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TMS and fMRI studies investigating the neural mechanisms of tool perception

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Summary

Human occipitotemporal cortex (OTC) is organized into distinct areas that are selectively activated by the visual presentation of different categories, like faces (Kanwisher et al., 1997), bodies (Downing et al., 2001), tools (Chao et al., 1999), hands (Bracci et al., 2010), and places (Epstein et al., 1999). However, the precise role of these areas in processing their preferred stimulus category is still unclear. Moreover, the debate on how such brain organization originates is still unresolved. In order to investigate these issues, we focused on one of these selective areas: a region in left lateral-occipitotemporal cortex (lLOTC) that has been shown to be selective to both hands and tools and that is functionally connected to left intraparietal sulcus (IPS) and premotor cortex (PM), regions involved in action processing (Bracci et al., 2012). Although tools and hands are visually very different, they are both involved in object-directed actions. Thus, lLOTC might process both categories in order to efficiently communicate with IPS and PM. Alternatively, however, tool selectivity in lLOTC may simply reflect epiphenomenal association of tools with hands, for example related to mental imagery. Furthermore, the role of feedback projections between IPS and lLOTC in shaping tool selectivity in lLOTC has not yet been investigated.

Using fMRI, we found that lLOTC was more strongly activated when participants processed action-related properties of tools (deciding on the typical hand action associated with a tool), as compared to when they processed contextual properties of tools (deciding on the typical location associated with a tool). Importantly, TMS over lLOTC led to a specific decrease in accuracy for action judgments but not for contextual judgments, supporting a causal role for lLOTC in processing tool actions. We investigated lLOTC-IPS connections by interleaving repetitive TMS with short fMRI scans. In a first experiment, we applied TMS over left IPS and found a decrease in BOLD signal in left IPS, confirming that TMS suppressed neural processing in the target area. Moreover, BOLD responses also decreased in left dorsal PM and right IPS, suggesting that these two areas receive projections from left IPS. However, no significant change was found in lLOTC, indicating that tool selectivity in lLOTC does not require feedback from IPS. In a second study, we stimulated lLOTC. In this

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study, however, no significant TMS-induced BOLD changes were found in the target region, possible reflecting insufficient statistical power.

In conclusion, our results show that lLOTC is critical in processing action-related properties of tools, indicating that tool selectivity in this area reflects processes that are necessary for understanding tool actions. As a consequence, the finding that lLOTC is causally selective for both tools and hands, even if these two categories are visually very different, supports the hypothesis that the functional organization of OTC partly reflects non-visual organizational principles. Finally, the fact that we did not find significant back projections from IPS to lLOTC suggests that the role of lLOTC in processing tools does not arise as a consequence of activity in higher areas, but that lLOTC is relatively autonomous in processing action-related properties of tools.

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1. Introduction

Vision is our main source of external information in our daily life. We are able to process visual information coming from our surrounding so quickly and automatically that we are not aware at all of how these pure visual inputs are organized and processed in our brain, in order to be able to take any decision on how to interact with our environment. Once our eyes have focused on the object of interest from its surroundings, axons coming from the retina of each eye meet at the optic chiasm, where fibers carrying information from the left and right visual hemifields are divided and project to the contralateral occipital cortex through the lateral geniculate nucleus. Here, the first elaboration of the visual information is carried on, and then passed to higher more specific cortical areas for further elaboration according to what we perceive, but also based on our intentions and needs, projecting towards the temporal and parietal cortex. This processing is organized hierarchically, so that low-level inputs are transformed into more detailed representations through successive stages. The further we go from the first area in the occipital cortex, the bigger the neuronal response latencies and the average receptive field sizes are, contributing to a more complex and fine neuronal response (Smith et al., 2001). While the lower level visual cortex is able to discriminate between basic properties of the visual stimuli, like orientation, shape, motion and color (Zeki et al., 1991; Ungerleider and Haxby, 1994), higher level cortex is organized in more selective areas, that are selectively activated by different categories, in particular categories that are extremely

important in our lives, like faces (Kanwisher et al., 1997), bodies (Downing et al., 2001), words (Dehaene and Cohen, 2011) and tools (Bracci et al., 2012).

Let's take an example that nowadays is very common in our lives: assembling furniture at home by ourselves. A good starting point would be to read the instruction manual. The first level of elaboration about the words that we perceive on the booklet, like shape, color and orientation, is certainly done in the occipital cortex, but later other areas involved in the language/semantic network conduct further processing of what we perceive (Dehaene, 2009). This will eventually consent us to read the instructions, associating a sound to the single letters and a meaning to the whole word and sentence, allowing us to give a meaning to our environment and to react accordingly to reach our goal and building our bookshelves. In the likely case that we were not able to understand the instructions, sometimes it is easier to simply have a look at the pictures and at the drawing of the tools we have to use. Certainly, even simple pictures of tools, like a hammer or a screwdriver, are very immediate to process and they can automatically activate the right motor pattern we would have to perform in order to handle them, even when we are not consciously planning to use them (Makris et al., 2011). Indeed, the simple visual presentation of tools is able to activate the parietal and the premotor cortices (Chao et al., 1999; Chao and Martin, 2000; Bracci et al., 2010; Bracci et al., 2012) implicated in action processing and motor programming, after receiving information from visual areas. The pathways underlying visual information processing have been widely studied, in both healthy participants and patients with restricted lesions, with particular focus on areas that are more selective for specific categories. Nonetheless, it is still unclear where the visual cortex ends and where action and semantic networks start. Is there really a boundary between the visual areas and the higher cortical regions? Why is the brain organized in these specific selective areas?

In order to shed light on these questions, we conducted a series of studies focusing on one of these selective areas in occipital temporal cortex that preferentially responds to the visual presentation of tools (Bracci et al, 2010; 2012), during viewing of pictures. One of the goals was to investigate if this occipitotemporal tool area is purely a vision region, or if it is already involved in processing more complex properties specific to tools (Study 2). Moreover, the study of connections between this lower area and higher areas of the same functional network

may help us to better understand the origin of brain organization. Thus, we explored how this occipitotemporal tool area is connected with higher areas in the downstream parietal/premotor network involved in action processing, in particular on possible back projections from the parietal cortex (Study 3). In order to efficiently investigate both the causal role of this tool area and its functional connections with other areas, we utilized both fMRI and TMS, implementing them in separate experiments as well as combining them in the same experiment. However, since it is also still unclear how TMS affects neuronal activity, in the first study we aimed to better understand how TMS effectively modulates neuronal activity in the stimulated brain region, allowing us to test the models proposed here. Studying the role of this selective area for tools within the tool network can help us to better understand the relevance of non-visual object properties in driving brain organization. But before focusing on this tool area, the next section discusses the path that visual information takes from the external world trough our eyes to the selective areas in occipitotemporal cortex, and the main models that have been proposed to explain such brain organization. Afterwards I will review the main findings about the cortical network involved in tool use, and finally I will illustrate the use of TMS in interfering with cortical activity and how its use can be efficiently combined with fMRI in order to study the role of a specific area within a network.

1.1 Processing of visual information

1.1.1 Organization of visual object knowledge

Visual stimuli are decoded in different steps in the brain passing by several regions while the encoding progressively becomes more complex. The first cortical area to process projections coming from the eyes is the occipital lobe, extending to the temporal lobe, which is the area usually referred to as the visual cortex. The visual cortex is hierarchically divided into five different regions, called V1, V2, V3, V4 and MT (middle temporal), sometimes also named V5 (Zeki et al., 1991). Visual information passes through these regions in an orderly manner, becomeing more and more complex as we proceed within the different levels of visual cortex. V1, or primary visual cortex, represents the biggest visual area covering most of the visual cortex and is also the most studied region in the brain. Cells in V1 have elongated

receptive fields, and thus respond best to elongated stimuli, namely bars and edges (Hubel and Wiesel, 1963; Wiesel and Hubel, 1963a, b), making them very sensitive to stimulus orientation and shapes. In fact, Hubel and Wiesel found that as one goes deeper into the cortex through successive layers perpendicular to the surface, all cells that have orientation tuning prefer the same orientation. In contrast with this, moving across the surface of the cortex, orientation preference changes in an organized way (Hubel and Wiesel, 1974), from which the name of orientation columns to describe V1 architecture derives. Moreover, although most cells in V1 are driven by information coming from both eyes, most of them show an ocular dominance, with a preference for a specific eye, structured in a way similar to the orientation preference (Swisher et al., 2010). While the role of V2 is still mainly confined to the lower visual properties such as orientation, in V3 there are also cells that are tuned to motion and to depth, whereas V4 mostly contains cells that are sensitive to colors (Lueck et al., 1989). Most cells in V5, or MT, are tuned to motion, and it is organized in columns selective for different direction and types of motion (Tootell et al., 1995). These different regions are not only linked by forward connections, but there are also back projections from the higher levels to the lower ones, for example from V5 back to V1 (Shipp and Zeki, 1985). From the occipital cortex, then visual information can flow through two main pathways: the ventral and the dorsal stream (Goodale and Milner, 1992; Ungerleider and Haxby, 1994). The ventral stream is crucial for the visual identification of objects, like when we have to recognize objects, faces, letters and words. It projects from primary visual cortex to the lateral and ventral surfaces of occipital cortex, through to anterior ventral temporal cortex. In contrast, the dorsal stream is involved in processing the spatial relationships among objects and mediates online object-directed action, like visual guidance of movements for using a tool, a network mainly lateralized in the left hemisphere (Figure 1.1). The dorsal stream projects from the primary visual cortex to the dorsal occipital and the lateral temporal cortex, through to parietal cortex (Ungerleider, 1995).

From the very first neuropsychological studies on patients it has been suggested that occipitotemporal cortex (OTC), which is part of both the ventral and the dorsal cortices, is organized in selective areas that are preferentially activated by specific semantic categories, documenting the existence of dissociable neural systems that are specialized for representing

knowledge of different conceptual domains (Warrington and McCarthy, 1987; Capitani et al., 2003).

More recently, several functional imaging studies (Kanwisher et al., 1997; Chao et al., 1999; Peelen and Downing, 2005; Downing et al., 2006), have also found selective areas in different domains in healthy individuals. One of the first categorizations is between an intact object and visual noise, with areas in the lateral occipital cortex showing greater activation with perception of intact objects compared to their scrambled counterparts (Malach et al., 1995).

Further in the ventral occipito-temporal cortex, an area encompassing mid-fusiform and the adjacent occipito-temporal sulcus (OTS), has been proposed to be activated by the visual presentation of words, referred to as the visual word form area (Dehaene and Cohen, 2011; Price and Devlin, 2011). In addition, in our daily life there are also two other categories that are extremely important for our social interaction: faces and bodies. So unsurprisingly there are specific areas that are tuned for these stimuli separately, both are predominately lateralized to the right hemisphere: a face area in the fusiform gyrus (Kanwisher et al., 1997), known as the fusiform face area (FFA); and an area selective for bodies in the extrastriate cortex (EBA), and the fusiform gyrus (FBA), overlapping with FFA (Peelen and Downing, 2005). Other examples of this cortical organization can be seen in the parahippocampal gyrus (Epstein et al., 1999; Downing et al., 2006). Moreover, left lateral occipitotemporal cortex (lLOTC) is preferentially activated by visual presentation of hands and tools (Frey, 2008; Bracci et al., 2012), which is the main focus of the present thesis.

1.1.2 Origin of brain organization

Although many studies have investigated both the dorsal and the ventral streams, from which the selective architecture of the occipitotemporal cortex has been mapped out, it is still unclear what drives such brain organization (Mahon and Caramazza, 2009). After the initial studies investigating cortical architecture, the main approach has been to put emphasis on the sensory-motor properties of visual stimuli that activate a specific area in the brain, with little emphasis on how this area is interacting with other areas involved in processing the same

stimulus (Warrington and McCarthy, 1983; Warrington and Shallice, 1984; Warrington and McCarthy, 1987).

It is clear that the very first elaboration, at least in the first regions of visual cortex, are determined by the type of visual input. Nevertheless, processing of stimuli in further areas is more and more susceptible also to our interpretation of the environment, including our previous knowledge of the stimulus, our beliefs and our actual needs. This process obviously cannot take place in an isolated region, but it must involve a more complex network of cortical areas that need to be efficiently connected. When we see an object, it is very important that we are very proficient in sending this visual input to the areas that are most appropriate for processing that specific stimulus, like the parietal cortex for tools and the temporal/frontal areas for words. This implies that these selective areas in the occipitotemporal cortex are structured in order to project in a parsimonious way to the corresponding higher areas. The importance of this connectivity between visual and higher areas is such that it has been proposed as being responsible for driving brain organization of object knowledge (Mahon and Caramazza 2009). In contrast, to support models that posit the origin of brain organization on the sensory and motor systems, it has been argued that objects that require foveation (e.g. like faces and words) are processed more in the ventral-lateral occipital temporal cortex, compared to other categories that are viewed in the periphery and are processed preferentially in the ventral-medial OTC. Shape has also been proposed to be responsible for visual cortex organization, based on the fact that objects that share form features activate overlapping areas, such as animals, or similar-looking unfamiliar shapes (Haxby et al., 2000; Op de Beeck et al., 2008). Moreover, regions in the occipitotemporal cortex and the parahippocampal cortices also have been shown to be selective for different real-world size of the viewed objects (Konkle and Oliva, 2012). However, also congenitally blind people have shown a similar organization for animate-non living stimuli (Mahon et al., 2010), words (Buchel et al., 1998) and tools (Mahon et al., 2010). A recent study also confirmed the existence of a separated ventral and dorsal stream in blind individuals comparable to the organization in sighted participants (Striem-Amit et al., 2012). Therefore, these results suggest that visual experience is not required for driving OTC organization, and the foundation for cortical architecture may be found internally to the brain rather than

externally. Indeed, an alternative explanation is to assume that the occipitotemporal cortex is organized in order to optimize functional connectivity to the specific downstream networks required by the different categories. This would explain why also people with no visual experience show a similar brain organization to sighted people. Additional support for this functional connectivity account comes from a recent study about left lateral occipital-temporal cortex (lLOTC) that is selective for both hands and tools, which is functionally connected to action processing areas in parietal cortex (Bracci et al., 2012).This is quite a surprising finding considering visual differences between hands and tools, and suggests the possibility that visual cortex organization also reflects action properties of objects, with LOTC possibly being a region that encodes hand-action properties of tools, as we will show in the second study of this thesis.
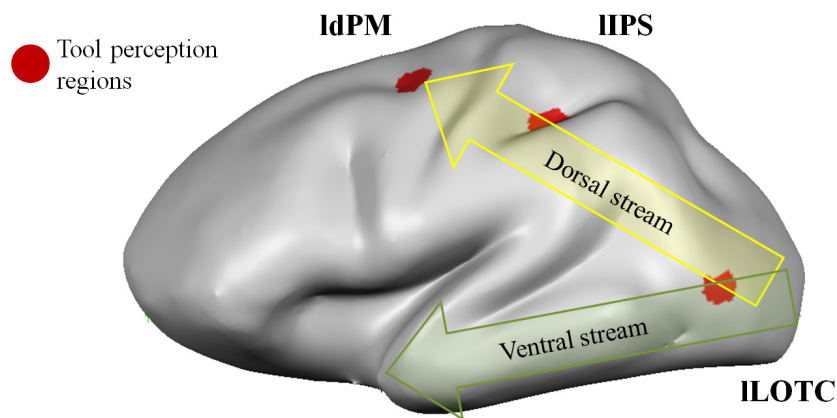
Figure 1.1. Dorsal and ventral pathways and tool perception areas. Yellow arrow depicts the path of the dorsal stream covering occipital cortex and going through parietal and frontal cortex. Green arrow represents the ventral stream, also originating in occipital cortex but then heading towards temporal regions. Red regions represent the three main areas that are activated by the simple presentation of pictures of tools (Bracci et al., 2012). ldPM: left dorsal premotor cortex; lIPS: left intraparietal sulcus; lLOTC: left lateral occipitotemporal cortex.

1.2 A tool network in the brain

1.2.1 Tool use and cortical lesions

Tools, here meant as handheld objects that are typically manipulated by the dominant hand to achieve action goals, physically and functionally extend our body, allowing us to achieve a wide range of goals that would not be possible with our bodies alone. Much progress has

recently been made in understanding the neural architecture that supports complex tool use. As it is for language, the tool-use network is mainly lateralized to the left hemisphere, represented in the brain as an extension of networks that are more generally involved in hand actions. Neuropsychological and imaging studies showed a temporo-parieto-frontal pattern for action knowledge elaboration, with the exact cortical locations slightly differing depending on the type of the task (see for example (Lewis, 2006). Tools, as well as other categories, are composed of different types of information. Indeed, in the last century, neuropsychological studies have indicated that at least two properties about tool knowledge are dissociable, supporting the idea that different features about tools are processed in separated cortical regions and that knowledge is spread across different areas. Indeed, there is evidence that skills in using a tool are processed separately from conceptual knowledge about the same tool. For example, specific damage to areas in the left hemisphere, in specific the posterior parietal and premotor cortex, can selectively impair the ability to correctly manipulate a tool or imitate its use, while leaving intact the capacity to name or describe the tool and its functional purpose (Ochipa et al., 1989; Leiguarda and Marsden, 2000; Rumiati et al., 2001; Koski et al., 2002). In contrast, lesions in more posterior areas, in particular the left posterior temporal-parietal junction, lead to the opposite pattern, with patients well able to correctly handle a tool, while conceptual knowledge like recognizing what it is for is compromised, sometimes putting the patient in funny situations like using a toothbrush as a razor blade, or in dangerous situations such as the opposite one (Leiguarda and Marsden, 2000; Johnson-Frey, 2004; Mahon and Caramazza, 2005). A more recent clinical study also investigated which type of information depended directly on parietal lobe lesions, and which were maintained relatively intact (Goldenberg and Spatt, 2009). The authors tested three different components of tool knowledge: functional knowledge from semantic memory (Functional Associations), where patients had to select the appropriate recipient for the tool (e.g. hair for a comb), or to indicate a substitutive tool that could be used for the same purpose; mechanical problem solving using new tools (Novel Tools); and, use of everyday tools and objects (Common Tools). Whereas the frontal lesions had an adverse influence on all experimental tests, parietal lesions impaired Novel and Common Tools, but did not have an adverse effect on the Functional Associations. Instead, temporal lesions seemed to be

linked to impairments in the Functional Associations test, but this association did not show up in the whole group analysis. Another study showed that lesions to the middle temporal gyrus lead to impairment in retrieving conceptual knowledge for actions, together with lesions in the left premotor/prefrontal sector, and in the left parietal region (Tranel et al., 2003). Interestingly, in a previous study by the same authors (Tranel et al., 1997) it was also shown that defective recognition of tools, compared to animals and persons, was associated with maximal lesion overlap in the occipitotemporal-parietal junction of the left hemisphere. Another way to selectively study the causal role of an area is by creating a "temporary lesion" by applying TMS to the specific area of interest during specific tasks, partially confirming findings coming from clinical studies. TMS over left posterior parietal cortex impaired the capacity to perform online adjustments that are necessary for visuo-motor task actions (Glover et al., 2005; Tunik et al., 2005). These studies also suggested that TMS over left parietal cortex showed that this area is more involved in tool use compared to the right parietal cortex, which instead is more responsible for imitation of the action of others. A recent study combined TMS and electromyography (EMG), showing that motor-evoked potentials (MEP) recorded from hand muscles are modulated by the handle orientation of passively viewed objects (Buccino et al., 2009), confirming that the tool network is activated also when there is no action planning involved, and simple tool presentation is enough to trigger also motor areas.

In conclusion, clinical studies suggest a causal role of MTG/lLOTC in tool perception, but it is not possible to exclude that this impairment in tool knowledge is due also to lesion in other areas. Moreover, whereas TMS studies showed the involvement of parietal and premotor cortex in tool processing, even where there is no overt action required, so far there are no TMS studies on healthy participants that show a causal role of lLOTC in tool knowledge. Our study in chapter 2 showed for the first time the causal role of lLOTC during tool perception in healthy subjects.

1.2.2 Tool use in healthy participants

Functional neuroimaging first of all confirmed the temporo-parietal network involved in tool use, and also demonstrated that the sensory and motor systems are both automatically

engaged during the conceptual processing of tools (Lewis, 2006). Besides, functional imaging studies converge with neuropsychological data in showing that different tool dimensions are elaborated in separated regions (Boronat et al., 2005; Canessa et al., 2008). Mirroring clinical studies, there is also imaging evidence that the temporal and not the parietal cortex is involved in representing the functional knowledge of tools (Canessa et al., 2008).

A PET study also showed that functional judgments about manipulable objects compared to non-manipulable ones, activated a network that included ventral premotor cortex (vPMC), posterior middle temporal gyrus (pMTG), and intraparietal sulcus (IPS). The left vPMC and pMTG were activated during both implicit and explicit retrieval of tool knowledge, while IPS clearly showed a preference for the explicit task (Kellenbach et al., 2003).

Tool knowledge has been widely investigated with fMRI also using different presentation modalities. Several studies have investigated the cortical regions involved in pantomiming tool use (Choi et al., 2001; Inoue et al., 2001; Grèzes et al., 2003; Johnson-Frey et al., 2005; Fridman et al., 2006). Although tool pantomiming may also include some abstraction that real tool use does not require, it still approximates quite well the planning of tool action and has the additional benefit to be more testable in the scanner. Activation of areas in the left hemisphere, primary motor cortex (M1) and in primary somatosensory cortex, were likely due to hand and arm movements independent of tool. Nevertheless, the general pattern of activation found in these studies still provided a good estimate of cortical regions involved in pantomiming tool use, like the left superior parietal lobule (SPL). In addition, other regions showed more bilateral activation, including the dorsolateral premotor cortex, ventral premotor cortex, the inferior parietal lobule (IPL), and again the posterior portions of the middle temporal gyrus (for review see Lewis, 2006).

Similarly, studies aiming to examine more conceptual aspects of tool use had participants only imagine using the tool or preparing an action with it, finding that a similar network was activated, which was more left lateralized (Decety et al., 1997; Grèzes et al., 2003; Creem-Regehr and Lee, 2005; Fridman et al., 2006). Several studies also showed that hearing hand tool sounds compared to sounds of different origins, like animal sounds, activated areas that overlapped those involved during tool use, and including again the left IPL, SPL, vPMC,

middle frontal cortex, and the bilateral pMTG (Aziz-Zadeh et al., 2004; Beauchamp et al., 2004; Lewis et al., 2005).

Other studies, where participants viewed and named several objects, showed activation in Wernicke's language perception area (Wise et al., 2001; Binder et al., 2003). But tools, compared to other categories of objects, activated more portions of the left posterior MTG, the inferior temporal cortex (ITC) and frontal cortex areas, as well as the left ventral premotor cortex and the left posterior parietal cortex, consistent with a left network involved in language related processing (Damasio et al., 1996; Vandenberghe et al., 1996; Tranel et al., 1997; Chao and Martin, 2000). Moreover, the same cortical network is also activated when deaf signers have to name tools (Emmorey et al., 2004). Notably, the overall pattern of activation for naming tools was remarkably similar to the pattern for viewing tools.

1.2.3 Tool perception in lateral occipitotemporal cortex

Within the tool network, most neuropsychological and imaging studies have focused on higher cortical areas in the temporo-parietal cortex, while more recently, imaging studies have shown that there is also a tool-selective area in the occipitotemporal cortex. That is, even when no overt action elaboration is required, an area in OTC, mostly lateralized in the left hemisphere, is selectively activated by tools compared to non-manipulable objects and other categories (Bracci et al, 2010; 2012), in addition to intraparietal sulcus and dorsal premotor cortex (Figure 1.1). As we partially reviewed in the previous section, the left lateral occipitotemporal cortex, including the middle temporal gyrus (MTG), is activated in different types of task involving tool perception from different sensory modalities, with the exact location slightly differing according to how it is localized (for an anatomical delineation of lateral OTC see (Weiner and Grill-Spector). In this section we focus on the studies on LOTC, the focal point of the thesis.

