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A TMS-EEG study on the modulation of perceptual bias in the right Posterior Parietal Cortex

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The right posterior parietal cortex (rPPC) is involved in visuo-spatial processing, as neglect patients (Vallar, 1998) and TMS studies revealed (Fierro et al., 2000; Bjoertomt et al., 2002; Ellison et al., 2004; Fierro et al., 2006; Ricci et al., 2012). Within this framework, one of the most frequently used research tasks is the Landmark Task (LT, Milner et al., 1992, 1993), a line bisection judgments task whose neural correlates are well known (Fink et al., 2000, 2001; Çiçek et al., 2009). Remarkably, it affords to disentangle perceptual and response biases (Bisiach et al., 1998).

Given this background, by combining EEG and TMS, we want to investigate the behavioral (i.e. modulation of perceptual bias, PB) and neurophysiological (i.e. brain activity changes) effects of single pulse TMS over rPPC.

The experiment followed the subsequent steps: (i) hunting procedure (Salatino et al., 2014), delivering ten single pulses for each of the 9 points of a grid centrally located over P6, while the subject was performing the LT; (ii) administration of the LT while recording EEG with (TMS ON) and without (TMS OFF) stimulation of the parietal hotspot. In both conditions, symmetrically and asymmetrically bisected lines were used.

Participants were divided in three different groups depending on the modulation of the PB on the TMS ON condition as compared with the TMS OFF condition: the Neglect-like bias group (n=16, PB TMS ON> PB TMS OFF), the Pseudoneglect-like bias group (n=14, PB TMS ON< PB TMS OFF), and the No Bias group (n=14, PB TMS ON= PB TMS OFF). We also performed a spatio-temporal analysis on the difference between asymmetrical vs symmetrical lines for the TMS ON and the TMS OFF conditions, separately on each groups (Groppe et al., 2011a & 2011b). In an early stage of processing we have found a significant effects in O2 and P8 electrodes in the TMS OFF condition in two groups (Pseudoneglect and No Bias), that was still present, in the same sites, in the TMS ON condition only for the No Bias group. Later in time there was a significant interaction effect of the TMS condition on the type of stimuli for only the No Bias group in the electrodes of the left hemisphere. Finally, between 200 and 430 ms, in all the groups, the difference waves were significant in almost all electrodes.

The present data thus show that rPPC is involved in magnitude estimation of line length. Generally we could conclude that the TMS induces different type of modulation of PB. Indeed TMS could not modulate the PB in a group of participants (No bias), probably due to preexisting differences between participants, as our results in the early time window in the TMS OFF condition would suggest. One possibility is that the effects of the TMS are determined not only by the properties of the stimulus or by the TMS itself, but also by the state of the cortex during the task execution (Silvanto & Pascual-Leone, 2008). These results seem to suggest that our groups are different in the perceptual processing of the stimuli.

Recently we are testing differences between groups. These would help us to clarify if, at a neural level, the no bias group is significantly different from the neglect and pseudoneglect like bias group. We are also trying to better understand the pre-existing difference found in our participants.

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1. THE POSTERIOR PARIETAL CORTEX

1.1 Visuo-spatial function of the Posterior Parietal Cortex

One of the most crucial areas involved in visuo-spatial perception is the posterior parietal cortex (PPC), specifically an important role is played by the right one (Sack et al., 2010).

An important line of research that has contributed to acquire evidence in favor of this assumption derives from patients. Indeed, a lesion in this area can induce spatial neglect (Vallar, 1998), although damage to the inferior parietal lobule, superior temporal sulcus, ventrolateral frontal cortex, and subcortical nuclei may also cause this syndrome (Karnath & Rorden, 2012).

Spatial neglect (NSU) is often defined as the difficulty or inability to report the presence of stimuli or to carry out an action in the space contralateral to the lesion (Heilman & Valestein, 1979). Among its manifestations, neglect usually induces a distortion of space that can be measured by a great variety of visuo-spatial tasks. For example, a rightward bias can be observed in line bisection tasks, wherein neglect patients usually bisect the line by shifting the real center toward the right (Heilman & Valenstein, 1979; Bisiach et al., 1983). Another version of this task is the end point task in which the patient is given the center of a horizontal line previously seen and she/he has to point to both ends. Typically the patient tends to put the right endpoint leftward (Bisiach et al., 1994). More evidence of this abnormal representation of space can be inferred from size matching: neglect patients underestimate the size of an object placed in the contralesional side of space (i.e. the neglected space) compared to the right one (Milner et al., 1995, 1998).

