to the right FEFs, immediately after presentation of an arrow cue indicating where to allocate attention (i.e., prior to the onset of the visual stimulus), induced a change in the baseline EEG signal and in the event-related cortical response recorded over posterior visual areas. The TMS-induced effect occurred at the same time as ERPs were shown to be modulated by visuospatial attention.

Finally, another possible approach is the use of TMS within the broader perspective on brain rhythms that EEG provides. This approach can be used to investigate how rhythmic brain stimulation can be used to modify brain functions as well as how TMS interacts with oscillatory brain activity (see for a review Thut and Miniussi, 2009).

One interesting characteristic that should not go unnoticed is that the result of these “on-line” approaches differs from the simple sum of the two single techniques, and researchers should therefore take extra care in designing experiments (i.e., appropriate control task, sham or control site, intensity of stimulation), collecting data (i.e., use of neuronavigation system, coil orientation, adopt careful timing of interleaved TMS stimulus and echo-planar images acquisition), and interpreting data (i.e., to take into account nonspecific task effects such as auditory and somatosensory responses, choice of artifact correction strategy during data analysis).

8. State-dependent TMS paradigms

As mentioned earlier, the neural impact of an external stimulus is determined not only by the properties of that stimulus but also on the initial state of the activated brain region. In accordance with this view of state-dependent effects of TMS, new methods have been devised. The first one, called TMS-adaptation paradigm (TMSA) is based on the hypothesis that TMS acts differentially on neurons according to their initial neural activation state, with attributes encoded by the adapted neural populations within the stimulated region being more susceptible to the effects of TMS. An adapting stimulus, presented for a long time (usually 40–60 s), is used to induce habituation in a subset of cells that code particular stimulus features, therefore making them a selective target for TMS. According to this paradigm, if TMS delivered over a cortical area – supposedly containing neurons that code for the adapting stimulus – improves behavioral performance for these stimuli, this indicates that neurons in that area were indeed adapted by and tuned to the adapting stimulus. Thus, by using adaptation to manipulate neural activation states prior to the application of TMS, one can control which neural populations are preferentially facilitated by TMS.

The validity of this TMSA paradigm has been confirmed in studies with stimulation of visual areas such as V1/V2 and V5/MT in which the receptive properties are relatively well known (Silvanto and Muggleton, 2008). Moreover, recent studies confirmed the validity of this paradigm in the language (Cattaneo et al., 2009a, 2009c), number (Cohen Kadosh et al., in press) and motor acts observation domains (Cattaneo et al., in press). With an elegant design, Cattaneo et al. (in press) were able to exploit the specificity of this paradigm to investigate the neural representation of observed motor behavior in the inferior parietal lobule (IPL), PMv and in the cortex around the superior temporal sulcus (STS). Participants were shown adapting movies of a hand or a foot acting on different objects and were asked to compare to the movie a motor act shown in test pictures. The invariant features between adapting and test stimuli fitted a 2 × 2 design: same or different action made by the same or different effector. TMS over the left and right PMv and over the left IPL induced a selective shortening of RTs to stimuli showing a repeated (adapted) action, regardless of the effector performing it. In a second experiment TMS applied over the left STS induced shortening of RTs for adapted actions but only if also the effector was repeated. In conclusion, these results indicate that observed motor behavior is encoded unitarily with the body part that performs it in the frontal lobe. A hierarchically higher level of representation is carried by neural populations in the parieto-frontal regions, where acts are encoded as their meaning.

Another psychophysical method for manipulating the initial activation state is priming. Preliminary data suggest that when TMS is applied after priming, TMS facilitates target detection when the target has not been primed, whereas performance is unaffected if the target has been primed (Campana et al., 2002; Cattaneo et al., 2008, 2010). For instance, Cattaneo et al. (2010) investigated the role of left PMd and PMv in semantic processing. Priming to a category name (either “Tool” or “Animal”) was used to modulate the initial activation state of these brain areas prior to application of TMS and the presentation of the target stimulus. When the target word was an exemplar of the “Tool” category, the effects of TMS over PMv (but not PMd) interacted with priming history by facilitating RTs on incongruent trials but not on congruent trials. This TMS by congruency interaction suggests that the “Tool” and “Animal” primes had a distinct effect on the initial activation state of the left PMv and implies that this cerebral region is one neural locus of category-specific behavioral priming for the “Tool” category. TMS delivered over PMv had no behavioral effect when the target stimulus was an exemplar of the “Animal” category, regardless of whether the target word was congruent or incongruent with the prime. In conclusion, results demonstrate the causal role of category specificity in the left PMv in the encoding of words describing graspable tools.