As aforementioned, activity related to tool processing in MTG was also found in response to pictures and written names of tools (Chao et al., 2002; Phillips et al., 2002; Kellenbach et al., 2003; Kable et al., 2005a). The same region was also activated when imagining tools (Creem-Regehr and Lee, 2005), hearing to tools associated sounds (Lewis et al., 2004; Lewis et al., 2005; Tranel et al., 2005) and in response to spoken tool names (Noppeney et al.,

2006). Activity in MTG was also differently modulated by the type of information that participants had to retrieve about an object, irrespective of whether this was presented as a picture or a written name. When the task required to generate words regarding an action on the object, activity in MTG was higher in comparison to the task requiring to name the color of the object (Martin et al., 1995). In another imaging study that supported the involvement of the left pMTG in action properties processing by using a noun-verb homonym production task, participants had to produce a single word in response to pictures of objects (saying "saw" to a picture of a saw) and actions (saying "saw" to a picture of a person sawing). Results showed that left pMTG was more active when noun-verb homonyms like "hammer" and "comb" were generated in comparison to name actions than when used to name objects (Tranel et al. 2005b). An area selective for visual presentation of tools is usually also found in the anterior medial fusiform gyrus (Chao et al., 1999; Chao et al., 2002; Whatmough et al., 2002; Fairhall et al., 2011), in contrast to other categories like animals and faces. Enhanced activity in the medial fusiform gyrus using tool pictures and/or their written names has also been reported by Devlin et al. (2005), Mechelli et al. (2006), Noppeney et al. (2006), and Whatmough et al. (2002).

The involvement of MTG/LOTC in tool knowledge arises quite clearly from imaging studies, while the causal role of this area in tool processing is still unclear. Indeed, as we reviewed above, there is little evidence that lesions selective to LOTC lead to impairment in tool processing. Even studies on healthy subjects are lacking for this finding, and as to our knowledge no TMS studies have tested the causal role of MTG while elaborating manipulable objects. Moreover, this area in lLOTC has been found to be selective also for hands more than other body parts. Obviously tools and hands are visually very different, but they both share the property of being involved in object-directed actions. Thus, this finding raises the possibility that lLOTC is not simply a visual area, but is already involved in processing action-related properties of both hands and tools, as also suggested by the significant functional connectivity between lLOTC and the parietal cortex (Bracci et al., 2012). Alternatively, selectivity to tools in hand-selective lLOTC could reflect epiphenomenal associations between tools and hands, for example through mental imagery of hands when viewing tools. Another point that is unclear about tool knowledge in LOTC, is

how this area is connected with the downstream fronto-parietal network, and if LOTC is a necessary step in order to further process tool information in higher areas.

1.3 Use of TMS and fMRI

In the present thesis we make use of both TMS and fMRI, in separate studies as in the second experimental chapter, or in combination in the third experimental chapter, where we used repetitive TMS followed immediately by an imaging session. In order to have a better interpretation of the TMS-fMRI results, here we review the main findings on how TMS interacts with neuronal activity and how its use can be combined with imaging. Indeed, TMS and fMRI have become two of the main standard tools in human cognitive neuroscience. fMRI achieves a satisfactory spatial resolution in fMRI to the order of millimeters, by assessing neuronal activity indirectly based on the detection of local changes of the blood oxygenation level. This is the reason why its temporal resolution is only in the order of seconds. However, one of the biggest limitations of fMRI in the study of cognitive functions is that with fMRI no causal relationships between the activity in a given brain area and the behavior under investigation can be achieved, providing only correlative information. On the other hand, TMS can directly interfere with ongoing neural activity creating transient so called 'virtual lesions', and thus can precisely provide the information on causal relations between brain and behavior that cannot be shown by imaging techniques alone (Walsh and Rushworth, 1999). Moreover, although TMS lacks in spatial resolution, it has a fine temporal resolution, allowing researchers to investigate more in detail the timing of the flow of information within a network. Thus, it is clear that TMS and functional imaging are two techniques that can be even more informative when used in combination in the study of the link between brain and behavior, assessing both spatial resolution and the causal role of an area. In addition, the TMS-fMRI approach can be extremely useful in order to investigate how different cortical regions are connected and to study network reorganization when activity in one area is impaired. However, despite the wide usage of TMS in any aspect of human cognition, its exact mechanisms of action and modulation of ongoing neural activity remain unclear. This is why in our first study we wanted to assess the different models that have been proposed in order to explain its effect. In the next section we will introduce these

main models that we will test in chapter 2. Next, we present how TMS can be combined with fMRI in order to investigate cortical networks.

1.3.1 Neural mechanisms of TMS-induced effects

TMS is able to give information about the causal role of an area by interfering with electrical activity of the underlying neuronal population, by using the principles of electromagnetic induction to generate electrical currents in the brain (Barker et al., 1985). The TMS stimulator delivers a large electrical current in a brief period of time, and the current flowing in the TMS coil produces a magnetic field that lasts for a few milliseconds, which is in turn able to reach the surface of the cortex given appropriate parameters like TMS intensity and coil orientation. This rapidly changing magnetic field can penetrate the scalp and skull and induce an electrical field sufficient to stimulate neuronal activity. Although the underlying mechanisms of this modulation are still far from clear, TMS is thought to interfere with neuronal activity at the level of the axons, rather than the cell bodies of cortical neurons (Ridding and Rothwell, 2007). The level at which TMS acts on the neuron could be very relevant for example in studies that want to investigate the connection between the stimulated cortical region and distal regions of interest. But how does TMS change this activity? Usually, the TMS effect is described as a "virtual lesion", with a general impairment of neuronal activity (Walsh and Rushworth, 1999; Harris et al., 2008). Recent findings, though, have demonstrated that TMS effects are not fixed, but state-dependent: brain states determine the direction of behavioral effects induced by TMS. Psychophysical paradigms, such as adaptation or priming, can increase the functional resolution of TMS and improve predictions about its behavioral consequences. Specifically, TMS adaptation (TMSa) predicts that TMS improves processing of perceptual attributes that are adapted, while decreasing performance for non-adapted attributes. In a series of studies, Silvanto and colleagues clearly showed that TMS is brain state dependent (Creem-Regehr and Lee, 2005; Silvanto et al., 2007a; Silvanto and Pascual-Leone, 2008). One of the first studies on TMS adaptation showed that after visual adaptation to the color green, we usually experience a red visual afterimage, meaning that neurons coding for green colour are less active then neurons coding red. However, TMS over occipital cortex induced phosphenes that looked like vivid green blobs within the

afterimage (Silvanto et al. 2007). This was one of the first pieces of evidence that effect of TMS depend on the prior activation of the stimulated neurons. On one side, this finding is extremely relevant because it opens new possibilities for using TMS in cognitive neuroscience. For example, TMS has a spatial resolution of about 1 cm, but the fact that its effect depends on the neurons activity, with appropriate paradigms and designs it is possible to measure differently the response of two close or even overlapping neuronal populations, given that they are activated by different stimuli. In this case it is possible to pre-activate only one of this population by adaptation of priming prior to the TMS stimulation (Silvanto and Muggleton, 2008; Cattaneo et al., 2010; Ruzzoli et al., 2010).

On the other hand, the study of brain-state TMS can help us to better understand the underlying mechanisms of TMS. The most popular proposed explanations are based on neuronal suppression and noise addition. Some findings seem to support the model for which TMS suppresses neural signals (Harris et al., 2008), while others would suggest that it adds random neural activity to the ongoing processing (Ruzzoli et al., 2010; Walsh and Cowey, 2000). Others again propose that TMS effects are better explained by a combination of each, with the exact balance depending on stimulation intensity but also on the anatomo-functional characteristics of the stimulated tissue (see Miniussi et al., 2010; Siebner et al., 2009a). Although there is still no agreement on how TMS interferes with neuronal activity, they clearly show that its effect is much more complex than the "virtual lesion" approach proposed at the beginning. As a consequence, also interpreting BOLD modulation due to TMS stimulation becomes more difficult, asking for additional investigation to put light on how TMS affects neural mechanisms.

1.3.2 TMS combined with fMRI

TMS and fMRI can be combined in three different ways (for review see (Siebner et al., 2009). The most traditional way is to use neuroimaging to functionally map the area to be targeted with TMS in a subsequent session (map-and-perturb approach). The other two methods both interleave TMS and fMRI measurement, one using single pulse or short TMS trains online in the scanner (perturb-and-measure), whereas the third method consists of using neuroimaging after conditioning with TMS in order to map brain areas that are

connected with the stimulated one, either during resting state or during a specific cognitive task. Interleaved TMS/fMRI combines the advantages of the two single techniques: fMRI offers whole-brain coverage at good spatial and temporal resolution, while TMS allows us to establish causal structure-function links. The perturb-and-measure approach was first introduced by Paus (2005) as a method for mapping neural connections in the live human brain. Authors used TMS to perturb directly a selected cortical area, while simultaneously they measured changes in brain activity using PET. In their first study, Paus et al (Paus, 2005) applied TMS to the left frontal eye fields (FEF) and found a significant positive correlation between the number of TMS pulses and CBF at the stimulation site. Most importantly, they found a change in CBF also in the superior parietal and medial parieto-occipital regions, consistent with the known anatomical connectivity of monkey frontal eye fields. Since then an increasing number of studies have used a similar TMS-fMRI approach to investigate neuronal connectivity, mainly targeting the motor system. Bestmann and colleagues (Bestmann et al., 2005; Bestmann et al., 2008) for example applied repetitive TMS over left dorsal premotor cortex (dPM), and found that it led to a decrease in BOLD response in regions that are connected with left dPM, including the right dorsal premotor, bilateral ventral premotor cortex, supplementary motor area, somatosensory cortex, cingulate motor area, left posterior temporal lobe, cerebellum, and caudate nucleus.

A more recent paper investigated functional connections between the posterior superior temporal sulcus (pSTS), activated when observing biological motion, and the rest of the action observation system. They applied low frequency TMS over pSTS and then measured BOLD activity while participants observed a hand holding an object or a control condition without grasping (Arfeller et al.). Results showed a change in BOLD in the lateral temporo-occipital cortex, anterior intraparietal region and the ventral premotor cortex during the holding of the objects, suggesting the role of pSTS as a pivotal region during observation of goal-directed actions.

So far few studies have investigated other networks than motor networks combining TMS and fMRI, like the impact of lamotrigine on prefrontal-limbic circuits (Li et al., 2004) and the effect of TMS over parietal cortex in a visuo-spatial imagery task while simultaneously assessing the impact on the task-related activity in the stimulated area and in interconnected

frontal regions (Sack et al., 2007). Two other studies investigated top-down effects of frontal and parietal regions on early visual areas (Ruff et al., 2006; Ruff et al., 2008). Here authors applied TMS over intraparietal sulcus, a site previously implicated in attention, and found that stimulation elicited a pattern of activity-changes in visual cortex, depended on the current visual context. TMS over IPS affected the BOLD signal in V5/MT only when moving stimuli were presented, whereas BOLD signal changes due to IPS TMS were observed in areas V1-V4 only when there was no visual input, showing directly that parietal and frontal regions can indeed have distinct patterns of causal influence upon functional activity in human visual cortex (Ruff et al., 2008). In contrast, TMS over FEF led to activity increase for retinotopic representations of the peripheral visual field, but to activity decrease of the central field, in areas V1- V4.

In general, the above studies illustrate well how the new approach of concurrent TMS-fMRI can reveal causal interactions between remote but interconnected areas of the human brain.

2. Occipital TMS has an activity-dependent suppressive effect

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Abstract

The effects of transcranial magnetic stimulation (TMS) vary depending on the brain state at the stimulation moment. Four mechanisms have been proposed to underlie these effects: (i) virtual lesion–TMS suppresses neural signals; (ii) preferential activation of less active neurons–TMS drives up activity in the stimulated area, but active neurons are saturating, (iii) noise generation–TMS adds random neuronal activity and its effect interacts with stimulus-intensity; (iv) noise generation–TMS adds random neuronal activity and its effect depends on TMS-intensity. Here we explore these hypotheses by investigating the effects of TMS on early visual cortex on the contrast response function while varying adaptation state of the observers. We tested human participants in an orientation discrimination task, in which

performance is contingent upon contrast sensitivity. Before each trial, neuronal activation of visual cortex was altered through contrast adaptation to two flickering gratings. In a factorial design, with or without adaptation, a single TMS pulse was delivered simultaneously with targets of varying contrast. Adaptation decreased contrast sensitivity. The effect of TMS on performance was state-dependent: TMS decreased contrast sensitivity in the absence of adaptation but increased it after adaptation. None of the proposed mechanisms can account for the results in their entirety, in particular, for the facilitatory effect at intermediate to high contrasts after adaptation. We propose an alternative hypothesis: TMS effects are activity-dependent, so that TMS suppresses the most active neurons and thereby changes the balance between excitation and inhibition.

2.1 Introduction

Transcranial magnetic stimulation (TMS) is applied on a cortical region of interest to disrupt the ongoing cerebral activity and test its causal role in a cognitive task, thus inducing a 'virtual lesion' (Walsh and Rushworth, 1999; Harris et al., 2008). Behavioral effects of TMS are not fixed, their direction is determined by the brain state during stimulation (Silvanto and Muggleton, 2008). Thus, psychophysical paradigms–adaptation or priming–can increase TMS functional resolution and improve predictions about its behavioral consequences (Cattaneo and Silvanto, 2008; Silvanto et al., 2008a; Cattaneo et al., 2010). TMS-adaptation (TMSa) paradigm predicts that TMS improves processing of perceptual attributes that are adapted while decreasing performance for non-adapted attributes. The underlying mechanisms of TMS and TMSa are still debated.

Here we manipulated the brain state using contrast adaptation–a decrease in visual contrast sensitivity produced by repeated exposure to high contrast stimuli (Dao et al., 2006; Pestilli et al., 2007)–to investigate the mechanisms of TMSa. We assessed how TMS affects the behavioral contrast response functions (CRF), which depict spike rate (McAdams and Maunsell, 1999) or discrimination performance (Pestilli et al., 2009) as a function of stimulus contrast (Figure 2.1a). We tested four hypotheses on how TMS modulates perception:

(i) Signal suppression (Naka and Rushton, 1966; Amassian et al., 1989) predicts that TMS decreases the sensitivity of the underlying cortex independent of state; a rightward shift of the CRF regardless of adaptation (Figure 2.1b).

(ii) The "preferential activation of less active neurons" (Silvanto et al., 2008b) predicts that as activity increases, TMS activates signal-bearing neurons less than noninformative neurons. Increasing stimulus contrast, causes only informative neurons, not non-informative, to increase their firing rate. At high contrast levels informative neurons are already highly active, so TMS would preferentially excite noninformative neurons. Hence, TMS would decrease the activation difference between informative and noninformative neurons and this SNR, and thus performance, decrement would be directly proportional to contrast, modulating the asymptote (Figure 2.1c). This argument holds if one assumes suppression of the most active neurons. Due to a floor effect, noninformative neurons cannot be further suppressed, and the SNR would decrease with stimulus contrast, decreasing the asymptote.

(iii) A noise generation hypothesis predicts different effects of TMS as a function of either stimulus intensity (Simonotto et al., 1997; Ruzzoli et al., 2010) or (iv) TMS and stimulus intensity (Schwarzkopf et al., 2011). Based on opposite TMS effects depending on the system's state it has been argued that noise induction in a nonlinear system leads to stochastic resonance (Stocks, 2000; Schwarzkopf et al., 2011). According to (iii), stochastic resonance would result in a nonlinear alteration of the effective threshold of near-threshold stimuli: noise would boost representations of stimuli just below threshold improving sensitivity, but it would reduce representations of stimuli just above threshold decreasing sensitivity (Simonotto et al., 1997; Ruzzoli et al., 2010). A parsimonious explanation predicts the same effect for the CRF after adaptation (Figure 2.1d). According to (iv), with high TMS-intensity, sensitivity should decrease regardless of stimulus intensity, but with low TMS-intensity, sensitivity would increase, especially for low stimulus values. To explain previous effects of TMS-adaptation, Schwarzkopf et al. (2011) claim that the adaptation effect is akin to reducing TMS-intensity. Consequently, stimulating with high intensity after adaptation and stimulating with low intensity without adaptation yield the same prediction (Figure 2.1e).
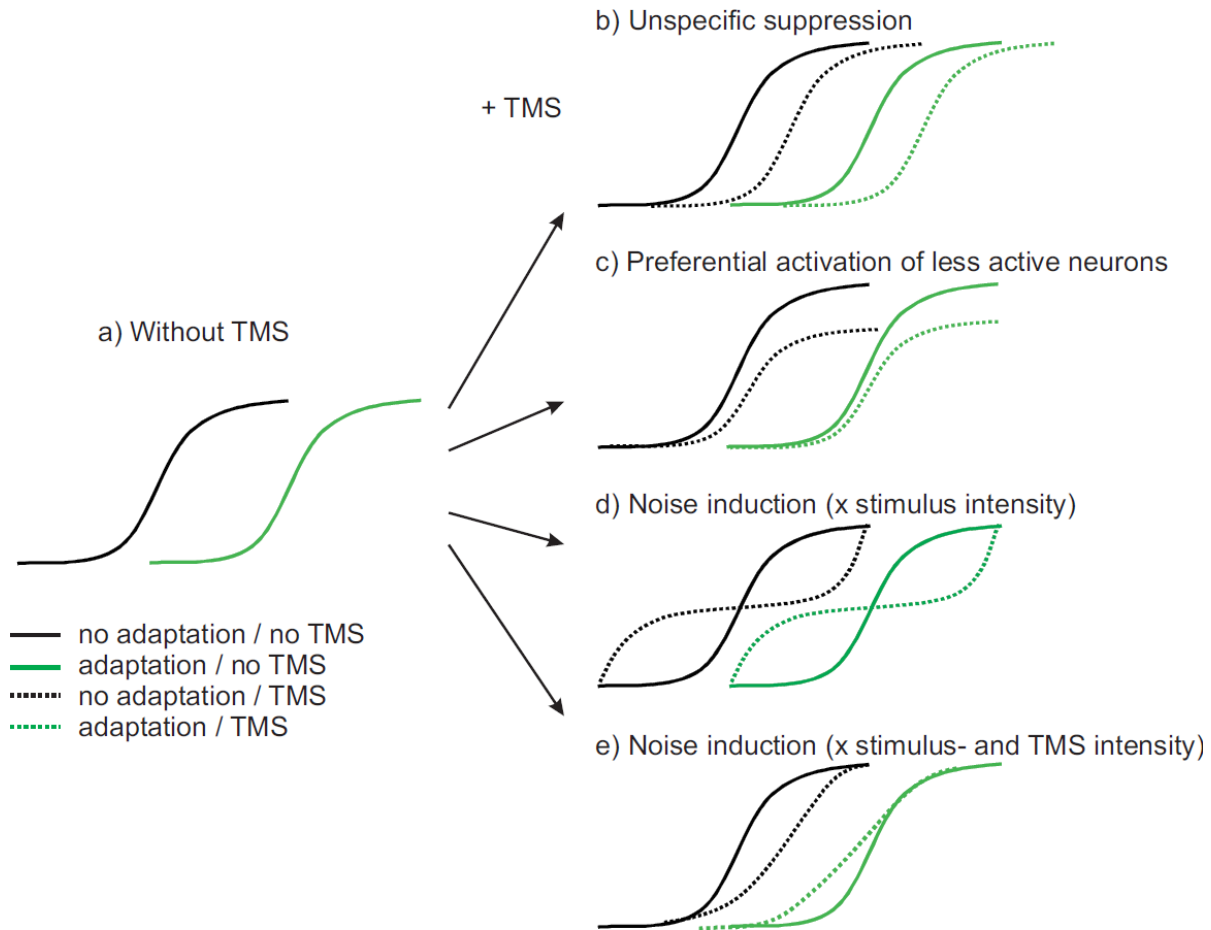
Figure 2.1. Contrast Response Function (CRF) without (a) and with TMS (b-e). a). Increasing stimulus contrast leads to increased accuracy of behavioral reports yielding a CRF (black solid line). Adaptation reduces contrast sensitivity, leading to a rightward shift of the CRF (green solid line). b-e), describe putative effects of TMS on behavioral performance. **b)**. A simple signal suppression account predicts that TMS decreases the sensitivity of the underlying cortex no matter which state it is in, thereby leading to a rightward shift of the CRF without (black dotted line) or with adaptation (green dotted line). **c)**. The "preferential activation of less active neurons" view (Silvanto and Pascual-Leone, 2008) postulates that TMS adds activity to the stimulated population of neurons, but that already active neurons are subject to saturation. The account predicts that, as activity increases, signal-bearing neurons get less activated by TMS than noninformative neurons. This would lead to a detriment in performance that becomes stronger as contrast increases, resulting in a change of asymptote of the CRF. **d)**. A noise-induction account in combination with stochastic resonance predicts that TMS particularly affects perithreshold stimuli, given that the predicted effect is a function of stimulus intensity (Ruzzoli et al., 2010). Neural representations just below threshold are pushed across threshold while signals just above threshold are pushed downwards. **e)**. An alternative noise induction account in combination with stochastic resonance predicts that TMS affects responses as a function of TMS- and stimulus intensity (Schwarzkopf et al., 2011). Without adaptation, when TMS-intensity is high, sensitivity would decrease regardless of stimulus contrast; with adaptation, akin to reducing TMS intensity, high-intensity TMS would have a facilitatory effect only for stimuli at low to intermediate contrast levels with TMS effects being inversely proportional related to contrast. Note that b-d predict qualitatively the same effect of TMS with and without adaptation. In contrast, for *e* TMS should modulate the shape of the adapted and not adapted CRF in a different way.

2.2 Materials and Methods

Six participants performed a 2AFC orientation discrimination task, following contrast adaptation to two flickering Gabors with (adapted) or without (non-adapted) adaptation (henceforth referred to as *adaptation* or *no-adaptation* blocks). To assess contrast sensitivity, we measured performance in an orientation discrimination task as a function of stimulus contrast (Nachmias, 1967; Carrasco et al., 2000; Cameron et al., 2002; Herrmann et al., 2010).

The experiment consisted of six different sessions. The first session was only behavioral, with a two-fold aim: to reach a stable performance and to determine stimulus-parameters so that participants' behavior would encompass the whole CRF to enable testing the different models. TMS was applied and behavior was measured concomitantly using the parameters obtained in the behavioral session.

2.2.1 Participants

Six healthy participants (4 females and 2 males, aged 26 to 36, including two of the authors F.P. and L.C.) with no history of neurological or psychiatric illness were recruited for the study. Participants were selected given their ability to detect the presence of phosphenes induced by occipital TMS. Four observers were naïve to the purpose of the experiment. All participants underwent a screening for TMS contraindications and a short informal debriefing after each session to assess short-term side-effects. All participants gave informed consent before participating in the study. The study was approved by the ethics committee of the University of Trento; participants were treated in accordance with the Helsinki declaration.

2.2.2 Apparatus

Stimuli were presented on a 17-inch gamma-corrected LCD monitor (DELL 1908FP-BLK) in a dimly lit room. The background luminance was set to the middle of the monitor range at 59 cd/m2. The stimuli were generated and presented on a standard PC using Matlab 2007b (Mathworks, Massachusetts) and ASF (Schwarzbach, 2011), an add-on to the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997).