Usually NSU is more frequent and severe after right brain damage (Stone et al., 1993); this has led to point for a right hemisphere dominance for spatial processing and attention (Corbetta & Shulman, 2002).

According to this, it is easy to understand the importance of the parietal lobe, in particular the right hemisphere, in space representation. However, this conclusion was determined by observing patients with brain damage, and therefore has to be taken carefully, as many confounding factors, like plasticity, can intervene after a lesion, thus making it difficult to draw inference about normal brain functioning.

In order to overcome this problem, an extensive use of transcranial magnetic stimulation (TMS) has been made. The TMS is a noninvasive technique that generates a changing magnetic field by means of a coil that induces electrical currents in the brain which modulates, for short period of time, neural activity. Given these characteristics, it has proved to be a useful tool for interfering with ongoing cognitive processes in order to study the involvement of specific areas. Even though this "virtual lesion" idea has been an attempt to prove the involvement of some cortical regions into cognitive behavior, this approach is inadequate to explain the wide range of effects induced by the stimulation. Actually the modulation of neural activity can be of two different types: facilitatory or disruptive, depending on the initial activation state of the cortical region. The first one is obtained when the TMS pulse is applied shortly before the beginning of the cognitive process, when all the neural population is at the same baseline level of activity, with no difference. Alternatively, the disruptive effect happens when the TMS pulse is delivered during the cognitive process. During a cognitive task, indeed, the neurons are at different activation states due to their different preferential tuning (Silvanto & Muggleton, 2008).

Due to these advantages, a few studies have shown that TMS applied to PPC can induce a perceptual bias in visuo-spatial tasks similar to those present in spatial neglect (Fierro et al., 2000; Bjoertomt et al., 2002; Ellison et al., 2004; Fierro et al., 2006; Valero- Cabré et al., 2006).

It is well known that also neurologically normal people systematically show misjudgements errors towards the left side of the true centre during line bisection or similar spatial tasks; this phenomenon is referred to as pseudoneglect by analogy to the performance of neglect patients (Jewell & McCourt, 2000). Also, these effects have been mostly reported for right cortical stimulation (Fierro et al., 2000).

One of the first studies carried out within this line of research is the one by Fierro and colleagues (2000). They applied repetitive TMS (rTMS) over the left and right PPC (P5 and P6 accordingly to the 10/20 EEG system) in order to disrupt, in healthy participants, high order cognitive processes during relative length judgment of pre-bisected lines. The stimulation protocol consisted in 10 stimuli delivered at the repetition frequency of 25 Hz for a stimulation time of 400 ms.
They showed the presence of a rightward bias in the visuo-spatial task when the TMS was applied over the parietal cortex; this effect was side specific for the right hemisphere, while left and sham stimulation failed to induce any behavioural changes (fig.1). The main contribution of this study consists of the finding of a side–specific contralateral visuospatial deficit induced in healthy participants only after right parietal cortex stimulation.

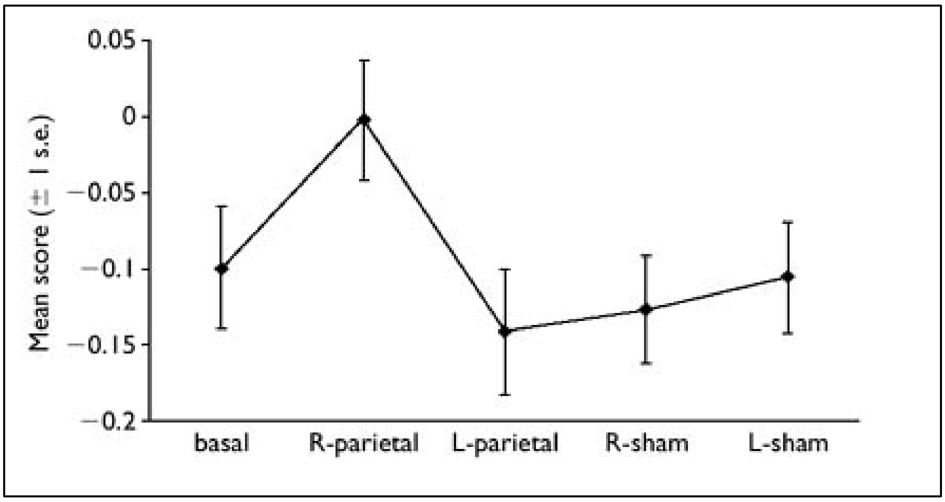


Fig.1 Mean subjects' score according to stimulus type. Positive score represents a rightward bias; negative score stands for a leftward bias (from Fierro et al., 2000).