A differential TMS effect when the target stimulus is primed vs. unprimed (or adapted vs. nonadapted) can reveal neural selectivity because it shows that the stimulated brain area contains a neural representation that was affected by the initial state manipulation.

Although the fact that preconditioning modulates the effects of subsequent TMS (see for a review Silvanto et al., 2008) and that a lot of state-dependency ideas in the form of adaptive coding (see Duncan, 2001) have been known for a long time, we believe that these paradigms are an important and useful step forward for the study of higher level cognitive processes.

These new methods could reveal something that ‘virtual lesion’ TMS would not (naturally considering only situations where they can be used to ask the same question). Imagine the case where a region contains functionally overlapping populations of neurons that are selectively crucial for function X vs. Y. Subjects receive a task that measures both X and Y. Normal TMS reveals a deficit of both tasks, which does not tell us whether it is a single population of neurons that do both functions, or intermingled specific populations. But by preconditioning subjects to X or Y (and then showing that the TMS effect differs for the two functions), some degree of specificity is revealed in a way that normal TMS cannot
access. Therefore, in these new paradigms the functional resolution of TMS is increased, allowing differential interventions on distinct, even spatially overlapping, neural populations within the stimulated brain region (see for reviews Silvanto et al., 2008; Silvanto and Pascual-Leone, 2008).

Finally, we do not believe that these new paradigms will be less useful to cognitive neurosciences because the physiological basis of state-dependent effects are unknown (Siebner et al., 2009a), as the same problem applies to other more common paradigms (including standard ‘virtual lesion’ TMS, fMRI adaptation, etc.).

9. Safety guidelines

Methodological issues and technological advances cannot be entirely separated from safety and ethical considerations; we will thus briefly summarize and discuss the implications of the newest safety guidelines (Rossi et al., 2009). The new guidelines are meant to update the ones (Wassermann, 1998) which have regulated TMS application during its first exciting decade of experimentation in laboratories all over the world.

The last decade has seen a rapid increase in the use of TMS to study cognitive functions, brain–behavior relation and in the treatment for a variety of psychiatric and neurological disorders. In particular, new protocols have been introduced, changes in devices have been implemented, TMS is being increasingly combined with other neuroimaging techniques and many normal subjects and patients are being investigated in longer stimulation sessions. The necessity to revisit the safety guidelines, update the recommendations of practice and improve the discussion of ethical aspects was the motivation behind the consensus conference in Certosa di Pontignano (Italy) on March 7–9, 2008. As in the NIH consensus conference (June 5–7, 1996 see Wassermann, 1998), the 2008 meeting brought together some of the leading scientists who are currently using TMS in basic research and for clinical applications. The 2008 consensus conference led to the development of new safety guidelines, which build, whenever possible, on the old guidelines (Wassermann, 1998).

Because the aim of our review is about research experiments in normal subjects, we will only summarize the safety guidelines strictly related to studies regarding cognitive functions. According to Rossi et al.’s (2009) classification, these studies belong to class 3, as they provide only indirect benefits for the subject (no immediate personal relevance) and the risk to which the subject is exposed is low. Given the inevitable presence of (a minimal) risk, these studies should be carried out when they can be expected to yield data of outstanding scientific or clinical value on brain physiology or safety.

In general, risk is defined as the product of threats, vulnerabilities and possible consequences implied by an action (see e.g., Aven, 2008). In the TMS environment, the most severe consequence that may be caused by stimulation on healthy participants is acute seizure induction. Based on the 9 new cases reported in the literature after the publication of Wassermann’s (1998) guidelines, acute seizure during TMS experiments is an extremely rare event. In addition, a few of the reported episodes are dubious, and their clinical description matches with syncope more than a seizure (see video available at www.brainstimjrn.com/content/mmc_library). It is, however, clear that pro-epileptogenic drugs (in patient studies) and the use of TMS parameters exceeding safety guidelines or experimenting with new TMS protocols for which no guidelines are available (in patient and healthy subjects) increase threats and/or individual vulnerability, and therefore amplify risk. Following these considerations and years of research experience from several leading laboratories in the world, Rossi et al. (2009) estimate a probability of less than 0.01 of inducing a seizure in healthy subjects when using established high-frequency rTMS parameters.

The wide hands-on experience of the consensus group guarantees that such estimate takes into account the possible hidden figure of cases that did not make it into the relevant literature. Considerations regarding the following main points should be consulted when designing a TMS or an rTMS study – they are all exhaustively discussed in section 7 of Rossi et al.’s (2009) guidelines:

(1) Ethical and regulatory issues.