The task was a 2AFC orientation discrimination task on one of two tilted peripheral Gabors (sinusoidal gratings embedded in a Gaussian distribution; Figure 2.2), while fixating a central fixation cross (0.5˚ x 0.5˚ visual angle). Trials were divided in adaptation and non-adaptation blocks of 20 trials each. At the beginning of each adaptation block, there were two vertical Gabors displayed 3˚to the left and right and 1.5˚ below the fixation cross, flickering at 7.5 Hz during 70 s. No-adaptation blocks started with a blank gray screen lasting for 20 s. Each trial started with a 4 seconds top-up phase, which consisted of two flickering Gabors in adaptation blocks (and a blank screen with only fixation cross in non-adaptation blocks). After the presentation of a central white square subtending 1° x 0.4° visual angle, which served as a fixation point, participants were presented with two static Gabor patches (5 c/deg subtending 4˚ of visual angle in diameter; σ of 1.5˚). In TMS-trials, a single pulse of 120% of the phosphene threshold was applied at stimulus onset. The test Gabors were displayed at the same locations as the adapting Gabors. To equate participants' performance, the test Gabors were slightly tilted (1.25˚ to 2.5°), clockwise or counter-clockwise, from vertical. Each Gabor had an independent, randomly chosen tilt. In each trial, both test Gabors had one out of six different contrast levels (2.5, 5, 10, 20, 30, 80%). Following presentation of the test Gabors, a response cue (white square) appeared 0.5° to the left or right of the fixation cross, indicating the side of the target Gabor. Participants responded within a 1-s allotted time, and got an acoustic feedback (a high or low pitch for correct or incorrect responses, respectively).

2.2.3 Procedure and Design

Participants were trained for about an hour and the tilt of orientation was adjusted for each observer and kept constant in the following five TMS sessions. Each of these sessions lasted 90 min, two no-TMS runs and two TMS runs. No-TMS and TMS runs were interleaved, and the first run of each session was counterbalanced. Adaptation and no-adaptation blocks were alternated within each run, with half of the runs starting with an adaptation-block. In no-TMS runs the coil was directed to the same location as in TMS runs, but moved away from the head by 1-2cm such that cortex would not be stimulated, while keeping the somatosensory and auditive experience for the participants constant. Contrast levels of the test Gabors, their respective tilts, and position of the response cue were randomized and counterbalanced

throughout the whole experiment. There were 1920 trials per participant, leading to 80 data points per cell and observer.
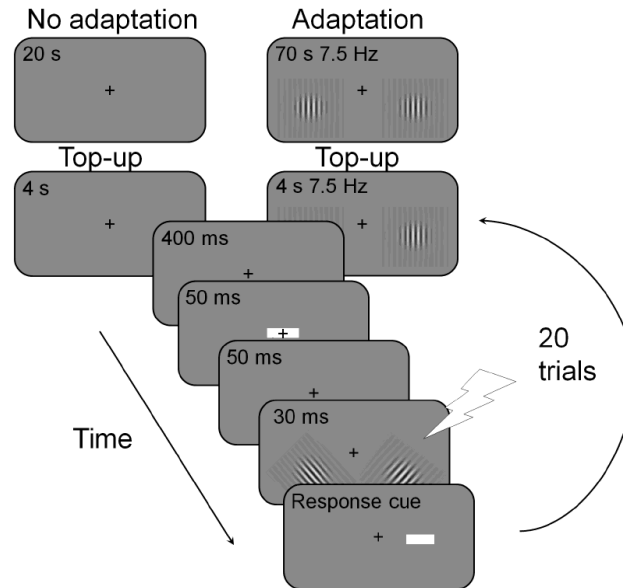

Figure 2.2. Trial procedure. At the beginning of each block of 20 either adapation or no adaptation trials, participants were presented with a long adaptation phase. In the no-adaptation blocks, participants saw a 20-s-long blank screen, while fixating the central cross. In the adaptation-blocks participants saw two vertical Gabors at 100% contrast flickering for 70 s. After this adaptation phase, there were 20 trials starting with a 4 s top-up phase. Following the presentation of a 50 ms central cue and a 50 ms blank screen, two tilted Gabors were presented for 30 ms. In TMS-trials, a single pulse of 120% of the phosphene threshold was applied at stimulus onset. Observers had to discriminate orientation of the target Gabor, whose location was indicated by a subsequent 1-s response cue (task adapted from Pestilli et al., 2007).

2.2.4 TMS methods

Prior to the experiment, a high-resolution T1-weighted scan (magnetization-prepared rapid gradient echo sequence (MPRAGE), 176 slices, in-plane resolution 256 x 224, 1 mm isotropic voxels, Generalized Autocalibrating Partially Parallel Acquisition (GRAPPA) with acceleration factor of 2, time to repeat (TR) = 2700 ms, time to echo (TE) = 4.18 ms, time to inversion (TI) = 1020 ms, flip angle = 7°) of the brain of each participant was obtained using a MedSpec 4-T head scanner (Bruker BioSpin, Ettlingen, Germany) with an 8-channel array head coil (USA Instruments, Aurora, Ohio, USA). We coregistered the position of the TMS coil with the participants' reconstructed head and marked the location of TMS-stimulation on the reconstructed pial surface of each individual brain using BrainVoyager Neuronavigator

(Brain Innovation BV, The Netherlands, version 2.1) combined with a Zebris CMS20S measuring system for real-time motion analysis (Zebris Medical GmbH, Isny, Germany). Average distance between occipital cortex and scalp was 14.5 mm (range 11-21 mm).

At the beginning of the first TMS session we determined the stimulation site by placing the coil over the occipital pole and moving the coil until a single pulse would generate a phosphene at the center of the visual field, where the two Gabors would later be presented. Although the main target of stimulation was V1, surrounding visual areas such as V2 and V3, where also probably stimulated (Thielscher et al., 2010; Salminen-Vaparanta et al., 2011). The coil position was stored for use by the neuronavigator in the remaining sessions. Biphasic TMS pulses were applied with a figure-of eight coil (MC-B70) and a MagPro x 100 stimulator (MagVenture A/S, Denmark). The individual threshold of cortical excitability was established as the lowest stimulation intensity applied over visual cortex necessary for inducing phosphenes after dark-adaptation. The stimulation intensity used during the experiment was set at 120% of the individual dark-adapted phosphene threshold on early visual cortex, which ranged from 70% to 80% of the maximal stimulator output. Participants wore earplugs throughout the whole experiment.

2.3 Results

We found that none of these prevalent hypotheses could account for the data we report in its entirety, that is, for the respective shifts of the contrast response function (CRF) induced by TMS with and without prior adaptation. Instead, we observed a decrease of contrast sensitivity with TMS in the no adaptation state, but an increase of sensitivity with TMS in the adaptation state for contrast levels at the dynamic range of the CRF.

The effects of adaptation, TMS, and contrast on the mean accuracy are depicted in Figure 2.3. We submitted accuracy to a three-way repeated measures ANOVA with the application of *TMS* (TMS/no TMS), *Adaptation* state (adaptation/no-adaptation) and *Contrast* (2.5, 5, 10, 20, 30, 80%), as within-subject factors. Accuracy was contrast dependent ($F_{5,25}=96.195$, p<.0001) with higher contrast levels leading to higher accuracy. Moreover, adaptation led to a strong reduction in performance, with an average accuracy of 75% for non-adapted and 64% for adapted gratings ($F_{1,5}=32.038$, p=.002).
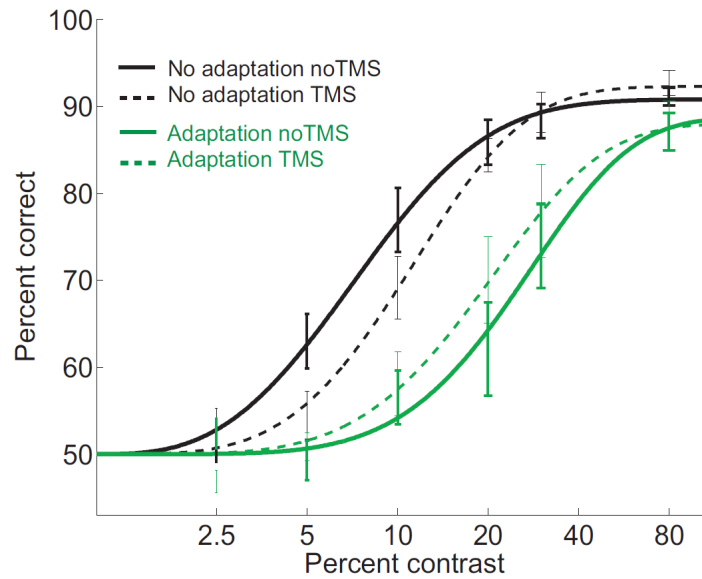
Figure 2.1: Adaptation and TMS effects. Accuracy was higher for the non adapted (black lines) then the adapted (green lines) condition. TMS (dotted lines) had a different effect depending on the state of adaptation: it decreased accuracy for the non adapted condition for intermediate contrast levels (dotted vs. continuous black lines), but increased performance in the adapted condition (dotted vs. continuous green lines). Bars are standard error of the mean.
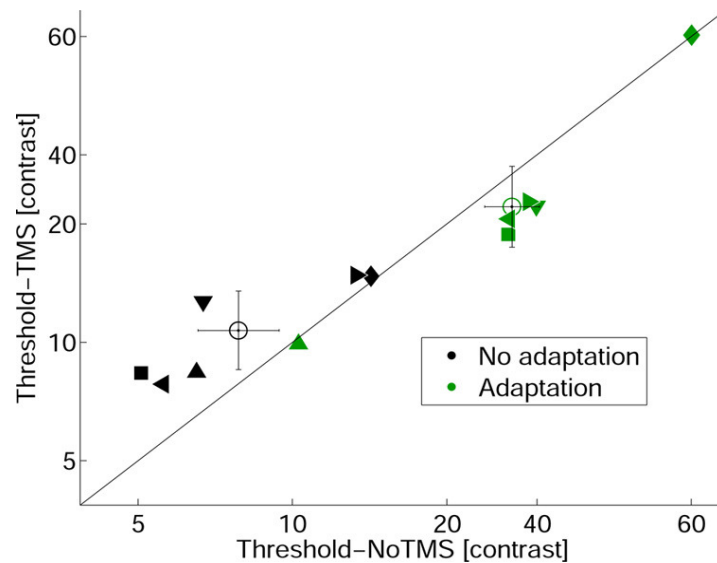
Figure 2.2: Scatter plots of thresholds for TMS and no-TMS conditions. Individual fitted threshold values are plotted for the no TMS (x axes) versus the TMS (y axes) conditions. Black symbols represent non-adapted conditions and green symbols represent adapted conditions. Each symbol depicts one observer. Open circles correspond to the averages of the six participants, with error bars representing the respective standard errors of the mean. TMS systematically increased individual contrast thresholds in the non-adapted condition while it decreased contrast thresholds in the adapted condition.

This decrease due to adaptation was stronger for intermediate contrast levels (adaptation*contrast: $F_{5,25}=5.117$, p=.02), confirming previous findings on contrast-adaptation (Dao et al., 2006; Pestilli et al., 2007). In addition, TMS had a different effect on accuracy depending on contrast level and adaptation (adaptation*TMS*contrast level: $F_{5,25}=6.108$, p=.001).

To differentiate among the three models (signal suppression and the two noise induction accounts), we obtained four psychometric contrast response functions (CRF) for each observer (adaptation-no TMS, no adaptation-no TMS, adaptation-TMS, and no adaptation-TMS) by fitting Weibull functions (Weibull, 1951) to the data using the maximum likelihood procedure implemented in *psignifit* (Wichmann and Hill, 2001b, a) a freely available toolbox for MATLAB (Mathworks, Inc.). For each observer, we estimated contrast thresholds at a fixed performance level of 70% (halfway between chance and asymptote). We analyzed these fitted thresholds and asymptotic performance to assess the magnitude of the adaptation and TMS effect with or without prior adaptation, using a two-way repeated measures ANOVA with *TMS* (TMS/no TMS) and *Adaptation* state (adaptation/no-adaptation). TMS had a differential effect on thresholds with respect to adaptation state (TMS*adaptation, $F_{1,5}=12.68$, p=.016). TMS significantly increased thresholds in non adapted conditions ($t_5= -3.315$, p=.02), but decreased thresholds after adaptation ($t_5= 3.321$, p=.02). These effects are also present in the individual thresholds (Figure 2.4). TMS did neither affect the asymptote nor the slope in any condition (TMS*adaptation, $F_{1,5}<1$, p=.62; TMS*adaptation, $F_{1,5}<1$, p =.86, respectively; Figure 2.3).

2.4. Discussion

We assessed the effect of TMS on the behavioral contrast response function in six participants with and without prior contrast adaptation. Without TMS, we replicated the finding that contrast adaptation leads to a decrease of contrast sensitivity (Ohzawa et al., 1982; Bonds, 1991; Pestilli et al., 2007), yielding a rightward shift of the CRF (Figure 2.2).With TMS we found clear evidence for state dependency. Applying TMS decreased contrast sensitivity across the dynamic range when there was no adaptation, but TMS increased contrast sensitivity across the dynamic range when participants were contrast-

adapted. In addition, similarly to adaptation effects (Pestilli et al., 2007), TMS affected only the thresholds of psychometric functions, leaving asymptotes unchanged. According to the well established Naka-Rushton function (Naka and Rushton, 1966), which describes the CRF, such left-right shift of the curve without affecting the asymptotes can only be explained if the variable introduced in the system works at the input level of the function. In our study, this suggests that TMS acts at the input level of neuronal processing, i.e. at the synaptic level, via an input-gain mechanism. In order to understand how TMS differently affected behavioral sensitivity we focus first on adaptation and then on TMS.

2.4.1 Behavioral and neural effects of adaptation on contrast sensitivity

The finding that adaptation shifts the CRF to the right without changing the asymptote has been taken to suggest that adaptation is a form of reduced contrast gain (Dao et al., 2006; Pestilli et al., 2007) which has also been reported with functional imaging in early visual cortex (Gardner et al., 2005). A similar CRF shift was also found in a physiological study of contrast adaptation using intracellular recordings in simple and complex cells of cat primary visual cortex (Carandini and Ferster, 1997). These authors found that spike rate increases with contrast and that after adaptation the resulting CRF was shifted to the right. Further investigations on membrane conductivity led them to conclude that the effect of adaptation is best explained by lack of excitation rather than an increase of tonic suppression, suggesting that adaptation changes synaptic efficacy.

2.4.2 State-dependent effects of TMS

2.4.2.1 Pre-adaptation

In this study, we observed that in the non-adapted state TMS decreased contrast sensitivity. This finding is compatible with the predominant view that TMS creates virtual lesions (i) by suppressing neuronal activity (Walsh and Rushworth, 1999; Figure 2.1b) as well as with the noise-induction account of TMS-intensity dependent effects (Figure 2.1e) (iv). However, the TMS-induced decreased sensitivity in the non-adapted state is not consistent with the preferential activation of less active neurons view (Silvanto et al., 2008a) (ii) because this

would predict a change in asymptote (Figure 2.1c). Our data are also incompatible with the noise-induction account of stimulus-intensity dependent effects (iii), for this would predict that noise would push up performance for subthreshold stimuli and pull down performance for suprathreshold stimuli, thus have a beneficial effect for the former but a detrimental effects for the latter (Figure 2.1d). Note that the impairment in sensitivity brought about by TMS cannot be attributed to phosphene induction, because the effect would have been the same for the adapted and the non-adapted conditions.

Seemingly, our results are inconsistent with the conclusion reached by Ruzzoli et al. (2010). They conducted a study dealing with the effects of TMS in V5 on motion coherence and report that TMS leads to a decrement in sensitivity, depicted by a shallower slope. They interpret their results to be consistent with the hypothesis that TMS induces randomly distributed neural noise. We note, however, that their conclusion is based on average results, but that the results of only less than half of the participants are consistent with such hypothesis.[1]

2.4.2.2 Post-adaptation

In the adapted state, TMS increased contrast sensitivity across the entire dynamic range of the CRF, shifting it leftwards, because the effect was more pronounced at the intermediate levels. This finding is incompatible with all discussed hypotheses: (i) virtual lesion - it would always predict detrimental effects on behavior (Figure 2.1b), (ii) the preferential activation of less active neurons view (Silvanto and Muggleton, 2008) would predict a change in asymptote reflecting a stronger effect at higher contrast levels (Figure 2.1c), (iii) noise induction/stimulus intensity – it would predict beneficial effect for low stimulus contrast, but detrimental effects for high stimulus contrast (Figure 2.1d); (iv) noise induction/TMS and stimulus intensity – it would predict a facilitatory effect for low to intermediate stimulus-contrasts (Figure 2.1e) with a consequent change in slope of the CRF. Schwarzkopf et al. (2011) have proposed that after adaptation TMS is less effective activating the neurons because they are less susceptible, and that 'this is akin to reducing the TMS-intensity' (p. 3146). Thus, they reason, after adaptation the TMS would have a similar effect to that caused by reducing the TMS intensity, at which point stochastic resonance would be observed. According to this proposal, it would follow that without adaptation the TMS effect should be

strong and therefore detrimental, but with adaptation the effect should be weak and therefore beneficial. Moreover, if TMS effects became increasingly smaller as stimulus signal increases, one would expect a stronger facilitation for low to intermediate contrast stimuli than for intermediate to high contrast stimuli, leading to a shallower slope.[1] Future experiments should explicitly vary TMS intensity.

In sum, none of the proposed hypotheses can account simultaneously for the observed results with and without adaptation. Furthermore, phosphene induction cannot explain the present findings. It would decrease signal to noise ratio and consequently impair performance orientation discrimination in both adaptation conditions; however, accuracy increased after adaptation. Our results make it necessary to simultaneously explain the rightward shift without adaptation and the leftward shift with adaptation, suggesting that TMS may change the balance of excitation and inhibition. Moliadze and colleagues (Moliadze et al., 2003) have reported that TMS pulses of high intensity (exceeding 50% of maximal stimulator output), suppress predominant activity during the first 100–200 ms. Based on our findings we propose that TMS changes the balance of excitatory and inhibitory input, supporting the idea that TMS has a suppressive effect that is activity dependent, with more active neurons being more strongly affected. It has been shown that the predominant activation without adaptation is excitatory, but after adaptation excitation decreases (Carandini and Ferster, 1997). Thus, according to our hypothesis, in the non-adapted case the predominant excitatory input would be more suppressed by TMS than any inhibitory input, resulting in a rightward shift of the CRF and a decrease in performance. Conversely, in the adapted case, suppressing inhibitory activity by TMS would lead to disinhibition, yielding an increase of sensitivity and a leftward shift of the CRF.

Our hypothesis could also explain previous studies on state-dependent TMS while disambiguating the hypothesis put forward by Silvanto and colleagues in order to account for the observation that after adaptation a TMS pulse leads to the perception of the adapter, rather than its opponent. In their view 'TMS perceptually facilitates the attributes encoded by

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[1] We note two relevant differences between our study and the studies of Schwarzkopf et al. (2011) and of Ruzzoli et al. (2010): (i) they did not manipulate the state of the neurons prior to stimulation, as in TMSa studies; (ii) they used three TMS pulses instead of one single pulse, with stimulus duration overlapping with one or two pulses.

the less active neural population' (Silvanto et al., 2007b), which would leave open the possibility that this perceptual facilitation is achieved either by exciting the less active neurons or by inhibiting the more active neurons. However, as we have argued above, *unspecific* excitation of less active neurons or *unspecific* inhibition of more active neurons both predict in our experiment a change in asymptote of the CRF, which we did not observe. We propose to treat excitatory and inhibitory neuronal populations separately, and that TMS has an activity dependent suppressive effect on the inhibitory population. Consequently, we suggest that the increased contrast sensitivity brought about by TMS after adaptation results from an underlying mechanism of disinhibition rather than excitation.

2.5 Conclusion

The present study shows that TMS impairs perception when the visual cortex is not adapted, but facilitates perception after adaptation. These results cannot be accounted for by any of the current hypotheses underlying TMS effects. Here we propose that an activity dependent suppressive effect of TMS may underlie state dependency of TMS effects, with TMS suppressing more the excitatory or the inhibitory neuronal population depending on the neuronal state at the time of stimulation.

3. Left occipitotemporal cortex is causally and selectively involved in tool action discrimination: TMS and fMRI evidence

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Abstract

Functional neuroimaging studies have implicated a region in the left lateral occipitotemporal cortex (LOTC) in tool perception. However, evidence for a causal role for this area in tool perception in healthy humans is currently lacking. Here, we used fMRI and online double-pulse TMS to test whether left LOTC is selectively and causally involved in discriminating tool-associated actions. Participants viewed briefly presented pictures of kitchen and garage tools while they performed one of two tasks that were matched for difficulty: in the action task, they judged whether the tool is associated with a rotation action (e.g., screwdriver) or a squeeze action (e.g., garlic press), while in the location task they judged whether the tool is typically found in the kitchen (e.g., garlic press) or in the garage (e.g., screwdriver).

Contrasting fMRI responses between these two tasks showed a significant increase of activity during the action task in both tool- and hand-selective LOTC regions, which closely overlapped. No differences were found in nearby object- and motion-selective control regions. Importantly, effective TMS over the tool-/hand-selective LOTC region, relative to sham TMS, significantly reduced accuracy on the action task but not the location task. The effects of TMS in the action task were significant when the double pulse was delivered at 150/250 ms and 270/370 ms after stimulus onset, but not at 30/130 ms after stimulus onset, providing insight into the time window during which LOTC contributes to tool perception. These results indicate that left LOTC is critical for knowledge of tool-associated hand actions.

3.1 Introduction

Tools physically and functionally extend our body, allowing us to achieve a wide range of goals that would not be possible with our bodies alone. Much progress has recently been made in understanding the neural architecture that supports complex tool use. Evidence from multiple methods points to a left lateralized network of frontal, parietal, and occipitotemporal brain regions involved in tool use and tool perception (for reviews, see (Johnson-Frey, 2004; Lewis, 2006; Martin, 2007)). In the present study we used functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS) to investigate the role of the left occipitotemporal cortex in tool perception.

fMRI studies have provided evidence that viewing pictures of tools, relative to other object categories such as animals or chairs, activates the left lateral occipitotemporal cortex (LOTC; (Chao et al., 1999; Beauchamp et al., 2002; Valyear et al., 2007; Weisberg et al., 2007; Valyear and Culham, 2010; Bracci et al., 2012); but see (Downing et al., 2006)), suggesting a role for this region in tool action perception, for example by representing tool-specific attributes such as characteristic tool motion patterns (Beauchamp et al., 2002). Although patient studies have broadly supported a role for left posterior temporal cortex in conceptual action knowledge (Tranel et al., 2003; Campanella et al., 2010; Kalenine et al., 2010), it is not clear from these studies whether lesions to tool-selective LOTC, more anterior regions in middle temporal gyrus (MTG), or other co-lesioned regions caused these deficits. Evidence

for a causal role for tool-selective LOTC in tool action perception in healthy humans is currently lacking.

Moreover, recent fMRI evidence calls for a reevaluation of the putative role of left LOTC in tool perception by showing that tool-selective regions closely overlap with hand-selective regions, with the strongest responses observed for hands rather than for tools (Bracci et al., 2012). This finding raises the possibility that tool-selective fMRI activity in left LOTC may reflect activation of hand-selective regions through epiphenomenal associations between tools and hands. For example, viewing a tool may induce visual imagery of a hand, leading to activation of hand-selective cortex. On this account, hand-/tool-selective left LOTC need not be critical for understanding tool-associated actions. These considerations highlight the importance of causal evidence for an involvement of tool-selective LOTC in tool perception.

In the present study we used fMRI and TMS to test whether left LOTC is causally and selectively involved in discriminating tool-associated actions. Participants in both studies discriminated the actions associated with tools. Discrimination of tool-associated location was used as a control task. The fMRI study showed a strong and anatomically specific increase of activity in hand- and tool-selective LOTC regions for the action relative to the location task. Importantly, the TMS study showed that the discrimination of tool action, but not of tool location, was impaired when TMS was applied over hand-/tool-selective left LOTC.

3.2 Materials and Methods

3.2.1 fMRI experiment

3.2.1.1 Participants

Fourteen healthy adult volunteers (6 females; mean age: 26.8 years, age range: 20 years - 35 years) participated in the fMRI experiment. One participant was excluded because of low accuracy in the main experiment (> 3 standard deviations below the group mean). All participants were right-handed with normal or corrected-to-normal vision, and no history of neurological or psychiatric disease. Participants gave written informed consent for

participation in the study, which was approved by the human research ethics committee of the University of Trento.