Another useful advantage introduces by transcranial magnetic stimulation is the possibility to investigate when a brain area is making its critical contribution to behavior.

Given this opportunity by means of this technique and the findings of the previous research that have proved the involvement of the right parietal cortex in high visuo-spatial cognitive task, the same group of authors decided to explore the timing of frontal and parietal areas in visuo-spatial attention and whether their contribution to process information is the same or not (2001).

In order to address this issue, single-pulse TMS was delivered at three different time intervals (150 ms, 225 ms and 300 ms) to the right parietal and frontal areas.

More precisely, they tested healthy participants on a computerized visuo-spatial task, while performing the TMS at various time intervals over two different scalp regions: right posterior parietal cortex (P6, intraparietal sulcus) and frontal premotor cortex (F4, second frontal gyrus).

They showed that single-pulse TMS over the right parietal cortex could interfere with visuo-spatial processing when applied 150 ms after visual stimulus onset (fig.2). This interval corresponds to the time relative to the transfer of visual information from occipital to parietal cortex.

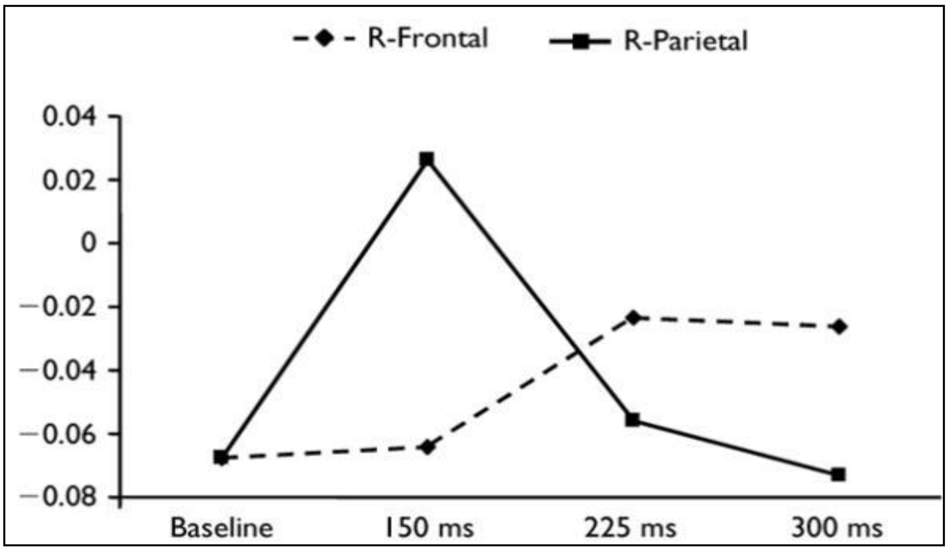


Fig.2 Mean subjects' score in baseline condition and during frontal (dotted line) and parietal (continuous line) TMS stimulation at different time intervals (from Fierro et al., 2001).

An additional study that can be placed in the same line of research of Fierro and colleagues (2000) is the one of Brighina et al. (2002). Their work can be viewed as a prosecution of the previous experiment, as their aim was to establish the contribution of frontal lobe in neglect syndrome and to differentiate the visuo-spatial deficits induced by the TMS over the frontal and parietal cortices. Again with an rTMS protocol and a line bisection judgment task, they tested a group of healthy participants. The stimulation was applied over the right frontal (F4) and parietal (P6) areas.

The results proved that the disruption of the frontal lobe could lead to visuo-spatial deficits; but the main finding concerned the induction of perceptual deficits for both frontal and parietal stimulation. They also confirmed that the parietal cortex was engaged in visuo-spatial processes around 150 ms after visual stimulus onset.

Further evidence about the role of rPPC in visuo-spatial processing could be derived from the study of Bjoertomt and colleagues (2002). The authors highlighted how the phenomenon of pseudoneglect is sensitive to viewing distance, precisely more prominent for peripersonal space than for extrapersonal space. This dissociation was also found for patients who showed neglect mostly for near-space but not for far-space. Thus, the authors suggested the idea that the PPC could be implied in near-space processing, instead inferior temporal cortex could be involved in far-space representation.