Research application must be governed by three fundamental ethical and legal requirements: informed consent, whereby participants receive all the relevant information about potential risks and discomfort with a terminology they can understand, and decide voluntarily to take part to the study; risk-benefit ratio, which has to be evaluated beforehand whether relevant data can be obtained and the scientific question can be answered without exposing any subjects to risks; equal distribution of the burdens and benefits of research, which prevents to conduct research on categories of individual made particularly vulnerable by economic, social or physical conditions.

(2) Stimulation parameters.

Conditions of increased or uncertain risk of inducing epileptic seizures are:

- Any “novel paradigm” (i.e., that is not a conventional method of high–low-frequency rTMS performed with a flat butterfly coil and biphasic stimulation) or TMS applied on more than a single brain region.

Conventional high-frequency protocol with parameters (intensity, frequency, train length or ITI) exceeding the safety limits reported in Rossi et al.’s Table 4; to prevent seizure occurrence when experimenting with new protocols and parameters, some precautions should be taken, such as continuous EMG monitoring, direct visual/video monitoring of the subjects by a qualified individual, and long-term neuropsychological monitoring.

(3) Setting, TMS team and management of potential adverse effects.

Class 3 studies that are conducted with known protocols and with parameters respecting the guidelines can be conducted in non-medical setting by expert personnel (not necessarily a licensed physician). Appropriate procedures should be planned, however, in case of medical emergency; the personnel should be adequately trained and instructed by a licensed physician on how to react in such emergencies (see Rossi et al., 2009, Section 7.2.6).

Finally, researchers combining TMS–fMRI studies have to know that if TMS is applied in the MR scanner, there are potential safety concerns which are related to the static magnetic field of the MR scanner, the radio frequency pulses and gradients applied during scanning, and the mechanic interaction between the TMS system and static magnetic field of the scanner (see Bestmann et al., 2008a; Siebner et al., 2009b). It will therefore be necessary to screen participants for additional exclusion factors (see Section 7.2.8 in Rossi et al., 2009).

In conclusion, this overview highlights the importance for experimenters to carefully screen their participants for potential exclusion factors and carefully consult the new safety guidelines when planning and designing a future TMS or rTMS study.

10. Conclusions

In this review we summarized the main technical and methodological considerations needed for the use of TMS in cognitive neuroscience, based on the corpus of studies and technical improvements that have become available in most recent years. All
of these aspects exemplified nicely how TMS can be used to investigate a variety of issues in the cognitive neurosciences in general, including location, timing, lateralization and the functional relevance of the neuronal correlates underlying cognitive functions. We provide crucial information for experimenters who want to choose carefully the most appropriate experimental design to test their hypotheses within a given theoretical framework. Moreover, we showed how the use of more than one control condition is crucial for determining the contribution made by a cortical area to a specific behavior. We also discussed the possible approaches to locate “behaviorally silent” areas and the complexity of the effects induced by TMS stimulation on these brain regions. This is due to the fact that, for this domain, the way in which TMS modulates behavior (facilitatory or disruptive effects), depends upon a number of variables such as stimulation parameters (intensity, duration, frequency and pattern of pulses applied), site of stimulation and type of task. Moreover, we should bear in mind that the neural impact of an external stimulus is not determined only by the properties of that stimulus but also on the initial activation state of the brain. Based on the state-dependent effects of TMS, new paradigms have been proposed (TMS-adaptation and TMS-priming) differing from the standard “virtual lesion” TMS which can reveal the necessity of a cortical region but cannot discriminate between functionally distinct neuronal representations in that region.

In addition, the introduction of a causal (i.e., interventional) dimension into noninvasive neuroimaging, shows that the effects may be related to the direct change of activity in the areas immediately underlying the stimulation site, or upon remote interconnected regions. This opens up a new field for research on the brain basis of cognition, in terms of interregional influences within networks. Finally, the increasing number of applications for new protocols (e.g., theta burst), the concurrent combination of TMS with other neuroimaging techniques and the investigation of normal subjects and patients (see for a reviews Dimyan and Cohen, 2010; Ridding and Rothwell, 2007; Minnissi et al., 2008) with longer stimulation sessions led to the development of the new safety guidelines which must be consulted when planning and designing a future TMS or rTMS study.

Acknowledgments

We would like to thank Leonardo Cohen and Chris Chambers for their insightful comments on a previous version of the manuscript, Paolo Ferrari for discussions about the combination of TMS with fMRI, Manuela Ruzzoli for useful suggestions about the neural mechanisms of TMS and two anonymous reviewers.

References


Snyder, A. 2009. Explaining and inducing savant skills: privileged access to lower level, less-processed information. Phil. Trans. R. Soc. B 364, 1399–1405.


Sparing, R., Hesse, M.D., Fink, G.R. 2010. Neuronavigation for transcranial magnetic stimulation (TMS): where we are and where we are going. Cortex 46 (1), 118–120.