3.2.1.2 Stimuli

The stimulus set (Figure 3.1) consisted of 5 different exemplars of 12 objects. Half of the objects are typically found in a kitchen, and the other half in a garage (or workplace). Half of the objects are manipulated by a wrist rotation movement, and the other half by a hand-squeeze movement.

Stimuli (400 x 400 pixels, 5 degrees) were presented centrally. Stimulus presentation was controlled by a PC running the Psychophysics Toolbox package (Brainard, 1997) in Matlab (Mathworks, Natick, MA, USA). Pictures were projected onto a screen and were viewed through a mirror mounted on the head coil.
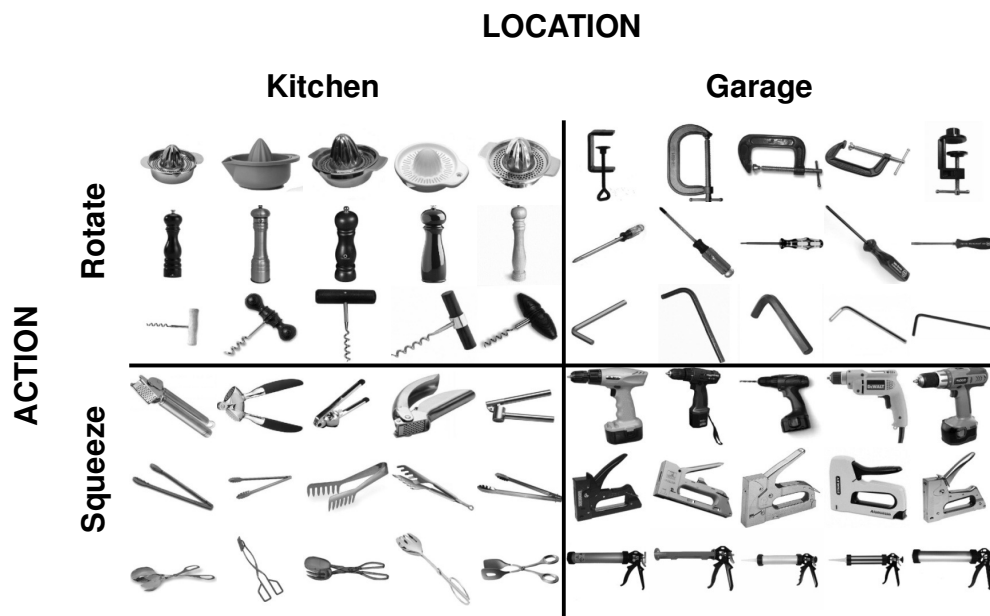
Figure 3.1. Stimuli used in the fMRI and TMS experiments.

3.2.1.3 Task and design of main fMRI experiment

On each trial, a picture of a tool object (Figure 3.1) was presented centrally for 1200 ms, followed by a 600 ms fixation period. Participants performed 6 runs of 120 trials each. Each of the 12 tool objects was presented 10 times within a run, in random order. The exemplar of the object that was presented (e.g., which of the 5 corkscrews; Figure 3.1) was randomly

selected on each trial. Participants performed a 1-back task, detecting repetitions of either the location (kitchen, garage) or the action (rotate, squeeze) dimension of the objects, in different runs. The order of the tasks was counterbalanced across participants. Participants pressed a response button with their right index finger when a task-relevant repetition occurred. Detection performance (1-back repetition detection) was high for both tasks (action task: mean = 97.2% correct; location task: mean = 97.9% correct; difference: $t_{12} = 2.1$, $P = 0.06$).

3.2.1.4 Functional localizers

Twelve participants additionally participated in 3 functional localizer experiments.

3.2.1.5 Category localizer

The category localizer consisted of two runs lasting 5 min each. The experiment consisted of four conditions: tools, animals, hands, and outdoor scenes. Stimuli (400 x 400 pixels, 12 degrees) were presented centrally and consisted of isolated objects on a white background (see (Bracci et al., 2012) for examples). One scanning run consisted of 21 blocks of 14 s each. Blocks 1, 6, 11, 16, and 21 were fixation-only baseline epochs. In each of the remaining blocks, 20 different stimuli from one category were presented. These stimuli were randomly selected from a total set of 40 stimuli per category. Each stimulus appeared for 350 ms, followed by a blank screen for 350 ms. Twice during each block, the same picture was presented two times in succession. Participants were required to detect these repetitions and report them with a button press (1-back task). Each participant was tested with two different versions of the experiment that counterbalanced for the order of the blocks. In both versions, assignment of category to block was counterbalanced, so that the mean serial position in the scan of each condition was equated.

3.2.1.6 Object localizer

Participants performed one run of a standard object-selective cortex localizer, lasting 5 min. Stimuli consisted of 20 intact and 20 scrambled objects, which were presented in alternating blocks. The block structure and task were identical to the object category experiment.

3.2.1.7 Motion localizer

To localize motion-selective cortex, visual displays of moving and stationary dot patterns were presented either in the left visual field (LVF) or in the right visual field (RVF). In the motion condition, dots shifted from the starting position toward the display's edge and back toward the fixation (0.5°/sec) alternating direction every 3 frames. In the static condition the dots remained still. The single localizer run lasted 8 min 48 s during which the four stimulus conditions (static dots in the LVF, moving dots in the LVF, static dots in the RVF and moving dots in the RVF), each lasting 16 s, were interleaved with fixation blocks (16 s). Each stimulus condition was repeated four times in a random order within the run. Fixation blocks also appeared at the beginning and end of each run. The fixation point alternated in color (red, yellow green, blue) every 500 ms. To maintain attention, participants were instructed to press a button with their right index finger whenever the central fixation point turned red.

3.2.1.8 fMRI data acquisition

Functional and structural MRI data were collected at the Center for Mind/Brain Sciences, University of Trento, Italy. All images were acquired on a Bruker BioSpin MedSpec 4-T scanner (Bruker BioSpin GmbH, Rheinstetten, Germany). Functional images were acquired using echo planar (EPI) T2*-weighted scans. Acquisition parameters were: repetition time (TR) of 2 s, an echo time (TE) of 33 ms, a flip angle (FA) of 73°, a field of view (FoV) of 192 mm, and a matrix size of 64 x 64. Each functional acquisition consisted of 34 axial slices (which covered the whole cerebral cortex) with a thickness of 3 mm and gap of 33% (1 mm). Structural images were acquired with an MP-RAGE sequence with 1 x 1 x 1 mm resolution.

3.2.1.9 fMRI data preprocessing

Data were analyzed using the AFNI software package (http://afni.nimh.nih.gov/) and MATLAB (The MathWorks, Natick, MA). Functional data were slice-time corrected, motion corrected, and low-frequency drifts were removed with a temporal high-pass filter (cutoff of 0.006 Hz). All data were spatially smoothed (4 mm Gaussian kernel) and transformed into Talairach space, which included resampling to 3 x 3 x 3 mm voxels.

3.2.1.10 fMRI data analysis

For each participant, general linear models were created to model the conditions in the experiment. All trials were included in the analyses. Regressors of no interest were also included to account for differences in the mean MR signal across scans and for head motion within scans.

3.2.1.11 Region of interest definition

Regions of interest (ROIs) were defined based on the independent localizer experiments in which 12 of the participants participated. Because not all participants had functional localizer data and because not all ROIs could be defined in those participants who had, ROIs were defined based on group-average data in random-effects analyses. These ROIs were then used to extract data for all participants in the main experiment. All ROIs were defined at a threshold of $P < 0.005$, $t_{11} = 3.5$.

The category localizer served to define our main ROIs. The contrast between tools and animals was used to define tool-selective regions in left LOTC (LOTC-Tool; volume=729 mm3), left and right fusiform gyrus (FG-Tool; 1215 mm3 and 621 mm3), and left intraparietal sulcus (IPS-Tool; 648 mm3). The contrast between hands and animals was used to define hand-selective regions in left LOTC (LOTC-Hand; 1647 mm3). The conjunction of these two contrasts (Bracci et al., 2012) was used to define a region in left LOTC selective for both hands and tools (LOTC-HandTool; 243 mm3). The contrast between scenes and the average of the other three categories (hands, tools, animals) was used to define the left and right parahippocampal place area (PH-Scene; 5238 mm3 and 5589 mm3). The OSC localizer (intact objects > scrambled objects) was used to define left and right object-selective regions in LOTC (LOTC-Object; 5427 mm3 and 7803 mm3). Finally, the MT/MST localizer (moving dots > static dots) was used to define left and right motion-selective regions in LOTC (LOTC-Motion; 2079 mm3 and 2673 mm3).

3.2.1 TMS experiment

3.2.1.1 Participants

Nine healthy adult volunteers (five females; mean age: 25.3 years; age range: 20 years - 29 years) participated in the TMS experiment. None of these volunteers participated in the fMRI experiment. All participants were right-handed with normal or corrected-to-normal vision, and no history of neurological or psychiatric disease. Participants gave written informed consent for participation in the study, which was approved by the human research ethics committee of the University of Trento.

3.2.1.2 Stimuli

The stimuli used in the TMS experiment were the same as those used in the fMRI experiment. Stimuli (280 x 280 pixels, 5 degrees) were presented on a 17-inch LCD monitor (DELL 1908FP-BLK) in a dimly lit room. Stimuli were presented on a standard PC using ASF (Schwarzbach, 2011), an add-on to the Psychophysics Toolbox package (Brainard, 1997) in Matlab (Mathworks, Natick, MA, USA). Masks (280 x 280 pixels, 5 degrees) consisted of static noise pattern of black and gray squares (7 x 7 pixels).

3.2.1.3 Task and design of TMS experiment

Participants performed two tasks. In the action task, participants indicated with a button press (using the index and middle finger of the right hand) whether a tool was associated with a rotation or a squeeze movement. In the location task, participants indicated with a button press (using the index and middle finger of the right hand) whether a tool was associated with a garage or a kitchen location.

Participants performed a total of 6 runs; 4 runs with effective TMS and 2 runs with sham TMS. In sham TMS runs the TMS coil was placed perpendicular to the scalp. For each participant, the order of the 4 effective and 2 sham TMS runs was randomized, with the constraint that the two sham TMS runs were never consecutive. The mean serial position of the sham TMS runs was 3.7, while the mean serial position of the effective TMS runs was 3.4 (difference: $t_8 = -0.8$, $P = 0.43$).

Each run was divided into two blocks, one block in which participants performed the action task, and one block in which they performed the location task. The order of blocks within runs was alternated such that half the effective TMS runs and half the sham TMS runs started with the action task. Four participants started with the location task, while five participants started with the action task. In total each participant performed 640 trials. Each trial started with a 1600 ms fixation cross, followed by the picture of a tool presented for 33 ms, which was immediately followed by a mask presented for 600 ms. The next trial started either 2700 or 3000 ms after the offset of the mask (with 50% probability).

3.2.1.4 TMS methods

For correct placement of the TMS coil, structural MRI images (MP-RAGE sequence with 1 x 1 x 1 mm resolution) were acquired for all participants. The position of the TMS coil was co-registered with the participant's reconstructed head, and the location of TMS-stimulation was marked on the reconstructed pial surface of each individual's brain using BrainVoyager Neuronavigator (Brain Innovation BV, The Netherlands, version 2.1) combined with a Zebris CMS20S measuring system for real-time motion analysis (Zebris Medical GmbH, Isny, Germany). TMS was applied over hand-/tool-selective left LOTC. For seven participants, LOTC coordinates were the group-average Talairach coordinates (-46, -68, -2) from a previous study that localized hand-/tool-selective LOTC (Bracci et al., 2012) contrasting hands > chairs and tools > chairs (the mean Talairach coordinates for these contrasts were identical). For the other two participants, LOTC was functionally localized with an fMRI localizer experiment in which pictures of tools, hands, and chairs were presented (for exemplars, see (Bracci et al., 2012)). Participants performed two runs, each containing six 14-second blocks per category. Left LOTC was localized by the conjunction of the contrast hands > chairs and the contrast tools > chairs. Talairach coordinates for these participants were: -43, -64, -1 and -46, -64, 5.

Biphasic TMS pulses were applied with a figure-of eight coil (MC-B65) and a MagPro x 100 stimulator (MagVenture A/S, Denmark). The stimulation intensity used during the experiment was set at 120% of the individual resting motor threshold measured as the intensity that elicited five visible hand movements out of ten stimulations. This resulted in a TMS intensity that ranged between 42% and 60% of the maximum stimulator intensity. On

each trial, two TMS pulses were applied with an interval of 100 ms. These pulses were delivered, on different trials, at three different timings relative to stimulus onset: 30/130 ms, 150/250 ms, and 270/370 ms. These three timings were used for both effective and sham TMS runs. The three TMS timings were each used on 1/3 of the trials, in random order. TMS timing was randomly paired with specific tool pictures.

Because of the small number of sham TMS trials for each of the 3 individual timings (only 2 of the 6 runs were sham TMS runs), and because no difference would be expected between sham stimulation at different time intervals, we collapsed data across the 3 sham timings within each task. This was supported by the results of within-subjects 2 x 3 ANOVAs on the sham data (Table 1), with Task (action task, location task) and Timing (30/130 ms, 150/250 ms, 270/370 ms) as factors. For both accuracy and RT, this analysis showed no main effect of Timing (Accuracy: $F_{2,16} = 1.0$, $P = 0.40$; RT: $F_{2,16} = 0.9$, $P = 0.41$), no main effect of Task (Accuracy: $F_{1,8} = 1.2$, $P = 0.30$; RT: $F_{1,8} = 0.0$, $P = 0.97$), and no interaction between Timing and Task (Accuracy: $F_{2,16} = 0.6$, $P = 0.58$; RT: $F_{2,16} = 1.6$, $P = 0.22$). These results show that, as would be expected, performance during sham conditions did not differ as a function of the time at which the sham stimulation was delivered. Furthermore, the absence of significant differences between the tasks shows that the tasks were equally difficult when no effective TMS was applied.

3.3 Results

3.3.1 fMRI results

A total of 12 ROIs were defined based on 3 functional localizer experiments (*Materials and Methods*). We tested whether activity in these ROIs differentiated between the action and location tasks.

Our main interest was in the left LOTC. Four nearby ROIs were defined in the left LOTC: left LOTC-Hand, left LOTC-Tool, left LOTC-Object, and left LOTC-Motion (Figure 3.2a-d). A 2 x 4 ANOVA with Task and ROI as factors showed a significant interaction ($F_{3,36} = 6.3$, $P = 0.002$), indicating that task-related modulations differed for the ROIs. Follow-up tests in each of the 4 ROIs showed a significantly stronger response during the action task then the location task in LOTC-Hand ($t_{12} = 3.0$, $P = 0.011$, Bonferroni corrected $P = 0.043$)

and LOTC-Tool (t_{12} = 2.3, P = 0.038, Bonferroni corrected P = 0.15), but not in LOTC-Object (t_{12} = 0.6, P = 0.57) or LOTC-Motion (t_{12} = 0.6, P = 0.58).

To test whether results differed in nearby hand- and tool-selective LOTC regions, we compared task-related modulation in left LOTC-Hand, left LOTC-Tool, and left LOTC-Hand/Tool (Figure 3.2e), a region defined as the overlap between LOTC-Hand and LOTC-Tool (*Materials and Methods*). A 2 x 3 ANOVA with Task and ROI as factors showed no interaction between Task and ROI ($F_{2,24}$ = 1.9, P = 0.18), a main effect of Task ($F_{1,12}$ = 9.1, P = 0.011), and a main effect of ROI ($F_{2,24}$ = 12.1, P < 0.001), indicating similar functional profiles of hand- and tool-selective LOTC regions.

To compare effects among tool-selective ROIs, we localized 3 tool-selective ROIs in addition to left LOTC-Tool: left FG-Tool, right FG-Tool, and left IPS-Tool (Figure 3.3). A 2 x 4 ANOVA with Task and ROI as factors showed a significant interaction ($F_{3,36}$ = 6.2, P = 0.002), indicating that the tasks modulated the ROIs to a different extent. Follow-up tests in each ROI showed a significantly stronger response during the action task then the location task in LOTC-Tool (t_{12} = 2.3, P = 0.038, Bonferroni corrected P = 0.15), but not in left FG-Tool (t_{12} = 0.2, P = 0.82) or right FG-Tool (t_{12} = -0.5, P = 0.63). The left IPS-Tool showed stronger activity during the action task than the location task (Figure 3.3d), but this difference did not reach significance (t_{12} = 1.7, P = 0.12).

We tested for task effects in 4 additional control regions: right LOTC-Object, right LOTC-Motion, left PH-Scene, and right PH-Scene (Figure 3.4). None of these regions showed a significant difference between the two tasks ($|t_{12}|$< 1.0, P > 0.34, for all ROIs).

Finally, a whole-brain random-effects group analysis (at P < 0.005, uncorrected) yielded one cluster for the contrast action task > location task, at the location of hand- and tool-selective LOTC (Figure 3.2f).

Table 3.1. Sham TMS data. Accuracy and reaction time (RT) for the sham TMS conditions, separately for each time window, are given. Standard deviations are given between brackets.

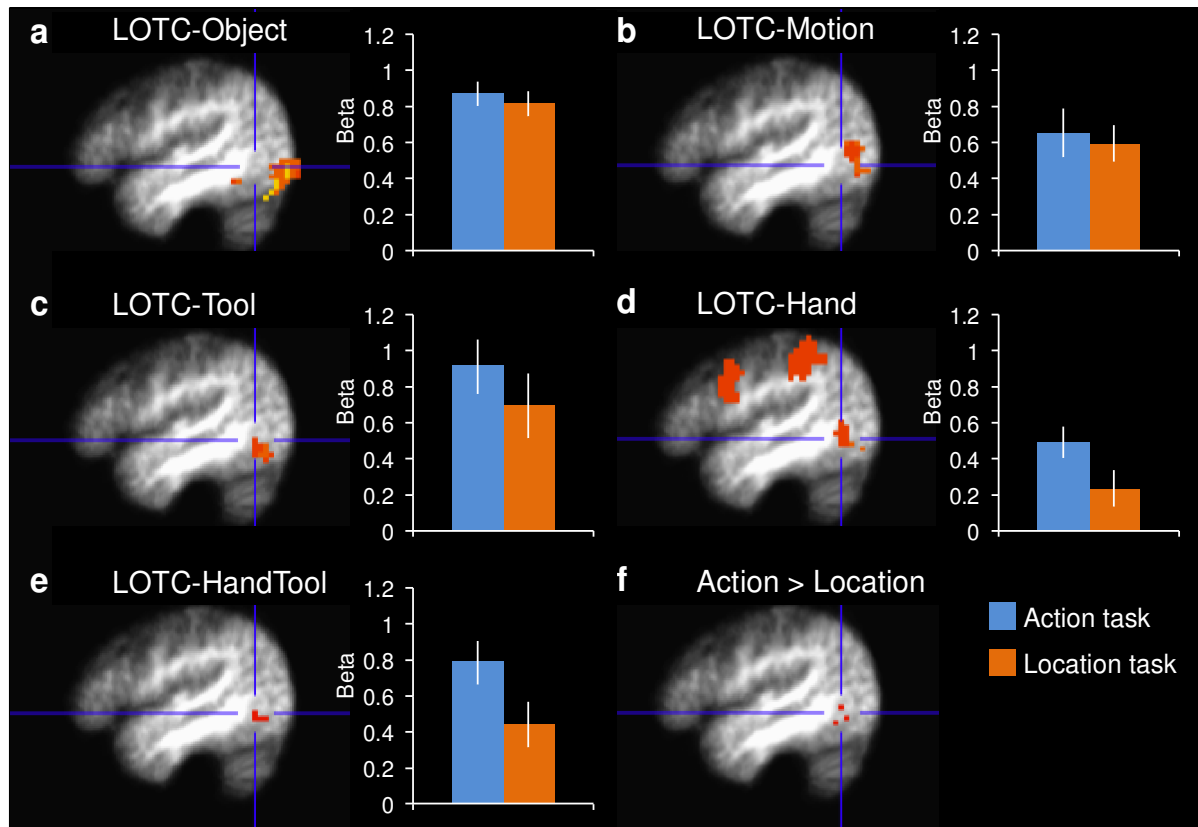|	30/130 ms		150/250 ms		270/370 ms	
	Accuracy	RT	Accuracy	RT	Accuracy	RT
Action task	95.24 (3.59)	741 (133)	93.37 (4.51)	774 (127)	94.61 (5.05)	749 (123)
Location task	94.26 (4.35)	753 (135)	92.89 (6.64)	752 (137)	91.63 (6.49)	756 (136)

Figure 3.2. fMRI results in functionally localized regions in left lateral occipitotemporal cortex. Bar graphs indicate activity in the displayed ROIs during the action (blue) and location (orange) tasks of the main experiment. a) LOTC-Object (intact > scrambled objects), b) LOTC-Motion (moving > static dots), c) LOTC-Tool (tools > animals), d) LOTC-Hand (hands > animals), e) LOTC-Hand/Tool (tools > animals AND hands > animals), f) the contrast action task > location task gave activity in left LOTC, overlapping with tool and hand-selective ROIs. All brain activity maps are shown at $P < 0.005$, displayed on the group-average anatomical scan, at x = -46. Blue crosshairs are added for spatial reference across panels. Error bars reflect s.e.m.
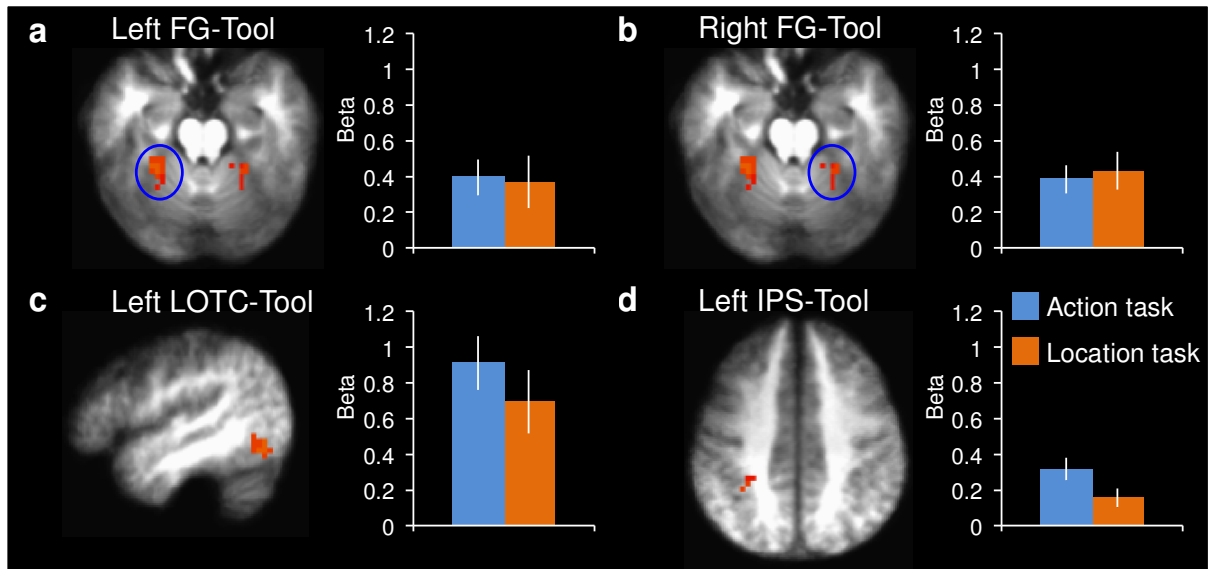
Figure 3.3. fMRI results in tool-selective regions, localized by contrasting tools with animals. Bar graphs indicate activity in the displayed ROIs during the action (blue) and location (orange) tasks of the main experiment. a) Left FG-Tool (z = -18), b) Right FG-Tool (z = -18), c) left LOTC-Tool (x = -46), d) Left IPS-Tool (z = 42). All brain activity maps are shown at $P < 0.005$, displayed on the group-average anatomical scan. Error bars reflect s.e.m.