So, in order to investigate distance difference in visuospatial perception, they first tried to replicate the gradient of pseudoneglect in near versus far-space in healthy participants and then applied rTMS protocol over rPPC to see if it could be decreased. The subjects were tested for both the experiments with a line bisection judgment task.

The results showed a dissociation between visuo-spatial perception in near and far space for healthy participants (fig.3).

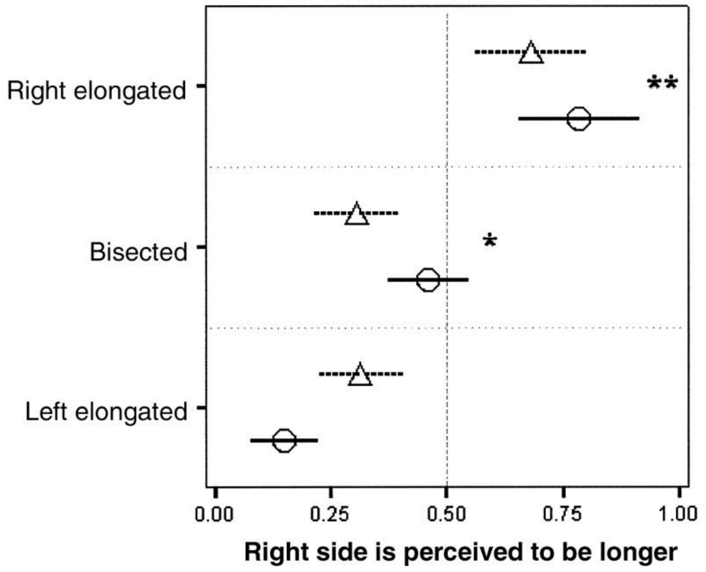


Fig.3 Different gradient of pseudoneglect depending on viewing distance (proportion of ‘right segment as longer’ response). Triangles for near space, circles for far space. For equally bisected lines the participants reported less than 50% right segment as the longer in near space; this could be interpreted as a decrease of pseudoneglect for this viewing distance (from Bjoertomt et al., 2002).

Also, when stimulating the right parietal area, the authors reported a less pronounced pseudoneglect as compared to baseline (fig.4).

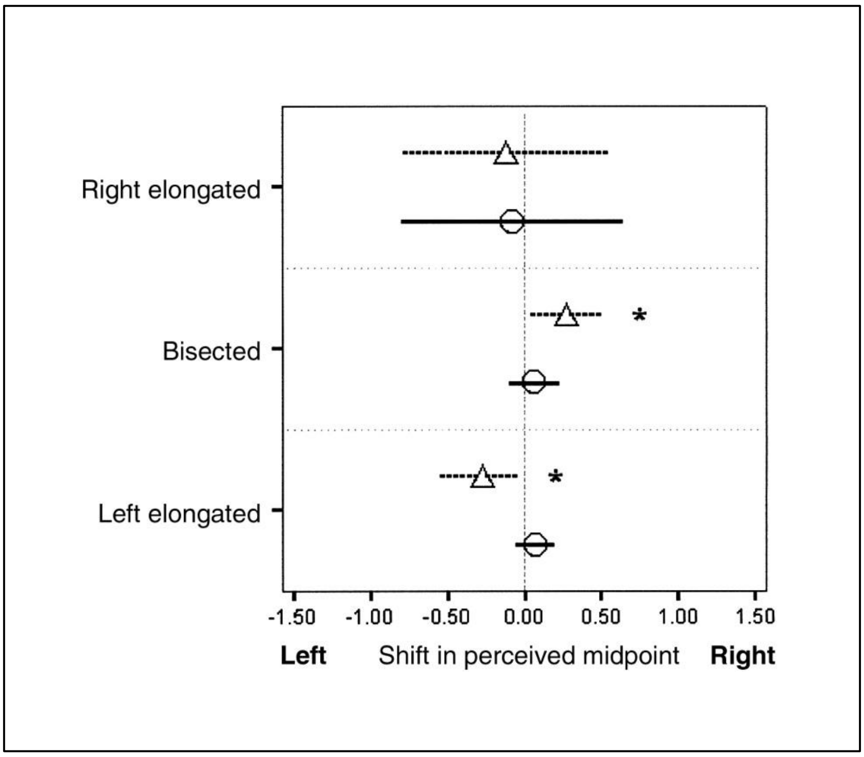


Fig.4 Effects of rTMS over the rPPC on subjects perception in near and far space. Triangles for near space, circles for far space. There is a shift in perception of equally bisected lines and left elongated lines in opposite direction in near space distance view (from Bjoertomt et al., 2002).