Figure 3.4. fMRI results in additional control regions. Bar graphs indicate activity in the displayed ROIs during the action (blue) and location (orange) tasks of the main experiment. a) Right LOTC-Object (intact > scrambled objects, x = -49), b) Right LOTC-Motion (moving dots > static dots, x = -49), c) left PH-Scene (scenes > hands + tools + animals, z = -9), d) Right PH-Scene (scenes > hands + tools + animals, z = -9). All brain activity maps are shown at $P < 0.005$, displayed on the group-average anatomical scan. Error bars reflect s.e.m.

3.3.2 TMS results
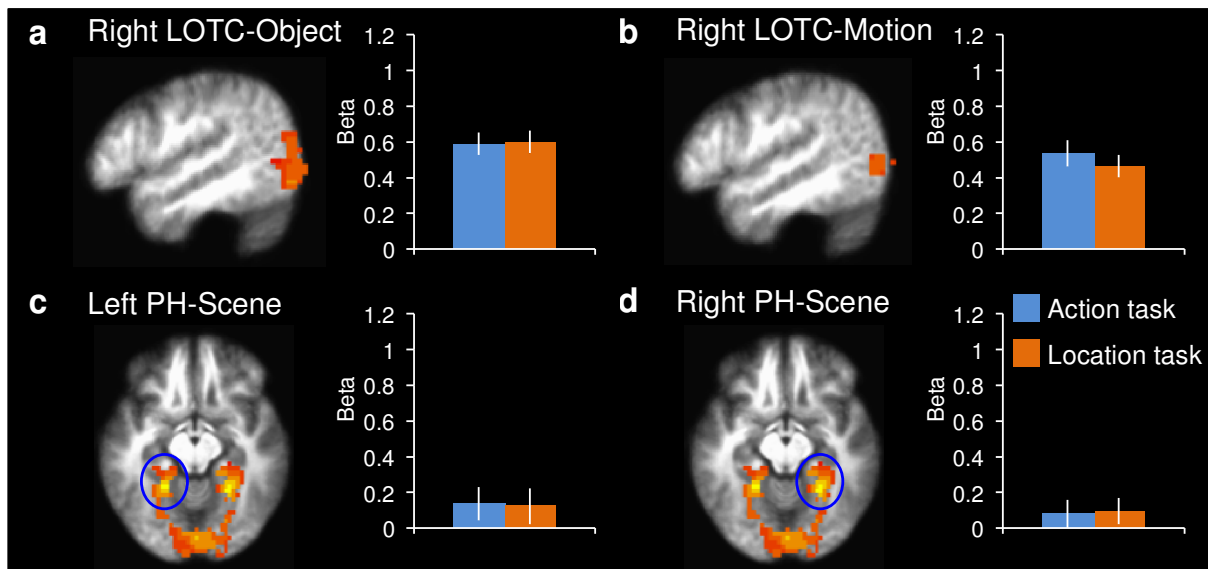
TMS was applied over left hand-/tool-selective LOTC (*Materials and Methods*), the region that was most strongly modulated by the action task in the fMRI experiment. Our main analysis focused on the effect of TMS on the accuracy with which participants could discriminate the action and location dimensions of the briefly presented tool pictures.

Accuracy was tested with a 2 x 4 within-subject ANOVA with Task (action task, location task) and TMS (sham, 30/130 ms, 150/250 ms, 270/370 ms) as factors. This analysis revealed a significant interaction ($F_{3,24} = 5.8$, $P = 0.004$), indicating that TMS differentially affected the two tasks (Figure 3.5a). For the action task, the main effect of TMS was significant ($F_{3,24} = 8.3$, $P = 0.0006$), while for the location task it was not ($F_{3,24} = 2.1$, $P = 0.12$). Pairwise t-tests on the action task data, comparing each of the 3 effective TMS conditions separately to sham stimulation, revealed highly significant effects of TMS at both the 150/250 ms ($t_8 = -4.8$, $P = 0.001$, Bonferroni corrected $P = 0.004$) and the 270/370 ms time windows ($t_8 = -3.4$, $P = 0.009$, Bonferroni corrected $P = 0.028$). No significant effects of TMS were observed for the action task at the 30/130 ms timing ($t_8 = 0.4$, $P = 0.69$).
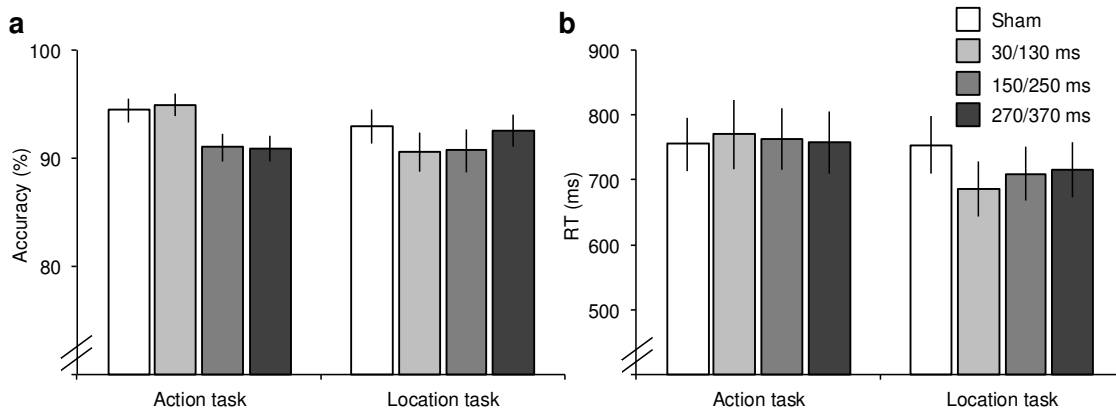
Figure 3.5. TMS results. a) Accuracy in the action (left) and location (right) tasks, comparing sham TMS (white bars) with effective TMS (grey bars) applied at 3 different timings relative to stimulus onset. b) Reaction times (RT) in the action (left) and location (right) tasks, comparing sham TMS (white bars) with effective TMS (grey bars) applied at 3 different timings relative to stimulus onset. Error bars reflect s.e.m.

Reaction time was tested with a 2 x 4 within-subject ANOVA with Task (action task, location task) and TMS (sham, 30/130 ms, 150/250 ms, 270/370 ms) as factors. This analysis revealed a significant interaction ($F_{3,24} = 4.3$, $P = 0.014$), indicating that TMS differentially

affected the two tasks (Figure 3.5b). For the location task, the main effect of TMS was significant ($F_{3,24} = 4.3$, $P = 0.014$), while for the action task it was not ($F_{3,24} = 0.4$, $P = 0.76$). Pairwise t-tests on the location task data, comparing each of the 3 effective TMS conditions separately to sham stimulation, revealed a significant *facilitative* effect of TMS for the location task at the 30/130 ms timing ($t_8 = -2.4$, $P = 0.044$, Bonferroni corrected $P = 0.13$), with faster RTs after effective TMS relative to sham TMS (Figure 3.5b). The facilitative effects for the location task at the 150/250 timing ($t_8 = -2.2$, $P = 0.056$) and the 270/370 ms timing ($t_8 = -1.5$, $P = 0.17$) did not reach significance.

3.4 Discussion

Using fMRI and TMS, we tested whether hand-/tool-selective regions in left LOTC are involved in tool action discrimination. Results from the fMRI experiment showed that functionally defined tool- and hand-selective LOTC regions, which partly overlapped (see also Bracci et al., 2012), were more active when participants discriminated the action associated with a tool than when they discriminated the location associated with a tool. Because the tool pictures presented in both tasks were identical, this result provides strong evidence that left LOTC is involved in action-related tool processing. It implies that tool selectivity in LOTC is driven by action-related features of tools (Weisberg et al., 2007), rather than by shape differences between tools and other objects (e.g., elongated tool shape; (Sakuraba et al., 2012)) or by the small size of tools relative to typically used control categories (Konkle and Oliva, 2012). Nearby object- and motion-selective LOTC regions, although strongly responsive to the tool pictures, showed no difference between the two tasks, indicating that the task effects in tool- and hand-selective regions did not reflect general object processing differences between the tasks, such as differences in attention.

To test whether the left LOTC is causally involved in tool action discrimination, we used online double-pulse TMS to disrupt activity in left hand-/tool-selective LOTC while participants discriminated either the action or the location associated with tools. Results showed a significant reduction (relative to sham TMS) in the accuracy of tool action judgments when the two TMS pulses were delivered at 150/250 ms or 270/370 ms after stimulus onset, but not when pulses were delivered at 30/130 ms after stimulus onset.

Reaction time data showed that the TMS effects for the action task could not be explained by a speed-accuracy tradeoff. Taken together, the present fMRI and TMS results converge to show that hand-/tool-selective left LOTC is selectively and causally involved in tool action discrimination.

How might left LOTC contribute to tool action discrimination? The close overlap between hand and tool responses in left LOTC (Bracci et al., 2012) raises the interesting possibility that the conceptual representation of tools may partly consist of the associated hand action representation. That is, discriminating the action of a tool (e.g., knowing that a screwdriver involves a hand rotation) may involve access to the tool-associated hand representation. Previous TMS studies have shown that TMS over the extrastriate body area (EBA; (Downing et al., 2001)), located about 0.5 cm posterior to hand-selective LOTC (Bracci et al., 2010; Bracci et al., 2012), selectively impairs discrimination of bodies (Pitcher et al., 2009) and body parts, including hands (Urgesi et al., 2004; Urgesi et al., 2007). It would be interesting for future studies to test, using fMRI and TMS, whether hand and tool action discrimination reflect the same underlying process in left LOTC, or whether these can be dissociated.

Our study focused on the hand-/tool-selective LOTC, functionally defined by contrasting activity to pictures of hands and/or tools with animals or chairs (both control categories give a similar localization of this region; (Bracci et al., 2012)), while participants performed a 1-back repetition detection task. Previous studies that localized tool-selective LOTC have often labeled this region pMTG. However, in our experience it is typically located just inferior and posterior to the MTG. It is possible that the location depends on the particular task used for localization, with more semantic tasks (e.g., learning facts about tools; (Simmons et al., 2010; Simmons and Martin, 2012)) shifting activity superiorly and anteriorly. Indeed, the MTG has been implicated in a variety of semantic tasks, including semantic control and conceptual processing (Whitney et al., 2011; Wei et al., 2012), verb processing (Perani et al., 1999; Shapiro et al., 2006; Willms et al., 2011; Peelen et al., 2012), action knowledge (Martin et al., 1995; Kable et al., 2005b), and access to functional object properties (Bach et al., 2010). It is presently unclear how these regions correspond to the hand-/tool-selective region investigated here. Recent studies have started to dissociate nearby verb-selective, action-selective, body-selective, motion-selective, and object-selective regions in posterior

temporal cortex (Downing et al., 2007; Bedny et al., 2008; Valyear and Culham, 2010; Peelen et al., 2012), but further research is necessary to investigate how hand-/tool-selective LOTC relates to more anterior parts of MTG implicated in other studies.

The present results showed that TMS disrupted tool action discrimination when TMS was applied at 150/250 or 270/370 ms after stimulus onset. The relatively late time window that resulted in task-specific impairment may suggest that the LOTC elaborates tool actions in concert with downstream regions such as the left dorsal premotor cortex and left IPS, to which it is functionally connected (Bracci et al., 2012). In the present study, tool-selective left IPS showed higher activity during the action task than the location task (Figure 3.3d), but this difference did not reach significance. A previous fMRI study that used a similar manipulation as the present study -- contrasting action knowledge with function knowledge of tools -- reported significant modulation in left dorsal premotor cortex and left IPS, but not in left LOTC (Canessa et al., 2008). Several differences may account for this discrepancy. Specifically, in the action knowledge task of the Canessa et al. study, participants were asked whether two objects (presented simultaneously for 4 seconds) were used with the same manipulation pattern (e.g., vacuum cleaner and metal detector), while in the present study participants made a hand rotate vs hand squeeze discrimination on a single object presented for 1.2 seconds. The strong focus on the specific hand action associated with the tool in our study (rotate vs squeeze) may have amplified responses in hand-/tool-selective left LOTC.

A recent study (Peelen and Caramazza, in press), found that multivoxel activity patterns in the anterior temporal lobes (ATLs) carry information about object-associated action (rotate versus squeeze); activity patterns were relatively similar for objects that are both associated with a rotation action (e.g., a corkscrew and a screwdriver). Because information was computed at the level of action category (rotate versus squeeze), these results likely reflected representations that generalize across specific visuomotor features. For example, while both a screwdriver and a corkscrew involve a wrist rotation movement, their specific motor patterns and hand postures are quite distinct and determined by the specific visual form of the objects in question. The present study, showing that the left LOTC is causally involved in tool action discrimination, complements these results: rather than housing generalized category-level representations of tool actions, LOTC may represent more specific visuomotor features

associated with individual tools. Access to such representations is required for performing higher-order categorization, and thus for performing the current action task. More generally, the two sets of results suggest that the functional roles of LOTC and ATL are related hierarchically, with LOTC reflecting an earlier, less abstract and less general level of representation than the ATL.

To conclude, the present study provides converging evidence from TMS and fMRI that the left LOTC is causally and selectively involved in the discrimination of hand actions associated with visually presented tools.

4. Connection between parietal cortex and lLOTC: rTMS interleaved with fMRI

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Abstract

Results from our previous study support the idea that left LOTC is not simply elaborating visual properties of the object, but is already involved in processing action-related features of tools, suggesting that this area may already be part of an action processing network together with parietal and premotor cortex. One possibility is that selectivity for action-related properties of tools in lLOTC arises from back projections coming from higher areas like the intraparietal sulcus. In order to test if and how lLOTC and parietal cortex are connected during tool perception, we interleaved repetitive TMS with fMRI while participants viewed pictures of tools, hands and chairs. In the first experiment we applied TMS over left IPS, and subsequently measured BOLD responses in several regions of interest including lLOTC. In a second experiment the target area for TMS was lLOTC. Results from the first experiment showed that BOLD activity in lIPS was effectively decreased after TMS, confirming that the

rTMS-fMRI procedure was effective. A significant decrease was also present in left dorsal premotor cortex and in right IPS. By contrast, there was no significant TMS effect in lLOTC, suggesting that back projections from IPS to lLOTC do not have a major influence on its response profile. Finally, rTMS over lLOTC did not lead to any significant TMS effect, not even in the stimulated region, suggesting that the TMS effect was too weak or that more participants are needed to test its effect.

4.1 Introduction

Both neuropsychological and functional neuroimaging studies support the involvement of a temporo-parietal network during tool use and perception (Lewis, 2006). The exact locations of the areas composing this tool network slightly vary according to the type of sensory modality of presentation of tools, and on the task performed by the participants. While in the past, especially neuropsychological studies, focused more on the role of higher areas in tool processing, more recently imaging studies have proposed that tool use knowledge is spread in the network, focusing more also on temporal and occipitals areas (Chao et al., 1999; Phillips et al., 2002; Kellenbach et al., 2003; Creem-Regehr and Lee, 2005). Indeed, different tool dimensions are elaborated in separated regions in the brain, with evidence for example that temporal, compared to parietal cortex, is more involved in representing functional knowledge of objects (Canessa et al 2008). In addition, our previous study in chapter 3 clearly showed that left LOTC is not a simple visual area, as it is already implicated in processing specific types of information regarding action associated with tools, compared to other properties not related with action like contextual information (Perini et al, submitted). However, our previous data do not address the possibility that processing of action properties in lLOTC takes place as a consequence of back projections from higher areas, like parietal cortex. This is why it is also important to investigate how lLOTC is connected with the downstream fronto-parietal network, and in particular with those areas that are also activated by simple passive perception of tools, i.e. left parietal sulcus, dorsal premotor cortex and fusiform tool area. Visual information proceed from occipital towards temporal and parietal cortex, so it seems quite obvious that lLOTC sends information about tools to higher areas, mainly to parietal cortex. For example, it could be that at first lLOTC is not processing selectively the

different properties about tools, but does it only after sending visual information to parietal and premotor cortex, and then receiving back projections from these areas that are stronger for action related properties, and hence for tools as well as hands. This could partially explain why in the previous study we found that TMS disrupts the action task only at the latest tested timings (150-370 ms after stimulus onset). In contrast, if lLOTC is actively involved in processing action features of tools from the beginning, then it does not necessarily need to send information to higher areas nor receiving back projections during tool perception. Furthermore, since even passive viewing of tools is able to activate the tool network until premotor cortex (Bracci et al, 2010; 2012), connections between occipitotemporal and parietal areas should be present also when there is no overt task on tool properties.

Thus, in order to investigate functional connectivity between lLOTC and lIPS during tool perception, we conducted two experiments combining TMS and fMRI. In the first experiment, we applied repetitive TMS over lIPS in order to interfere with neuronal activity in the stimulated region and the connected ones. Directly following TMS, we measured BOLD responses while participants viewed static pictures of tools, hands and chairs. If lIPS was automatically sending projections towards lLOTC, then activity in this area should be affected by TMS, as well as other connected regions to which IPS is sending projections, but not regions that don't receive feedback from IPS. By contrast, when stimulating with TMS over lLOTC, we expect to find a change also on IPS. Moreover, if connections between IPS and LOTC are specific for action-related properties also when we are not actively involved in processing tool use and tool-hand interaction, BOLD modulation due to TMS between these two regions should be selective for tools and hands, but not for chairs.

4.2 Methods

4.2.1 Participants

Twenty-one healthy volunteers (mean age 27.2 years, range 20-53, 13 females) participated in the first experiment. Eleven volunteers (mean age 25.1, range 19-36 years, 6 females) participated in the second experiment, four of whom also participated in the first experiment. All the participants of the first experiment and six participants of the second experiment were scanned at Center for Mind/Brain Sciences, University of Trento, Italy, while five

participants in the second experiment were scanned at the Centre for Cognitive Neuroscience, University of Bangor, UK. Informed written consent according to the Declaration of Helsinki was obtained from each participant prior to the experiment. The study was approved by the local ethics committee of the University of Trento and by the ethics committee of the University of Bangor.

4.2.2 Stimuli

Functional runs in both first and second sessions were the same and lasted 7.4 minutes each. The design included three categories: tools, hands and chairs. Stimuli were presented centrally, had a size of 12° x 12° (400 x 400 pixels), and consisted of isolated objects on a gray background (see Figure 4.1 for examples). One scanning run consisted of 18 blocks of 14 s each, interleaved by 10 seconds of fixation cross only. In each run there were 6 blocks per category, including 20 different stimuli each.

These stimuli were randomly selected from a total set of 60 stimuli per category. Each stimulus appeared for 350 ms, followed by a blank screen for 350 ms. Three times during each block, the same picture was presented twice in succession. Participants were required to detect these repetitions and report them with a button press (1-back task). Each participant was tested with two different versions of the experiment that counterbalanced for the order of the blocks. Each version was used twice, once in a sham run and once in a TMS run. Assignment of category to block was counterbalanced, so that the mean serial position in the scan of each condition was equated.

4.2.3 Experimental Protocol

The experiment consisted of two different sessions. In the first session, all participants underwent structural MRI acquisition plus two functional runs to be used for functional neuronavigation. The second session was divided into three different parts. In the first part participants were tested for individual dosing of TMS by assessment of the static resting motor threshold (rMT) by visual inspection. Third, the actual experimental session was performed, in which participants were targeted with 1-Hz rTMS for 13 minutes and immediately after were scanned with MRI while performing the 1-back task. Four blocks of

rTMS + fMRI were conducted for each participant. Site of stimulation was the same in all runs, but in two runs TMS intensity was only at 40% of the individual motor threshold, such that it is considered not to be effective on cortex, and was then regarded as sham condition. In contrast, in the other two runs TMS intensity was at 98% of rMT and is referred to as effective stimulation. The order of sham and effective TMS runs were always interleaved.
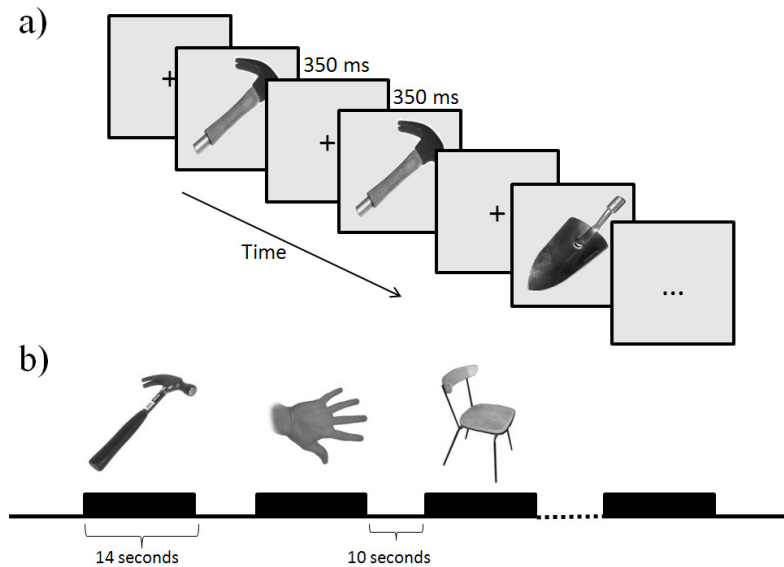

Figure 4.1. Procedure task. Both in the localizer and in the second sessions of both experiments, the task consisted of a 7.4 minute run during which participants had to make a key press only when the same picture was repeated twice in sequence (1-back task). Three different categories were presented divided in blocks: tools, hands and chairs. There were six blocks per category, each lasting 14 seconds and containing 20 pictures. Pictures were presented for 350 ms and interleaved by a 350 ms of fixation, while blocks were interleaved by 10 seconds of fixation.

4.2.4 fMRI data acquisition

For the structural and functional MRI data collected at the Center for Mind/Brain Sciences, University of Trento, all images were acquired on a Bruker BioSpin MedSpec 4-T scanner (Bruker BioSpin GmbH, Rheinstetten, Germany) with an 8-channel array head coil. A high-resolution T1-weighted magnetization-prepared rapid gradient echo sequence (176 axial slices, in-plane resolution 256 3 224, 1-mm isotropic voxels, generalized autocalibrating partially parallel acquisition with acceleration factor = 2, time repetition = 2,200 ms, time echo = 4.180 ms, time to inversion = 1,020 ms, flip angle = 7°). Functional images were

acquired using echo planar (EPI) T2*-weighted scans. Acquisition parameters were: repetition time (TR) of 2 s, an echo time (TE) of 33 ms, a flip angle (FA) of 73°, a field of view (FoV) of 192 mm, and a matrix size of 64 x 64. Each functional acquisition consisted of 34 axial slices (which covered the whole cerebral cortex) with a thickness of 3 mm and gap of 33% (1 mm).

For the functional MRI data collected at the University of Bangor, data were acquired using a 3T Philips MRI scanner with a SENSE phased-array head coil. Acquisition parameters for functional MRI were the same as data collected in Trento, with the only difference that the number of axial slices was 31, which still had a good coverage of the whole cerebral cortex. Parameters for T1-weighted anatomical scans were: 175 sagitally-oriented slices; 1 mm isotropic voxels; TR=8.4 ms, TE= 3.8 ms; flip angle = 8°.

For the neuronavigation, a 3D reconstruction of the scalp and brain surfaces was produced using the BrainVoyager software (Brain Innovation BV, The Netherlands) and the target locations of IPS and LOTC on the left hemisphere were identified individually on the basis of the functional runs collected in the first session.

4.2.5 fMRI data preprocessing

For both studies, data preprocessing and analysis were performed with Brain Voyager QX (version 2.20; Brain Innovation, Maastricht, The Netherlands). Three-dimensional motion correction was performed to correct for subject's head motion. After linear trend removal, functional data underwent high-pass temporal filtering (cutoff 3 cycles per time course). Functional volumes were spatially smoothed (4-mm full-width half-maximum isotropic Gaussian kernel). Manual coregistration was performed to align the functional images with the T1 anatomical images. Subsequently the anatomical images were transformed into Talairach stereotaxic space, and this transformation was applied to the aligned functional data, which was interpolated to 1 x 1 x 1 mm.

4.2.6 fMRI data analysis

For both studies, data were analyzed with the general linear model. Each GLM included the conditions of interest, as well as the six parameters from the motion correction procedure (*x*,

y, *z* for translation and for rotation). Predictors' time courses were modeled with a linear model of hemodynamic response using the default Brain Voyager QX "two-gamma" function. Before computing the GLM, functional runs were *z*-normalized.

	TMS over IPS				TMS over LOTC			
	x	*y*	*z*	*mm3*	*x*	*y*	*z*	*mm3*
left IPS	-34	-44	44	826	-36	-45	45	718
left dPM	-37	-16	43	675	-42	-9	39	546
right IPS	25	-44	45	766	32	-42	46	721
left LOTC	-46	-63	1	910	-47	-64	-1	896
left LOTC-Tool	-47	-65	-1	931	-46	-64	-2	918
left LOTC-Hand	-42	-63	4	959	-45	-63	0	869
right LOTC	45	-60	1	704	41	-60	-3	740
left FG-Chair	-25	-44	-6	714	-28	-46	-9	662
left FG-Tool	-34	-45	-16	640	-41	-43	-17	536

Table 4.1. Talairach coordinates for ROIs in study 1. Mean Talairach coordinates and cluster size are reported for individual-subject regions of interest (ROIs) localized with the comparison of tools > chairs (LOTC-Tool / FG-Tool); hands > chairs (LOTC-Hand) and the conjunction of these two contrasts for all the other ROIs (IPS, dPM, left and right LOTC, FG-Chair). Threshold $p < 0.05$, uncorrected. IPS: interparietal sulcus; LOTC: lateral occipitotemporal cortex; dPM: dorsal premotor cortex; FG: fusiform gyrus.

4.2.7 Regions of interest definition

The two runs from the first session were used in order to localize the two target ROIs for neuronavigation, i.e. left IPS in the first experiment and left LOTC in the second experiment. These ROIs were found with the conjunction of the contrast tools versus chairs and hands versus chairs (Bracci et al., 2012). In the first experiment, the mean average of the Talairach coordinates for left IPS to be targeted with TMS localized with the two runs in the first session was -33, -42, 42. In the second experiment the target area left LOTC had mean Talairach coordinates of -45, -64, -2. In contrast, regions of interest (ROIs) for further analysis were defined based on all four runs of the second sessions, because for some subjects it was not possible to correctly localize some of the ROIs using solely the two runs of the first session, in particular left dorsal premotor cortex and the tool fusiform gyrus. All ROIs were individually defined at a threshold of $P < 0.05$. The contrast between tools and chairs was used to define tool-selective regions in left LOTC (LOTC-Tool) and left tool

fusiform gyrus (FG-Tool). The contrast between hands and chairs was used to define hand-selective regions in left LOTC (LOTC-Hand). The conjunction of these two contrasts (Bracci et al., 2012) was used to define left and right intraparietal sulcus (IPS), left dorsal premotor cortex (ldPM), left and right lLOTC, and left fusiform gyrus for chairs (FG-Chair) using the opposite contrasts where activity for chairs was higher for tools and hands.
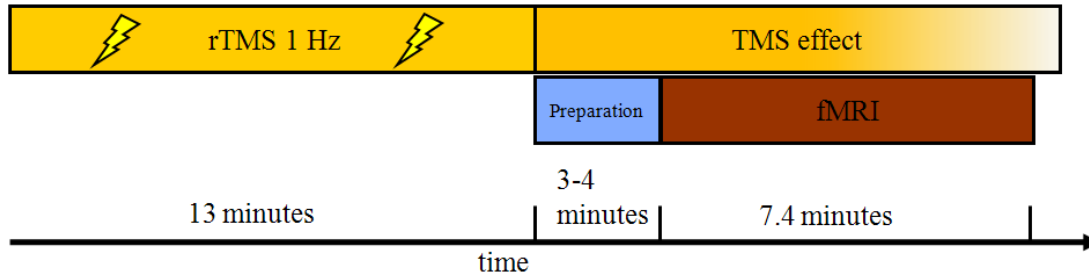

Figure 4.2. Repetitive TMS combined with fMRI. In both experiments we applied repetitive TMS at low frequency at 1 Hz over the target areas for 13 minutes, for a total of 780 pulses, while the participant was laying down on a gurney. Since TMS effect is supposed to last maximally as long as the stimulation, the gurney with the participant was taken as fast as possible inside the scanner, and then we started the functional scan for 7.4 minutes. Considering that the time between the end of effective stimulation and beginning of the functional run was on average 4.01 minutes in the first study and 4.16 in the second study, we were still inside the period of efficacy for the TMS effect.

4.2.8 TMS methods

In Italy, Biphasic TMS pulses were applied using a figure-of-eight coil (MC-B70 MagVenture A/S, Denmark) and a MagPro 3100 stimulator (MagVenture A/S, Denmark). For participants tested in the UK we used a MagStim Super Rapid (Magstim Whitland UK) with a 70-mm figure-eight coil. Right before the experiment, the individual resting Motor Threshold (rMT) was defined as the lowest stimulation intensity applied over the primary motor cortex capable of evoking a visible contraction in the relaxed right fingers in at least five out of ten consecutive stimuli. The stimulation intensity for the experiment was set to 98 % of the individual rMT (which on average corresponded to 50.7% of maximum stimulator intensity in the first experiment, and to 56.3% in the second experiment) in the two effective stimulation blocks and to 40 % of the rMT in the other two sham stimulation blocks. Such intensity has not been investigated in literature with 1-Hz stimuli but a review of previous works shows that the effects of 1-Hz rTMS on the motor cortex tend to disappear at intensity

approaching 80 % of rMT (Fitzgerald et al. 2006), so we used the two blocks at 40% as a sham condition.

During the actual experimental session, a single block of 13 min of rTMS was delivered in both effective and sham conditions prior to the functional run in the scanner for a total of 780 pulses per run. Effective and sham blocks were always interleaved. In the first experiment half of the participants started with a sham block, and the other half with an effective stimulation block. In the second experiment five subjects started with sham condition, and six with TMS condition. During rTMS, subjects were placed on a gurney in a room neighboring the MRI scanner. At the end of the rTMS train, the subject was moved as fast as possible in the MR scanner. In the first experiment with TMS over IPS, mean duration of the gap between the end of stimulation and start of the functional runs was 4.03 minutes in TMS condition, and 4.01 minutes in sham condition. In the second experiment, gap duration in TMS condition was 4.16 and in sham condition was 4.20.

4.5 Results

4.5.1 rTMS over left IPS

4.5.1.1 Behavioral results

We analyzed accuracy and reaction times in the 1-back task for the three different categories, tools, hands and chairs, and the two TMS conditions, effective and sham. A two ways ANOVA on reaction times showed that there was no general TMS effect ($F_{1,19} = 1.271$, $P = 0.27$), while reaction times depended on the type of category ($F_{2,38} = 9.537$, $P < 0.0001$). There was no different TMS effect on the different categories ($F_{2,38} = 0.748$, $P = 0.48$). A post hoc T-test showed that the 1-back task for hands yielded slower responses both compared to tools ($t = -3.797$, $P = 0.001$) and to chairs ($t = 2.637$, $P = 0.016$), with responses for tools faster also compared to chairs ($t = -2.170$, $P = 0.043$). Two ways ANOVA on accuracy confirmed the trend of reaction times, with no general TMS effect ($F_{1,19} = 0.430$, $P = 0.52$) nor an interaction with the type of category ($F_{2,38} = 0.420$, $P = 0.66$), but there was a significant difference for the various categories ($F_{2,38} = 14.885$, $P < 0.001$).

Accuracy for detection of hand repetition dropped in comparisons to tools ($t_{19} = 4.466$, $P < 0.001$) and chairs ($t_{19} = -4.246$, $P < 0.001$), but there was no difference between tools and chairs ($t_{19} = 1.021$, $P = 0.320$).
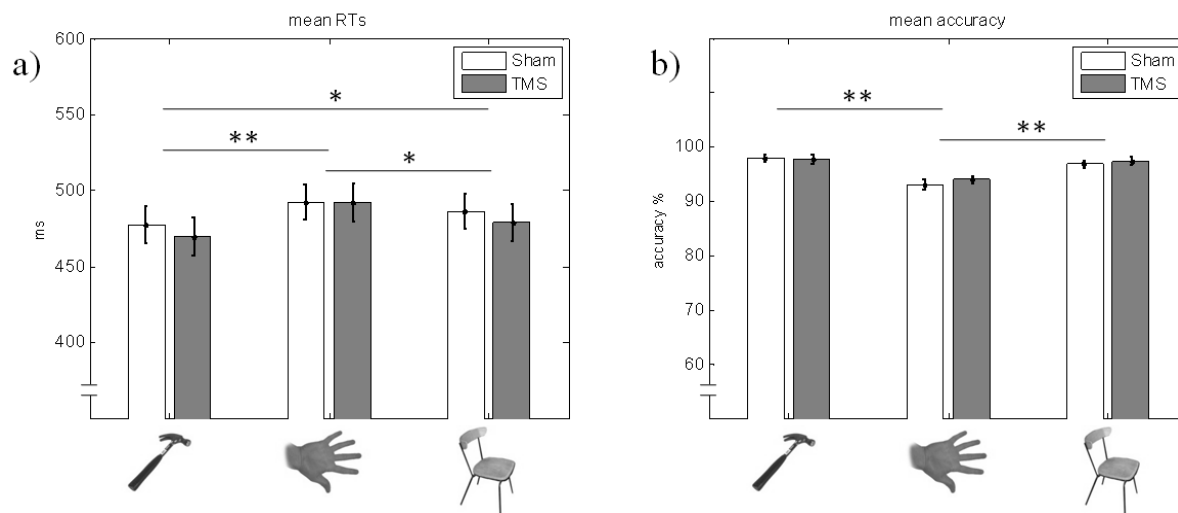

Figure 4.3. Behavioral results study rTMS over IPS. Mean performance from twenty participants in the one back task for the three different categories: tools, hands and chairs. White bars represent performance in sham condition, and gray bars depict performance after effective TMS stimulation. **a) Reaction times**. TMS had no effect on performance for any category, but performance depended on the category, with detection for tool repetitions leading to faster responses and detection of hand repetitions leading to slower responses. **b) Accuracy**. Accuracy analysis confirms RTs trend, with no TMS effect but showing that hand category was more difficult with the lowest accuracy level compared to both tools and chairs. ** $P < 0.01$; * $P < 0.05$. Error bars are 1-standard error from the mean.

4.5.1.2 fMRI results

In order to test if TMS had a different effect according to the category and to the cortical area, we applied a 3 ways repeated measures ANOVA on the beta values resulting from the different TMS and sham runs for all the nine regions of interest defined as described above (see table 4.1). The main effect of category was significant ($F_{2,38} = 75.875$, $P < 0.001$), and also general responses depended on the ROIs, ($F_{8,152} = 13.850$, $P < 0.001$). There was a main effect of TMS ($F_{1,19} = 5.101$, $P = 0.036$), but a significant interaction showed that this TMS effect depended on ROI ($F_{8,152} = 2.193$, $P = 0.031$). There was no interaction between TMS and type of category ($F_{2,38} = 0.988$, $P = 0.382$). A significant interaction between ROIs and category simply confirmed that we localized the ROIs contrasting the different categories ($F_{16,304} = 53.535$, $P < 0.001$). There was no significant three ways interaction between ROIs, category and TMS ($F_{16,304} = 0.480$, $P = 0.956$). In order to test in which areas the TMS

stimulation was efficient and if in some ROIs this was dependent on the category, we conducted a 2 ways ANOVA on beta values with TMS and category as factors for each ROI separately. In all the ROIs considered, beta values obviously depended on the category (all P values < 0.001). TMS effect on the stimulated left IPS was significant ($F_{1,19} = 7.165$, $P = 0.015$), confirming that we were effectively stimulating the target area (Figure 4.4). However, this decrease in activity in IPS did not depend on category ($F_{2,38} = 1.212$, $P = 0.31$). We found a decrease of BOLD also in left dorsal premotor cortex ($F_{1,19} = 7.647$, $P = 0.012$), and in the right IPS ($F_{1,19} = 6.697$, $P = 0.018$), but again no interaction with category in ldPM ($F_{2,38} = 0.081$, $P = 0.92$) nor in left IPS ($F_{2,38} = 0.966$, $P = 0.39$). No general TMS effect was found in lLOTC irrespective of how it was localized: in the lLOTC-tool ($F_{1,19} = 1.937$, $P = 0.18$), lLOTC-hand ($F_{1,19} = 0.953$, $P = 0.34$) or lLOTC with tools and hands in conjunction ($F_{1,19} = 1.302$, $P = 0.27$).
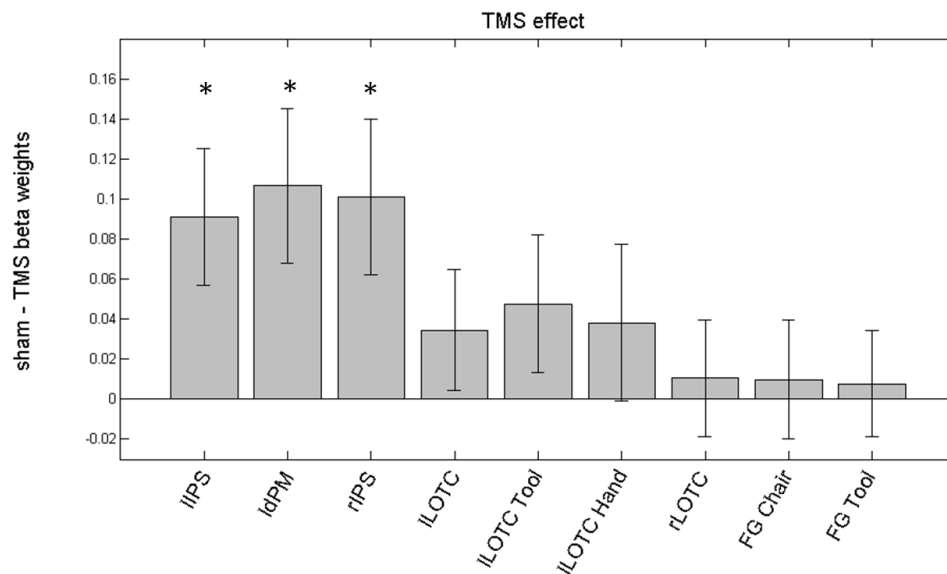

Figure 4.4. TMS effect in ROIs in study rTMS over IPS. Each bar represent the mean difference of beta weights in sham condition minus TMS condition, collapsed for all categories. TMS significantly suppressed activity in the stimulated IPS, in left dorsal PM and in right IPS, but not in the other ROIs. IPS: intraparietal sulcus; LOTC: lateral occipitotemporal cortex; dPM: dorsal premotor cortex; FG: fusiform gyrus. * P < 0.05. Error bars reflect standard error of the mean.

However, analysis of TMS effect size showed a trend for a decrease of BOLD, especially in lLOTC tool (1.4), while effect size was smaller for lLOTC localized as conjunction (1.1) for lLOTC hands (0.9). There was no difference between TMS and sham condition for the other

ROIs: left FG-Tool ($F_{1,19}$ = 0.084, P = 0.78), left FG-Chair ($F_{1,19}$ = 0.106, P = 0.785), or right LOTC ($F_{1,19}$ = 0.953, P = 0.341).
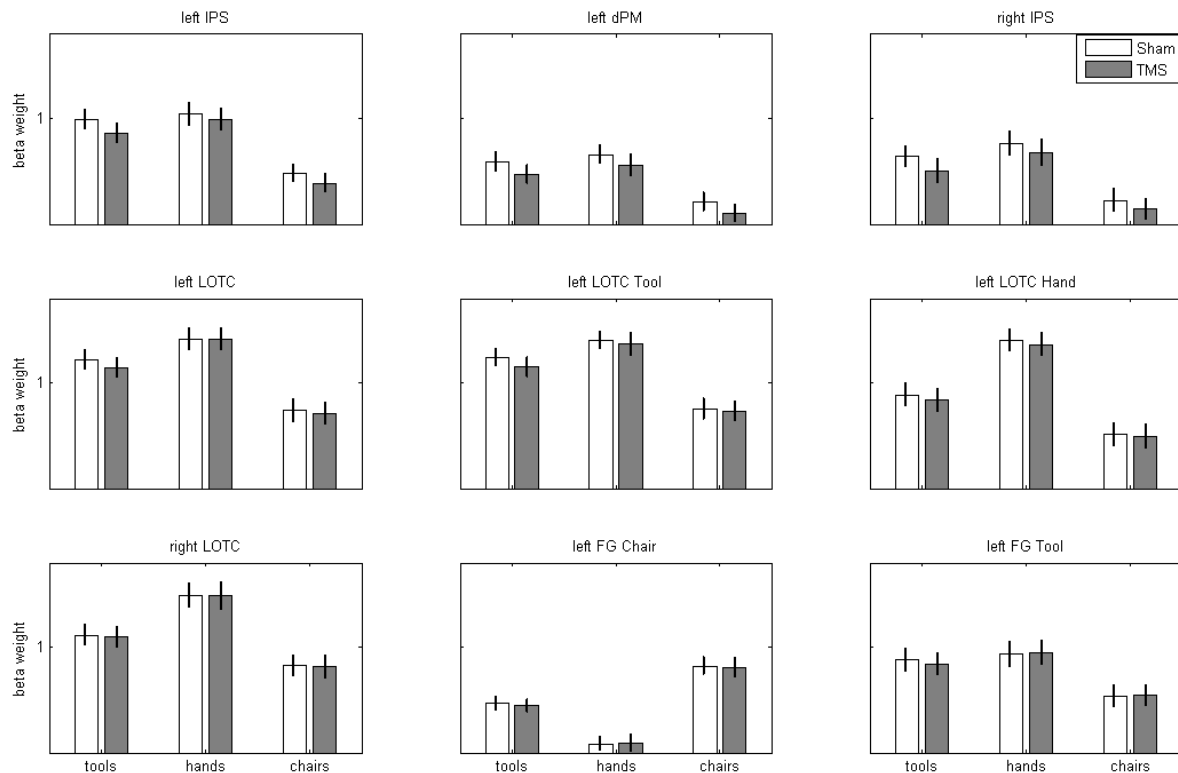

Figure 4.5. ROIs beta weights in study rTMS over IPS. Each panel presents the beta weights for each ROI defined in table 4.1 for the three categories tools, hands and chairs. White bars depict sham condition, while gray bars represent TMS condition. IPS: intraparietal sulcus; LOTC: lateral occipitotemporal cortex; dPM: dorsal premotor cortex; FG: fusiform gyrus. Error bars reflect standard error of the mean.

4.5.2 rTMS over left LOTC

4.5.2.1 Behavioral results

Behavioral results in the second experiment confirmed the trend seen in the first study, with task on hands more difficult than for the other two categories. A 2 x 3 two ways repeated measures ANOVA on reaction times showed that there was no general TMS effect ($F_{1,10}$ = 1.896, P = 0.199), while reaction times depended on the type of category ($F_{2,20}$ = 6.555, P = 0.006). There was no different TMS effect for the different categories ($F_{2,20}$ = 0.688, P = 0.51). A post hoc T-test showed that the 1-back task for hands yielded slower responses compared to tools (t_{10} = -3.909, P = 0.003), but not compared to chairs (t_{10} = 1.669, P =

0.13). There was no difference between tools and chairs (t_{10} = -1.828, P = 0.098). Two ways repeated measures ANOVA on accuracy confirmed that there was no main effect of TMS ($F_{1,10}$ = 0.040, P = 0.85) nor an interaction with the type of category ($F_{2,20}$ = 0.785, P = 0.47). There was neither a significant difference for the various categories ($F_{2,20}$ = 2.931, P = 0.077). Accuracy for detection of hand repetition dropped in comparisons to chairs (t_{10} = -2.306, P = 0.044), whereas there was no difference with tools (t_{10} = 2.144, P = 0.058), nor between tools and chairs (t_{10} = -0.387, p = 0.71).
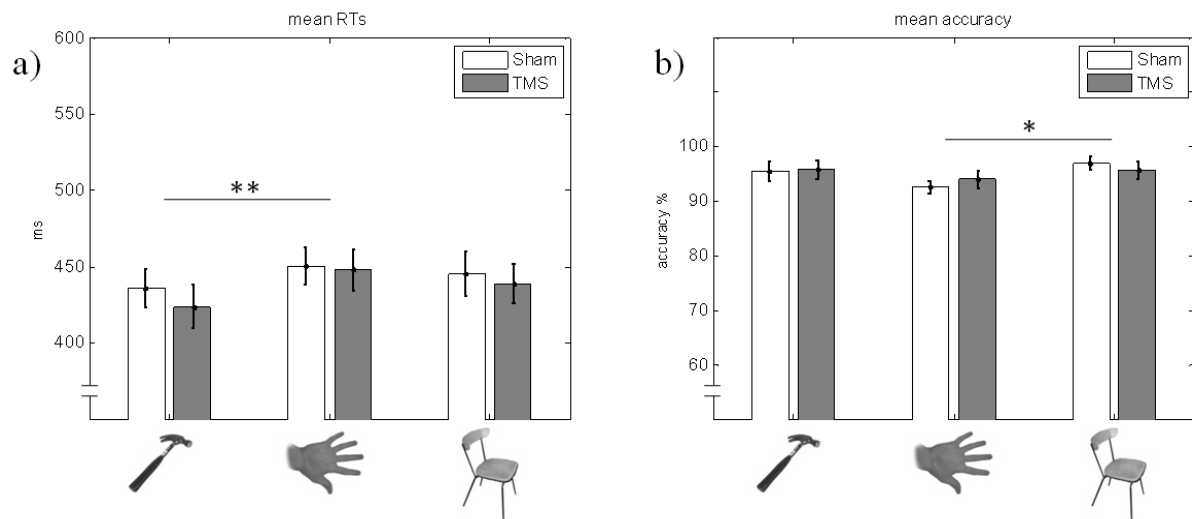

Figure 4.6. Behavioral results study rTMS over LOTC. Mean performance from eleven participants in the one back task for the three different categories: tools, hands and chairs. White bars represent performance in sham condition, and gray bars depict performance after effective TMS stimulation. **a) Reaction times**. TMS had no effect on performance for any category, but performance depended on the category, with detection of tool repetitions leading to faster responses compared then hands, while there was no difference with chairs, nor between hands and chairs. **b) Accuracy**. Accuracy analysis confirms RTs trend, with no general TMS effect but showing that hand category was more difficult with the lowest accuracy level compared to both tools and chairs. ** p < 0.01; * p < 0.05. Error bars reflect standard error of the mean.

4.5.2.2 fMRI results

In order to test if TMS had a different effect according to the category and to the cortical area, we applied a 3 ways repeated measures ANOVA to the beta values resulting from the different TMS and sham runs for all the nine regions of interest defined as described above (see table 4.1), in the same way as we did for the previous experiment. The main effect of category was significant ($F_{2,20}$ = 35.197, P < 0.001), and general responses also depended on the ROIs, ($F_{8,80}$ = 7.005, P < 0.001). There was no main effect of TMS ($F_{1,10}$ = 1.954, P =

0.19), nor an interaction between TMS effect and ROI ($F_{8,80}$ = 0.995, P = 0.446). The interaction between TMS and type of category was not significant ($F_{2,20}$ = 0.982, P = 0.392). The interaction between ROIs and category confirmed again that we localized the ROIs contrasting the different categories ($F_{16,160}$ = 36.774, P < 0.001). There was no significant three ways interaction between ROIs, category and TMS ($F_{16,160}$ = 0.782, P = 0.704). Separate two ways repeated measures ANOVA for each individual region showed main effects of category (P < 0.001, for all ROIs), but in none of the ROIs it showed a significant main effect of TMS (P > 0.065, for all ROIs), nor an interaction between TMS and category (P > 0.09, for all ROIs).

4.5.3. Discussion

In the first experiment, the fact that in the targeted left IPS BOLD activity decreased after effective TMS compared to sham condition, confirmed that we were effectively suppressing this area and hence that the rTMS-fMRI procedure worked. This is interesting also because several studies using a similar approach actually did not find a significant TMS effect on the stimulated region, but only in the connected ones, while others found a decrease of BOLD (Nowak Da and et al., 2008; Ward et al., 2010) and some also an increase (Bohning et al., 1999; Chouinard et al., 2003) depending on the task.

Furthermore, we found that also left dorsal premotor cortex showed a decrease of BOLD after TMS, indicating a connection between IPS and dorsal premotor cortex. Other rTMS-fMRI studies investigated connections to left dorsal PM, and also found that PM sends projections back to parietal cortex (Bestmann et al., 2005; Bestmann et al., 2008). So our data also confirm the opposite flow of information from IPS to PM. Because of the vicinity of PM to IPS, one could argue that the decrease of BOLD in PM was due to direct TMS stimulation and not to its connection to IPS. However, we have to note that on average there were 2.8 cm distance between these two areas, and considering that the induced electric field decreases very rapidly with the distance from the TMS coil, it is very unlikely that BOLD modulation in PM was due to direct stimulation. Moreover, also right IPS, which is further away from left IPS, showed a significant decrease of BOLD after TMS. Connections between left and right parietal cortex have been extensively investigated during visuospatial attention, and

they are usually proposed to be in competition (Kinsbourne, 1977, 1993), with one side controlling attention preferentially in the contralateral visual field (Bisiach et al., 1986; Karnath et al., 2002; Driver et al., 2004). However, in our task the attentional load was relatively low and was balanced for the two hemispheres, as the pictures were presented centrally on the screen, such that we did not put the two hemispheres in competition. The fact that TMS over left IPS showed a decrease in right IPS here indicates that the two areas are connected and that TMS is strong enough to transfer its effect to the opposite hemisphere. Interestingly, we did not find a significant TMS effect in lLOTC. The absence of a TMS effect did not seem to depend on how lLOTC was localized, although there was a trend for a decrease of BOLD, especially in the lLOTC-tools (Figure 4.4).

Additionally, TMS effects were not selective for particular categories: in those areas where TMS was effective, lIPS, ldPM and rIPS, TMS decreased activity for both the two preferred categories of these areas, tools and hands, as well as for the control category, chairs. However, in figure 4.5, by visual exploration we can see that TMS effect was somewhat larger for tools compared to the other two categories, although there was no significant TMS by category interaction.

Results from the second experiment did not show any interesting significant TMS effect, but the fact that we did not find an effect in the stimulated lLOTC would suggest that we did not have enough power, and we would probably need to test more participants. In fact, in the first study we tested 20 participants, while in the second study we had only 11 participants, due to technical problems and time limits. Moreover, five participants of the second study were tested in a different laboratory, where due to technical reasons the gap between the end of stimulation and the beginning of the functional run was longer (4.39 minutes) compared to the six participants tested in Italy (4.04 minutes). Since we don't know exactly how long TMS effects last, but we know that it decreases consistently in time, it is possible that we lost some power also for the long interval. Additional participants are needed in order to draw final conclusion of this second study.
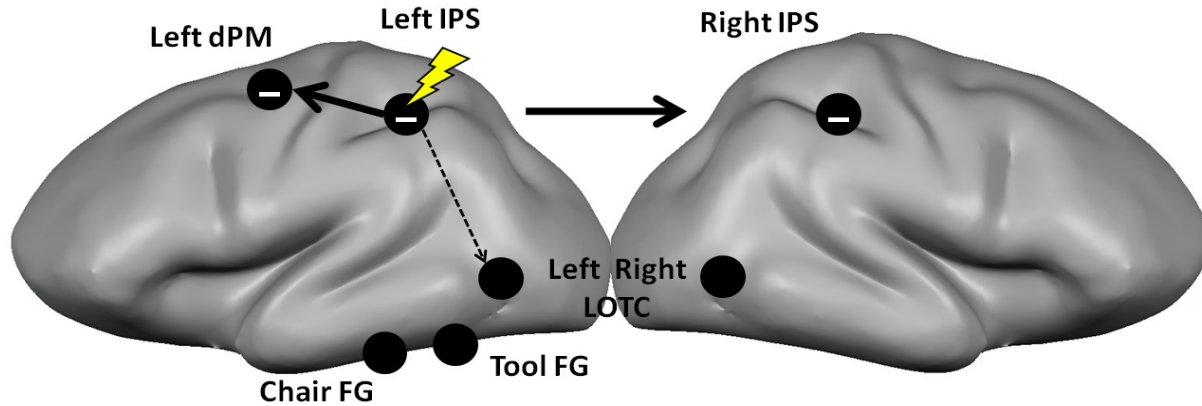
Figure 4.7. Overview rTMS over IPS. Figure represents a schematic summary of the main finding when stimulating over left IPS. Black circles stand for different ROIs. For visual purposes only, lLOTC localized in the three different ways are collapsed together. Minus sign inside a circle means that there was a decrease of BOLD after TMS in that ROI. Bold arrows mean that there was a significant connection between IPS and ldPM and rIPS, while dotted arrow toward lLOTC means that there was a trend but connection from IPS was not strong to reach significance.

To conclude, our results proves the existence of a feed forward flow of information from left IPS to left dPM, as well as inter hemispheric connection from left IPS to right IPS, but no evidence of a back propagation flow to lLOTC. This would suggest that connections from IPS to lLOTC are not automatic, although both areas are strongly activated by the simple presentation of static pictures of tools. This lack of back projections would support the hypothesis that the selectivity in lLOTC for processing tools, and in particular specific properties related to tool action, is not due to modulation coming from lIPS. This finding would integrate results coming from our previous study, where we showed the selective and causal role of lLOTC in processing action related features. Future research should investigate whether a different type of task that involves a more complex elaboration about tools, like discrimination of movement needed to use the tool versus contextual information (see chapter 3), would increase the connection between IPS and lLOTC.

5. General discussion and conclusion

5.1 Summary of principal findings

Tool use is essential in our daily life, such that our brain is organized with a specific network for it, lateralized in the left hemisphere and spreading from occipital up to frontal cortex (Johnson-Frey et al., 2005). Tools evoke such an automatic affordance that also passive viewing of static pictures of tools activate areas in this left network, namely lateral occipitotemporal cortex, interparietal sulcus and dorsal premotor cortex (Chao et al., 1999; Chao and Martin, 2000; Lewis, 2006; Bracci et al., 2012). While more is known about the involvement of parietal and frontal areas, the role of lLOTC in tool knowledge is unclear. Thus, the aim of this thesis was to investigate the neuronal mechanism of tool perception, with a focus on left LOTC, making use of the capacity of TMS to test the causal involvement of a specific area and its connection with other areas. Moreover, we also made use of the ability of fMRI to measure functional activation of an area in different experimental conditions. We conducted three different studies. The first study aimed to investigate how TMS affects neuronal activity, in particular why we can get different performance modulation according to the brain state at the time of the stimulation. The second study was composed of an fMRI experiment and a separate TMS experiment, both testing the role of lLOTC is processing different properties of tools. Finally, the scope of the third study was to investigate connections between left IPS and lLOTC during tool perception, interleaving TMS and fMRI.

In the first experimental chapter we assessed the effect of TMS on the behavioral psychometric response function with and without prior contrast adaptation. Without TMS, we replicated the finding that contrast adaptation leads to a decrease of contrast sensitivity (Ohzawa et al., 1982; Bonds, 1991; Pestilli et al., 2007). With TMS we found clear evidence for state dependency: TMS decreased performance when there was no adaptation, but after adaptation TMS increased accuracy. In addition, TMS affected only the thresholds of the psychometric functions, leaving asymptotes unchanged, suggesting that TMS acts at the input level of neuronal processing, i.e. at the synaptic level, via an input-gain mechanism (Naka and Rushton, 1966). These results cannot be accounted for by any of the current hypotheses underlying TMS effects (Walsh and Rushworth, 1999; Silvanto and Pascual-Leone, 2008; Ruzzoli et al., 2010; Schwarzkopf et al., 2011). In contrast, our results were better explained by our proposed model that TMS suppresses primarily the dominant neuronal population, depending on the neuronal state at the time of stimulation, leading to a facilitation of performance (when inhibitory neurons are more active, i.e. after adaptation) or an impairment (when excitatory neurons are more active, i.e. without adaptation).

In the second experiment, we tested whether LOTC is causally and selectively involved in tool action discrimination. Results from the fMRI experiment showed that functionally defined tool and hand selective LOTC regions, which partially overlapped (see also Bracci et al., 2012), were more active when participants discriminated the action associated with a tool than when they discriminated the location of the tool. Moreover, a separate TMS experiment showed that TMS over lLOTC impaired performance when participants were engaged in processing action related properties of the tool, while accuracy did not change during discrimination of the usual location of the tool. In addition, TMS in action task was effective only when applied at the later timings (150/250-270/370 ms after target onset) but not at the earliest timing (30/130 ms). These findings confirmed the causal role of lLOTC in processing action-related properties of tools.

In the third study, we applied repetitive TMS over left IPS and then measured BOLD response while participants were viewing pictures of tools, hand and chairs. Results showed that BOLD responses in the stimulated IPS decreased after effective TMS compared to sham condition. TMS decreased BOLD responses also in left dorsal premotor cortex and right IPS,

suggesting that these two areas received projections from left IPS. By contrast, we did not find a significant change in BOLD responses after TMS over left LOTC, although there was a trend for a decrease, suggesting that TMS effect over lLOTC was two weak and we should test additional participants.

5.2 Methodological implication

5.2.1 State-dependent TMS

The first study of the thesis aimed to better understand how TMS interferes with neuronal activity. As reviewed in chapter 2, there are several models that tried to explain how TMS interacts with neurons, as this is still unclear. Therefore, it is extremely important when we have to interpret results of a TMS experiment, and even more cautiously when we combine TMS with fMRI, to take into account what we know and what we still don't know about TMS effects. Indeed, our study on state-dependent TMS showed that none of the proposed models about TMS mechanisms could fully explain our data, while our proposed new model could better fit the data. However, only recently it has been shown that TMS effects also depend on TMS intensity (Schwarzkopf et al., 2011), and our suggested model should also be tested using differing TMS intensities. This noticeably shows that we are still away from deeply understanding the interaction between TMS and neuronal activity and we need further investigation, while it posits more attention to the fact that TMS effects can vary radically depending on the state at which cortex is at the time of simulation.

Based on our proposed model, TMS seems to suppress the most active neurons, with a consequent benefit for the less active neurons. Thus, we can try to understand under this account our findings from the second experiment. Here we stimulated with single pulse over lLOTC while participants were involved in two different tasks about tools: categorizing between type of movements or between different context of the tool. During the action task, TMS would decrease activity of neurons in lLOTC that are highly activated during action properties processing, as revealed by the fMRI experiment, leading to a consequent impairment in performance. However, fMRI results showed that lLOTC is activated also during the contextual task, even if in a lesser extent than for action task. If neurons in lLOTC were directly involved in the contextual task, then TMS would decrease their activity and

impair performance also during contextual processing, but this is not what we found. Thus, it seems more likely that activity during context task arose simply because tool action is still somewhat processed even during context task, but a decrease in activation of these neurons in context task is not relevant for the performance, further supporting that lLOTC is specific for processing action features of tools.

5.2.2 TMS combined with fMRI

There is an increasing number of studies that combine TMS and fMRI, and although it is established that this is a valid method to investigate regions connected to the stimulated one, it is still unclear why the stimulated region can either show an increase (Bohning et al., 1999; Chouinard et al., 2003) or a decrease (Nowak Da and et al., 2008; Ward et al., 2010) of BOLD, and sometimes no TMS effect at all, while still seeing a significant positive or negative effect on distal regions (O'Shea et al., 2007; van der Werf et al., 2010). Usually studies on state-dependent TMS, like our study in the first chapter, have involved single or short trains of TMS, but probably a similar approach in studying TMS effect has to be applied also to long trains of stimulation. 1 Hz TMS has usually been referred as an inhibitory paradigm, but the studies mentions above showed that this is not always the case. It would then be interesting to investigate the relation between BOLD change and magnetic stimulation. Another unclear point about the use of TMS arises from our work. That is, the duration of TMS effect on BOLD response, which seems to be different than the duration of the effect on performance, also considering that several studies found a change in BOLD but not on behavior (O'Shea et al., 2007). In our third study we used a common rTMS-fMRI paradigm where we stimulated for 13 minutes at 1 Hz TMS, followed by a 4 minutes gap before starting the functional MRI runs, which was then placed between 4-11.5 minutes after the end of the stimulation. It is usually thought that TMS effects last approximately as long as the duration of stimulation. For example, Boroojerdi et al. (2000) found that 15 minutes of 1 Hz stimulation over the occipital cortex at intensity of phosphene threshold decreased the excitability of the visual cortex (i.e. increased phosphene threshold) for about 10 minutes after the end of stimulation. However, many variables have to be taken into account when designing an experiment and to know how long the functional run can be. For example, apart

from the two main parameters of TMS of frequency and intensity, there is also a wide inter-individual variability, like thickness of the scalp at the location of stimulation, which should be taken into account when testing with TMS. Considering the increase of studies that combined TMS and fMRI, future investigation is needed to shed light not only on how TMS can modulates neuronal activity, but also which is its relation with BOLD response, in order to know how we have to interpret an increase or a decrease of BOLD and how this is related to the task that participants are performing during measurements of BOLD.

5.3 Cognitive implication

5.3.1 Tool perception in lLOTC

The second and the third study focused on the role of lLOTC in tool knowledge. Both studies confirmed that the role of this area in processing properties specific of tools and their use is more relevant than what was previously thought. Our results show that lLOTC has a selective and causal role in encoding action-related features of tools (study 2), while back projections from IPS seem not be relevant, at least during passing viewing of tools (study 3). These findings taken together suggest then that left LOTC is not simply a visual area, but is already causally and actively involved in a type of encoding about tools that goes beyond visual features: LOTC is causally and selectively involved in the discrimination of hand actions associated with visually presented tools.

Several imaging studies have shown activation in MTG/LOTC when processing tools from different sensory modalities, not just with visual presentation of pictures, but also during reading of tool names, pantomiming actions with tools and with the presentation of noises related to tool-use (for review, see (Lewis, 2006). However, clinical studies failed to demonstrate a clear causal role of this region in the processing of action-related features of tools. A specific syndrome, Balint's syndrome, may suggest the role of MTG/LOTC in encoding visually guided object-hand interaction. Balint's syndrome is a clinical desease which combines variously a set of complex spatial behavior disorders following bilateral damage to the occipito–parietal junction (slightly overlapping also LOTC) affecting the capacity of the patient to perform correctly visually guided action on objects, but keeping

intact semantic knowledge about the object (optic ataxia, for review see (Pisella et al., 2008)). Interestingly, apart from a wide region in occipito-parietal regions, other lesions of this syndrome covers a region in IPS and in premotor cortex, which correspond to the areas that are also activated by tool perception. Another study that investigated 90 patients with different lesions in the left and right hemisphere, showed that the regions of highest lesion overlap in subjects with impaired retrieval of conceptual knowledge for actions were in the left premotor/prefrontal sector, the left parietal region, and in the white matter underneath the left posterior middle temporal region (Tranel et al., 2003). Interestingly, in a previous study by the same authors (Tranel et al., 1997) it was also shown that defective recognition of tools, compared to animals and persons, was associated with maximal lesion overlap in the occipitotemporal-parietal junction of the left hemisphere. In contrast, defective recognition of people was associated with maximal lesion overlap in right temporal polar region and defective recognition of animals was associated with maximal lesion overlap in right mesial occipital/ventral temporal region. These studies also support the hypothesis that knowledge for categories from different conceptual domains depends on partially segregated neural systems. However, it is unclear from these neuropsychological studies what the causal role of lLOTC in tool perception is, since impairment of tool knowledge could also arise from concomitant lesions to other areas. Our study instead strongly suggests that MTG/LOTC is effectively and causally involved in the retrieval of conceptual knowledge for tool action, possibly without involving projections from parietal cortex.

5.3.2. Organization of occipitotemporal cortex

We know that this area in lLOTC is also selective for hands (Bracci et al., 2010; Bracci et al., 2012), but our second study shows that selectivity for tools is not due to a simple epiphenomenal association with hands, nor that this area encodes all tool properties in the same way, but it is specific for tool-hand interaction, such as the type of movement we have to make with the hand in order to act with the tool. This is extremely relevant because it suggests that occipital-temporal cortex is not organized based on visual properties of the stimuli alone (Mahon and Caramazza, 2011), since tools and hands are obviously very different visually. In contrast, our data would better support the hypothesis that brain

organization is driven by a principle of efficiency, like clustering together categories that share high-level properties relevant for interacting with our environment, in regions that are better located for sending information to other areas in charge of further action processing. In the case of tool perception, tools and hands may be processed in the same area to better decode the interaction between them and send information directly to higher areas of the tool network in parietal cortex. LOTC is hence able to distinguish a familiar tool from a non-manipulable object without receiving information from IPS. But how does the brain know that it is more efficient to specialize this area for elaborating tools? Since this cannot depend on visual information of the object, as there are no fixed visual characteristics that define what a tool is, and even blind patients show a similar cortical organization (Mahon et al., 2010), it would be interesting to investigate the role of back projections from parietal and other higher areas while we are learning to recognize a new unfamiliar object as a tool. It is indeed possible that interactions towards and from IPS, and possibly higher areas, are critical during the process of discriminating and storing a new item, but once knowledge about the item has been internalized, "visual" areas in occipital-temporal regions are independent in recognizing which domain the item belongs to, and this would explain why we did not find an effect on lLOTC when stimulating over IPS. Future research could investigate the role of IPS over lLOTC while learning to use a new object and categorizing it as a new tool, perhaps making use of TMS in order to disrupt connections headed to lLOTC, and viceversa.

5.4 Conclusions

In conclusion, in this thesis we investigated the role of left LOTC during tool perception, using TMS and fMRI. Our findings enhance our knowledge about how lower cortical areas in occipital-temporal cortex are not simply visual areas, but can be already involved in processing properties about the stimuli that go beyond visual information. In particular, we showed that this area in lLOTC that is selectively activated by visual representation of tools as well as hands is specific in coding interactions between tool and hands, rather than general features about tools. Moreover, activity in lLOTC does not seem to depend on back projections from higher areas like parietal sulcus, as TMS over IPS did not have a significant effect on BOLD responses in lLOTC, suggesting that lLOTC is relatively autonomous in

processing action-related properties of objects. In general, more attention should be given also to the role of "visual" occipitotemporal regions in extracting information about our external world, that go beyond purely visual information. Hence, although there is evidence for specific and selective areas coding for preferred categories (Kanwisher et al., 1997; Peelen and Downing, 2005), the representation about objects seems to be spreaded among different regions in the network, including also occipitotemporal areas.

6. References

Amassian VE, Cracco RQ, Maccabee PJ, Cracco JB, Rudell A, Eberle L (1989) Suppression of visual perception by magnetic coil stimulation of human occipital cortex. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section 74:458-462.

Arfeller C, Schwarzbach J, Ubaldi S, Ferrari P, Barchiesi G, Cattaneo L (2012) Whole-Brain Haemodynamic After-Effects of 1-Hz Magnetic Stimulation of the Posterior Superior Temporal Cortex During Action Observation. Brain Topography:1-14.

Aziz-Zadeh L, Iacoboni M, Zaidel E, Wilson S, Mazziotta J (2004) Left hemisphere motor facilitation in response to manual action sounds. European Journal of Neuroscience 19:2609-2612.

Bach P, Peelen MV, Tipper SP (2010) On the role of object information in action observation: an fMRI study. Cereb Cortex 20:2798-2809.

Beauchamp MS, Lee KE, Haxby JV, Martin A (2002) Parallel Visual Motion Processing Streams for Manipulable Objects and Human Movements. Neuron 34:149-159.

Beauchamp MS, Lee KE, Argall BD, Martin A (2004) Integration of Auditory and Visual Information about Objects in Superior Temporal Sulcus. Neuron 41:809-823.

Bedny M, Caramazza A, Grossman E, Pascual-Leone A, Saxe R (2008) Concepts are more than percepts: the case of action verbs. J Neurosci 28:11347-11353.

Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J (2005) BOLD MRI responses to repetitive TMS over human dorsal premotor cortex. NeuroImage 28:22-29.

Bestmann S, Ruff C, Blankenburg F, Weiskopf N, Driver J, Rothwell J (2008) Mapping causal interregional influences with concurrent TMS–fMRI. Experimental Brain Research 191:383-402.

Binder JR, McKiernan KA, Parsons ME, Westbury CF, Possing ET, Kaufman JN, Buchanan L (2003) Neural Correlates of Lexical Access during Visual Word Recognition. Journal of Cognitive Neuroscience 15:372-393.

Bisiach E, Vallar G, Perani D, Papagno C, Berti A (1986) Unawareness of disease following lesions of the right hemisphere: Anosognosia for hemiplegia and anosognosia for hemianopia. Neuropsychologia 24:471-482.

Bohning D, Shastri A, McConnell K, Nahas Z, Lorberbaum J, Roberts D, Teneback C, Vincent D, George M (1999) A combined TMS/fMRI study of intensity-dependent TMS over motor cortex. Biological Psychiatry 45:385-394.

Bonds AB (1991) Temporal dynamics of contrast gain in single cells of the cat striate cortex. Vis Neurosci 6:239-255.

Boronat CB, Buxbaum LJ, Coslett HB, Tang K, Saffran EM, Kimberg DY, Detre JA (2005) Distinctions between manipulation and function knowledge of objects: evidence from functional magnetic resonance imaging. Cognitive Brain Research 23:361-373.

Bracci S, Ietswaart M, Peelen MV, Cavina-Pratesi C (2010) Dissociable Neural Responses to Hands and Non-Hand Body Parts in Human Left Extrastriate Visual Cortex. Journal of Neurophysiology 103:3389-3397.

Bracci S, Cavina-Pratesi C, Ietswaart M, Caramazza A, Peelen MV (2012) Closely overlapping responses to tools and hands in left lateral occipitotemporal cortex. Journal of Neurophysiology 107:1443-1456.

Brainard DH (1997) The Psychophysics Toolbox. Spat Vis 10:433-436.

Buccino G, Sato M, Cattaneo L, Rodà F, Riggio L (2009) Broken affordances, broken objects: A TMS study. Neuropsychologia 47:3074-3078.

Buchel C, Price C, Friston K (1998) A multimodal language region in the ventral visual pathway. Nature 394:274-277.

Cameron EL, Tai JC, Carrasco M (2002) Covert attention affects the psychometric function of contrast sensitivity. Vision Res 42:949-967.

Campanella F, D'Agostini S, Skrap M, Shallice T (2010) Naming manipulable objects: anatomy of a category specific effect in left temporal tumours. Neuropsychologia 48:1583-1597.

Canessa N, Borgo F, Cappa SF, Perani D, Falini A, Buccino G, Tettamanti M, Shallice T (2008) The Different Neural Correlates of Action and Functional Knowledge in Semantic Memory: An fMRI Study. Cerebral Cortex 18:740-751.

Capitani E, Laiacona M, Mahon B, Caramazza A (2003) WHAT ARE THE FACTS OF SEMANTIC CATEGORY-SPECIFIC DEFICITS? A CRITICAL REVIEW OF THE CLINICAL EVIDENCE. Cognitive Neuropsychology 20:213-261.

Carandini M, Ferster D (1997) A Tonic Hyperpolarization Underlying Contrast Adaptation in Cat Visual Cortex. Science 276:949-952.

Carrasco M, Penpeci-Talgar C, Eckstein M (2000) Spatial covert attention increases contrast sensitivity across the CSF: support for signal enhancement. Vision Research 40:1203-1215.

Cattaneo L, Sandrini M, Schwarzbach J (2010) State-Dependent TMS Reveals a Hierarchical Representation of Observed Acts in the Temporal, Parietal, and Premotor Cortices. Cerebral Cortex 20:2252-2258.

Cattaneo Z, Silvanto J (2008) Time course of the state-dependent effect of transcranial magnetic stimulation in the TMS-adaptation paradigm. Neuroscience Letters 443:82-85.

Chao LL, Martin A (2000) Representation of Manipulable Man-Made Objects in the Dorsal Stream. NeuroImage 12:478-484.

Chao LL, Haxby JV, Martin A (1999) Attribute-based neural substrates in temporal cortex for perceiving and knowing about objects. Nat Neurosci 2:913-919.

Chao LL, Weisberg J, Martin A (2002) Experience-dependent Modulation of Category-related Cortical Activity. Cerebral Cortex 12:545-551.

Choi S, Na D, Kang E, Lee K, Lee S, Na D (2001) Functional magnetic resonance imaging during pantomiming tool-use gestures. Experimental Brain Research 139:311-317.

Chouinard P, Van Der Werf Y, Leonard G, Paus T (2003) Modulating neural networks with transcranial magnetic stimulation applied over the dorsal premotor and primary motor cortices. J Neurophysiol 90:1071 - 1083.

Creem-Regehr SH, Lee JN (2005) Neural representations of graspable objects: are tools special? Cognitive Brain Research 22:457-469.

Damasio H, Grabowski TJ, Tranel D, Hichwa RD, Damasio AR (1996) A neural basis for lexical retrieval. Nature 380:499-505.

Dao DY, Lu ZL, Dosher BA (2006) Adaptation to sine-wave gratings selectively reduces the contrast gain of the adapted stimuli. J Vis 6:739-759.

Decety J, Grèzes J, Costes N, Perani D, Jeannerod M, Procyk E, Grassi F, Fazio F (1997) Brain activity during observation of actions. Influence of action content and subject's strategy. Brain 120:1763-1777.

Dehaene S (2009) Reading in the Brain: Penguin Viking.

Dehaene S, Cohen L (2011) The unique role of the visual word form area in reading. Trends in cognitive sciences 15:254-262.

Downing PE, Wiggett AJ, Peelen MV (2007) Functional magnetic resonance imaging investigation of overlapping lateral occipitotemporal activations using multi-voxel pattern analysis. J Neurosci 27:226-233.

Downing PE, Jiang Y, Shuman M, Kanwisher N (2001) A cortical area selective for visual processing of the human body. Science 293:2470-2473.

Downing PE, Chan AW, Peelen MV, Dodds CM, Kanwisher N (2006) Domain specificity in visual cortex. Cereb Cortex 16:1453-1461.

Driver J, Vuilleumier P, Husain M, Gazzaniga M (2004) Spatial neglect and extinction. In: The new cognitive neurosciences III pp pp 589–606.: Cambridge: MIT.

Emmorey K, Grabowski T, McCullough S, Damasio H, Ponto L, Hichwa R, Bellugi U (2004) Motor-iconicity of sign language does not alter the neural systems underlying tool and action naming. Brain and Language 89:27-37.

Epstein R, Harris A, Stanley D, Kanwisher N (1999) The Parahippocampal Place Area: Recognition, Navigation, or Encoding? Neuron 23:115-125.

Fairhall SL, Anzellotti S, Pajtas PE, Caramazza A (2011) Concordance between perceptual and categorical repetition effects in the ventral visual stream. Journal of Neurophysiology 106:398-408.

Frey SH (2008) Tool use, communicative gesture and cerebral asymmetries in the modern human brain. Philosophical Transactions of the Royal Society B: Biological Sciences 363:1951-1957.

Fridman EA, Immisch I, Hanakawa T, Bohlhalter S, Waldvogel D, Kansaku K, Wheaton L, Wu T, Hallett M (2006) The role of the dorsal stream for gesture production. NeuroImage 29:417-428.

Gardner JL, Sun P, Waggoner RA, Ueno K, Tanaka K, Cheng K (2005) Contrast Adaptation and Representation in Human Early Visual Cortex. Neuron 47:607-620.

Glover S, Miall RC, Rushworth MFS (2005) Parietal rTMS Disrupts the Initiation but not the Execution of On-line Adjustments to a Perturbation of Object Size. Journal of Cognitive Neuroscience 17:124-136.

Goldenberg G, Spatt J (2009) The neural basis of tool use. Brain 132:1645-1655.

Goodale MA, Milner AD (1992) Separate visual pathways for perception and action. Trends in Neurosciences 15:20-25.

Grèzes J, Tucker M, Armony J, Ellis R, Passingham RE (2003) Objects automatically potentiate action: an fMRI study of implicit processing. European Journal of Neuroscience 17:2735-2740.

Harris JA, Clifford CW, Miniussi C (2008) The functional effect of transcranial magnetic stimulation: signal suppression or neural noise generation? J Cogn Neurosci 20:734-740.

Haxby JV, Ishai A, Chao LL, Ungerleider LG, Martin A (2000) Object-form topology in the ventral temporal lobe: Response to. Trends in cognitive sciences 4:3-4.

Herrmann K, Montaser-Kouhsari L, Carrasco M, Heeger DJ (2010) When size matters: attention affects performance by contrast or response gain. Nat Neurosci 13:1554-1559.

Hubel DH, Wiesel TN (1963) Receptive fields of cells in striate cortex of very young, visually inexperieced kittens. Journal of Neurophysiology 26:994-1002.

Hubel DH, Wiesel TN (1974) Sequence regularity and geometry of orientation columns in the monkey striate cortex. The Journal of Comparative Neurology 158:267-293.

Inoue K, Kawashima R, Sugiura M, Ogawa A, Schormann T, Zilles K, Fukuda H (2001) Activation in the Ipsilateral Posterior Parietal Cortex during Tool Use: A PET Study. NeuroImage 14:1469-1475.

Johnson-Frey SH (2004) The neural bases of complex tool use in humans. Trends in cognitive sciences 8:71-78.

Johnson-Frey SH, Newman-Norlund R, Grafton ST (2005) A Distributed Left Hemisphere Network Active During Planning of Everyday Tool Use Skills. Cerebral Cortex 15:681-695.

Kable JW, Kan IP, Wilson A, Thompson-Schill SL, Chatterjee A (2005a) Conceptual Representations of Action in the Lateral Temporal Cortex. Journal of Cognitive Neuroscience 17:1855-1870.

Kable JW, Kan IP, Wilson A, Thompson-Schill SL, Chatterjee A (2005b) Conceptual representations of action in the lateral temporal cortex. J Cogn Neurosci 17:1855-1870.

Kalenine S, Buxbaum LJ, Coslett HB (2010) Critical brain regions for action recognition: lesion symptom mapping in left hemisphere stroke. Brain 133:3269-3280.

Kanwisher N, McDermott J, Chun MM (1997) The Fusiform Face Area: A Module in Human Extrastriate Cortex Specialized for Face Perception. The Journal of Neuroscience 17:4302-4311.

Karnath H-O, Milner D, Vallar G, eds (2002) The cognitive and neural bases of spatial neglect. Oxford: Oxford University Press.

Kellenbach ML, Brett M, Patterson K (2003) Actions Speak Louder Than Functions: The Importance of Manipulability and Action in Tool Representation. J Cognitive Neuroscience 15:30-46.

Konkle T, Oliva A (2012) A real-world size organization of object responses in occipitotemporal cortex. Neuron 74:1114-1124.

Koski L, Iacoboni M, Mazziotta JC (2002) Deconstructing apraxia: understanding disorders of intentional movement after stroke. Current Opinion in Neurology 15:71-77.

Leiguarda RC, Marsden CD (2000) Limb apraxias: Higher-order disorders of sensorimotor integration. Brain 123:860-879.

Lewis JW (2006) Cortical Networks Related to Human Use of Tools. The Neuroscientist 12:211-231.

Lewis JW, Brefczynski JA, Phinney RE, Janik JJ, DeYoe EA (2005) Distinct Cortical Pathways for Processing Tool versus Animal Sounds. The Journal of Neuroscience 25:5148-5158.

Lewis JW, Wightman FL, Brefczynski JA, Phinney RE, Binder JR, DeYoe EA (2004) Human Brain Regions Involved in Recognizing Environmental Sounds. Cerebral Cortex 14:1008-1021.

Li X, Nahas Z, Anderson B, Kozel FA, George MS (2004) Can left prefrontal rTMS be used as a maintenance treatment for bipolar depression? Depression and Anxiety 20:98-100.

Lueck CJ, Zeki S, Friston KJ, Deiber MP, Cope P, Cunningham VJ, Lammertsma AA, Kennard C, Frackowiak RSJ (1989) The colour centre in the cerebral cortex of man. Nature 340:386-389.

Mahon BZ, Caramazza A (2005) The orchestration of the sensory-motor systems: Clues from Neuropsychology. Cognitive Neuropsychology 22:480-494.

Mahon BZ, Caramazza A (2011) What drives the organization of object knowledge in the brain? Trends in cognitive sciences 15:97-103.

Mahon BZ, Schwarzbach J, Caramazza A (2010) The Representation of Tools in Left Parietal Cortex Is Independent of Visual Experience. Psychological Science 21:764-771.

Makris S, Hadar AA, Yarrow K (2011) Viewing objects and planning actions: On the potentiation of grasping behaviours by visual objects. Brain and Cognition 77:257-264.

Malach R, Reppas JB, Benson RR, Kwong KK, Jiang H, Kennedy WA, Ledden PJ, Brady TJ, Rosen BR, Tootell RB (1995) Object-related activity revealed by functional magnetic resonance imaging in human occipital cortex. Proceedings of the National Academy of Sciences 92:8135-8139.

Martin A (2007) The Representation of Object Concepts in the Brain. Annual Review of Psychology 58:25-45.

Martin A, Haxby JV, Lalonde FM, Wiggs CL, Ungerleider LG (1995) Discrete cortical regions associated with knowledge of color and knowledge of action. Science 270:102-105.

McAdams CJ, Maunsell JH (1999) Effects of attention on orientation-tuning functions of single neurons in macaque cortical area V4. J Neurosci 19:431-441.

Moliadze V, Zhao Y, Eysel U, Funke K (2003) Effect of transcranial magnetic stimulation on single-unit activity in the cat primary visual cortex. J Physiol 553:665-679.

Nachmias J (1967) Effect of Exposure Duration on Visual Contrast Sensitivity with Square-Wave Gratings. J Opt Soc Am 57:421-427.

Naka KI, Rushton WA (1966) S-potentials from colour units in the retina of fish (Cyprinidae). The Journal of physiology 185:536-555.

Noppeney U, Price CJ, Penny WD, Friston KJ (2006) Two Distinct Neural Mechanisms for Category-selective Responses. Cerebral Cortex 16:437-445.

Nowak Da GCDM, et al. (2008) EFfects of low-frequency repetitive transcranial magnetic stimulation of the contralesional primary motor cortex on movement kinematics and neural activity in subcortical stroke. Archives of Neurology 65:741-747.

O'Shea J, Johansen-Berg H, Trief D, Göbel S, Rushworth MFS (2007) Functionally Specific Reorganization in Human Premotor Cortex. Neuron 54:479-490.

Ochipa C, Rothi LJG, Heilman KM (1989) Ideational apraxia: A deficit in tool selection and use. Annals of Neurology 25:190-193.

Ohzawa I, Sclar G, Freeman RD (1982) Contrast gain control in the cat visual cortex. Nature 298:266-268.

Op de Beeck HP, Torfs K, Wagemans J (2008) Perceived Shape Similarity among Unfamiliar Objects and the Organization of the Human Object Vision Pathway. The Journal of Neuroscience 28:10111-10123.

Paus T (2005) Inferring causality in brain images: a perturbation approach. Philosophical Transactions of the Royal Society B: Biological Sciences 360:1109-1114.

Peelen MV, Downing PE (2005) Selectivity for the Human Body in the Fusiform Gyrus. Journal of Neurophysiology 93:603-608.

Peelen MV, Caramazza A (in press) Conceptual Object Representations in Human Anterior

Temporal Cortex. Journal of Neuroscience.

Peelen MV, Romagno D, Caramazza A (2012) Independent Representations of Verbs and Actions in Left Lateral Temporal Cortex. J Cogn Neurosci.

Pelli DG (1997) The VideoToolbox software for visual psychophysics: transforming numbers into movies. Spatial Vision 10:437-442.

Perani D, Cappa SF, Schnur T, Tettamanti M, Collina S, Rosa MM, Fazio F (1999) The neural correlates of verb and noun processing. A PET study. Brain 122 (Pt 12):2337-2344.

Pestilli F, Viera G, Carrasco M (2007) How do attention and adaptation affect contrast sensitivity? J Vis 7:9 1-12.

Pestilli F, Ling S, Carrasco M (2009) A population-coding model of attention's influence on contrast response: Estimating neural effects from psychophysical data. Vision Research 49:1144-1153.

Phillips JA, Noppeney U, Humphreys GW, Price CJ (2002) Can segregation within the semantic system account for category-specific deficits? Brain 125:2067-2080.

Pisella L, Ota H, Vighetto A, Rossetti Y (2008) Optic ataxia and Bálint's syndrome: neuropsychological and neurophysiological prospects. In: Handbook of Clinical Neurology pp 393–415.

Pitcher D, Charles L, Devlin JT, Walsh V, Duchaine B (2009) Triple Dissociation of Faces, Bodies, and Objects in Extrastriate Cortex. Current Biology 19:319-324.

Price CJ, Devlin JT (2011) The Interactive Account of ventral occipitotemporal contributions to reading. Trends in cognitive sciences 15:246-253.

Ridding MC, Rothwell JC (2007) Is there a future for therapeutic use of transcranial magnetic stimulation? Nat Rev Neurosci 8:559-567.

Ruff CC, Bestmann S, Blankenburg F, Bjoertomt O, Josephs O, Weiskopf N, Deichmann R, Driver J (2008) Distinct Causal Influences of Parietal Versus Frontal Areas on Human Visual Cortex: Evidence from Concurrent TMSâ€"fMRI. 18:817-827.

Ruff CC, Blankenburg F, Bjoertomt O, Bestmann S, Freeman E, Haynes J-D, Rees G, Josephs O, Deichmann R, Driver J (2006) Concurrent TMS-fMRI and Psychophysics Reveal Frontal Influences on Human Retinotopic Visual Cortex. Current biology : CB 16:1479-1488.

Rumiati RI, Zanini S, Vorano L, Shallice T (2001) A Form of Ideational Apraxia as a Delective Deficit of Contention Scheduling. Cognitive Neuropsychology 18:617-642.

Ruzzoli M, Marzi CA, Miniussi C (2010) The Neural Mechanisms of the Effects of Transcranial Magnetic Stimulation on Perception. J Neurophysiol 103:2982-2989.

Sack AT, Kohler A, Bestmann S, Linden DEJ, Dechent P, Goebel R, Baudewig J (2007) Imaging the Brain Activity Changes Underlying Impaired Visuospatial Judgments: Simultaneous fMRI, TMS, and Behavioral Studies. 17:2841-2852.

Sakuraba S, Sakai S, Yamanaka M, Yokosawa K, Hirayama K (2012) Does the Human Dorsal Stream Really Process a Category for Tools? The Journal of Neuroscience 32:3949-3953.

Salminen-Vaparanta N, Noreika V, Revonsuo A, Koivisto M, Vanni S (2011) Is selective primary visual cortex stimulation achievable with TMS? Human Brain Mapping:n/a-n/a.

Schwarzbach J (2011) A simple framework (ASF) for behavioral and neuroimaging experiments based on the psychophysics toolbox for MATLAB. Behav Res Methods.

Schwarzkopf DS, Silvanto J, Rees G (2011) Stochastic Resonance Effects Reveal the Neural Mechanisms of Transcranial Magnetic Stimulation. The Journal of Neuroscience 31:3143-3147.

Shapiro KA, Moo LR, Caramazza A (2006) Cortical signatures of noun and verb production. Proc Natl Acad Sci U S A 103:1644-1649.

Shipp S, Zeki S (1985) Segregation of pathways leading from area V2 to areas V4 and V5 of macaque monkey visual cortex. Nature 315:322-324.

Siebner HR et al. (2009) Consensus paper: Combining transcranial stimulation with neuroimaging. BRAIN STIMULATION: Basic, Translational, and Clinical Research in Neuromodulation 2:58-80.

Silvanto J, Pascual-Leone A (2008) State-Dependency of Transcranial Magnetic Stimulation. Brain Topography 21:1-10.

Silvanto J, Muggleton NG (2008) New light through old windows: Moving beyond the "virtual lesion" approach to transcranial magnetic stimulation. NeuroImage 39:549-552.

Silvanto J, Muggleton N, Walsh V (2008a) State-dependency in brain stimulation studies of perception and cognition. Trends Cogn Sci 12:447-454.

Silvanto J, Muggleton N, Cowey A, Walsh V (2007a) Neural adaptation reveals state-dependent effects of transcranial magnetic stimulation. European Journal of Neuroscience 25:1874-1881.

Silvanto J, Muggleton NG, Cowey A, Walsh V (2007b) Neural adaptation reveals state-dependent effects of transcranial magnetic stimulation. Eur J Neurosci 25:1874-1881.

Silvanto J, Cattaneo Z, Battelli L, Pascual-Leone A (2008b) Baseline cortical excitability determines whether TMS disrupts or facilitates behavior. J Neurophysiol 99:2725-2730.

Simmons WK, Martin A (2012) Spontaneous resting-state BOLD fluctuations reveal persistent domain-specific neural networks. Soc Cogn Affect Neurosci 7:467-475.

Simmons WK, Reddish M, Bellgowan PS, Martin A (2010) The selectivity and functional connectivity of the anterior temporal lobes. Cereb Cortex 20:813-825.

Simonotto E, Riani M, Seife C, Roberts M, Twitty J, Moss F (1997) Visual Perception of Stochastic Resonance. . Physical Re-view Letters 78.

Smith AT, Singh KD, Williams AL, Greenlee MW (2001) Estimating Receptive Field Size from fMRI Data in Human Striate and Extrastriate Visual Cortex. Cerebral Cortex 11:1182-1190.

Stocks NG (2000) Suprathreshold stochastic resonance in multilevel threshold systems. Phys Rev Lett 84:2310-2313.

Striem-Amit E, Dakwar O, Reich L, Amedi A (2012) The large-Scale Organization of "Visual" Streams Emerges Without Visual Experience. Cerebral Cortex 22:1698-1709.

Swisher JD, Gatenby JC, Gore JC, Wolfe BA, Moon C-H, Kim S-G, Tong F (2010) Multiscale Pattern Analysis of Orientation-Selective Activity in the Primary Visual Cortex. The Journal of Neuroscience 30:325-330.

Thielscher A, Reichenbach A, Ugurbil K, Uludag K (2010) The cortical site of visual suppression by transcranial magnetic stimulation. Cereb Cortex 20:328-338.

Tootell R, Reppas J, Kwong K, Malach R, Born R, Brady T, Rosen B, Belliveau J (1995) Functional analysis of human MT and related visual cortical areas using magnetic resonance imaging. The Journal of Neuroscience 15:3215-3230.

Tranel D, Damasio H, Damasio AR (1997) A neural basis for the retrieval of conceptual knowledge. Neuropsychologia 35:1319-1327.

Tranel D, Grabowski TJ, Lyon J, Damasio H (2005) Naming the Same Entities from Visual or from Auditory Stimulation Engages Similar Regions of Left Inferotemporal Cortices. Journal of Cognitive Neuroscience 17:1293-1305.

Tranel D, Kemmerer D, Adolphs R, Damasio H, Damasio AR (2003) Neural correlates of conceptual knowdelge for actions. Cognitive Neuropsychology 20:409-432.

Tunik E, Frey SH, Grafton ST (2005) Virtual lesions of the anterior intraparietal area disrupt goal-dependent on-line adjustments of grasp. Nat Neurosci 8:505-511.

Ungerleider LG, Haxby JV (1994) 'What' and 'where' in the human brain. Current Opinion in Neurobiology 4:157-165.

Urgesi C, Berlucchi G, Aglioti SM (2004) Magnetic stimulation of extrastriate body area impairs visual processing of nonfacial body parts. Curr Biol 14:2130-2134.

Urgesi C, Candidi M, Ionta S, Aglioti SM (2007) Representation of body identity and body actions in extrastriate body area and ventral premotor cortex. Nat Neurosci 10:30-31.

Valyear KF, Culham JC (2010) Observing learned object-specific functional grasps preferentially activates the ventral stream. J Cogn Neurosci 22:970-984.

Valyear KF, Cavina-Pratesi C, Stiglick AJ, Culham JC (2007) Does tool-related fMRI activity within the intraparietal sulcus reflect the plan to grasp? Neuroimage 36 Suppl 2:T94-T108.

van der Werf Y, Sanz-Arigita E, Menning S, van den Heuvel O (2010) Modulating spontaneous brain activity using repetitive transcranial magnetic stimulation. BMC Neuroscience 11:145.

Vandenberghe R, Price C, Wise R, Josephs O, Frackowiak RSJ (1996) Functional anatomy of a common semantic system for words and pictures. Nature 383:254-256.

Walsh V, Rushworth M (1999) A primer of magnetic stimulation as a tool for neuropsychology. Neuropsychologia 37:125-135.

Ward NS, Bestmann S, Hartwigsen G, Weiss MM, Christensen LOD, Frackowiak RSJ, Rothwell JC, Siebner HR (2010) Low-Frequency Transcranial Magnetic Stimulation over Left Dorsal Premotor Cortex Improves the Dynamic Control of Visuospatially Cued Actions. The Journal of Neuroscience 30:9216-9223.

Warrington EK, McCarthy R (1983) Category specific access dysphasia. Brain 106:859-878.

Warrington EK, Shallice T (1984) Category specific semantic impairments. Brain 107:829-853.

Warrington EK, McCarthy RA (1987) Categories of knowledge: further fractiontations and an attempted integration. Brain 110:1273-1296.

Wei T, Liang X, He Y, Zang Y, Han Z, Caramazza A, Bi Y (2012) Predicting conceptual processing capacity from spontaneous neuronal activity of the left middle temporal gyrus. J Neurosci 32:481-489.

Weibull W (1951) A statistical distribution function of wide applicability. Journal of Applied Mechanics, 18:292–297.

Weiner K, Grill-Spector K (2011) Neural representations of faces and limbs neighbor in human high-level visual cortex: evidence for a new organization principle. Psychological Research:1-24.

Weisberg J, van Turennout M, Martin A (2007) A Neural System for Learning about Object Function. Cerebral Cortex 17:513-521.

Whatmough C, Chertkow H, Murtha S, Hanratty K (2002) Dissociable brain regions process object meaning and object structure during picture naming. Neuropsychologia 40:174-186.

Whitney C, Kirk M, O'Sullivan J, Lambon Ralph MA, Jefferies E (2011) The neural organization of semantic control: TMS evidence for a distributed network in left inferior frontal and posterior middle temporal gyrus. Cereb Cortex 21:1066-1075.

Wichmann FA, Hill NJ (2001a) The psychometric function: II. Bootstrap-based confidence intervals and sampling. Perception & Psychophysics 63:1314-1329.

Wichmann FA, Hill NJ (2001b) The psychometric function: I. Fitting, sampling, and goodness of fit. Perception & Psychophysics 63:1293-1313.

Wiesel TN, Hubel DH (1963a) Efeects of visual deprivation on morphology and physiology of cells in the cats lateral geniculate body. Journal of Neurophysiology 26:978-993.

Wiesel TN, Hubel DH (1963b) Single-cell responses in striate cortex of kitten deprived of vision in one eye. Journal of Neurophysiology 26:1003-1017.

Willms JL, Shapiro KA, Peelen MV, Pajtas PE, Costa A, Moo LR, Caramazza A (2011) Language-invariant verb processing regions in Spanish-English bilinguals. Neuroimage 57:251-261.

Wise RJS, Scott SK, Blank SC, Mummery CJ, Murphy K, Warburton EA (2001) Separate neural subsystems within `Wernicke's area'. Brain 124:83-95.

Zeki S, Watson J, Lueck C, Friston K, Kennard C, Frackowiak R (1991) A direct demonstration of functional specialization in human visual cortex. The Journal of Neuroscience 11:641-649.