Ellison and colleagues (2004) have approached the question of the involvement of the parietal cortex in high visuo-spatial processing from a different point of view.

In literature, the regions commonly associated with NSU are the inferior right posterior parietal lobe and the temporo-parietal junction (TPJ). However other authors have suggested the involvement of the right superior temporal gyrus (STG).

In order to test the possibility that more than one area is related with different neglect manifestation, Ellison et al. made use of TMS to get a hint on this research question. Two traditional neglect paradigms were used: a line bisection judgment task and visual search task, while the healthy subjects were stimulated on the right PPC and on the right STG.

From the comparison between the stimulation of these two areas the authors discovered a double dissociation between the brain site and the task: previous findings of neglect like symptoms on the line bisection judgment task when the TMS was applied over the rPPC were replicated, however no such effects were found over the right STG (fig.5). Instead, when difficult exploratory search through target was required, the right STG, and not the right PPC, was involved.

So the authors have concluded that the area involved in neglect-like symptoms is highly dependent on the task used.

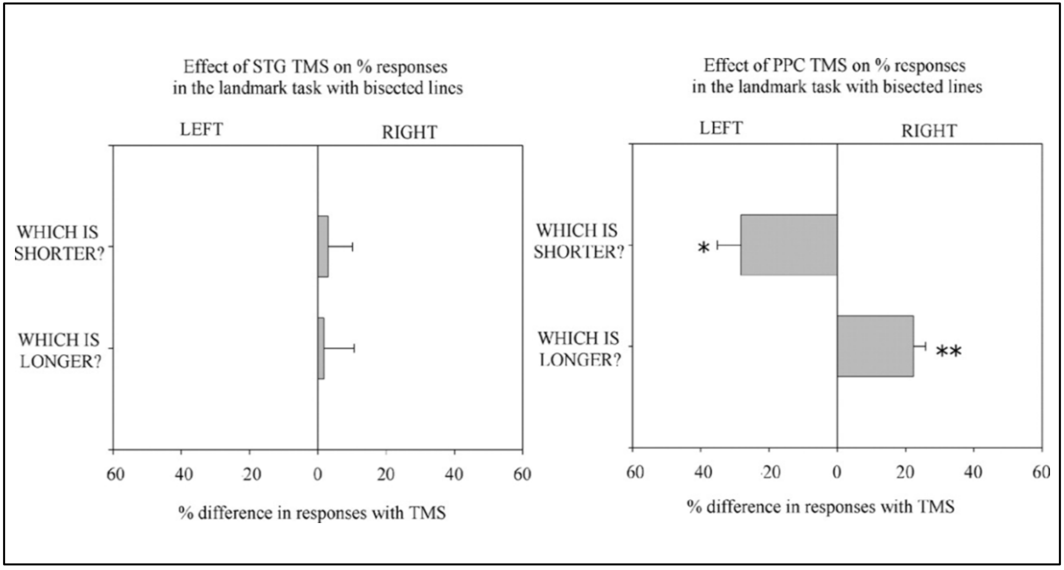


Fig.5 Effects of TMS over the rPPC and rSTG in line bisection judgment task. Only when the TMS was applied over the PPC, subjects showed neglect-like symptoms (from Ellison et al., 2004).

Consistent with these findings is the study of Olivieri and Vallar (2009). They used 25 Hz frequency rTMS stimulation protocol to assess the contribution in a visuo-spatial judgment task of three brain spots: the first was a posterior parietal site corresponding to the angular gyrus (ANG), the second posterior parietal site was located in the supramarginal gyrus (SMG) and the last one was the superior temporal gyrus (STG).

Their results were in line with the one of Ellison and colleagues (2004): the interference of the rTMS with the right posterior-inferior parietal region, SMG, led to a rightward deviation that can be considered as a reduction of the leftward pseudoneglect shown by the participants in the baseline condition (fig.6).

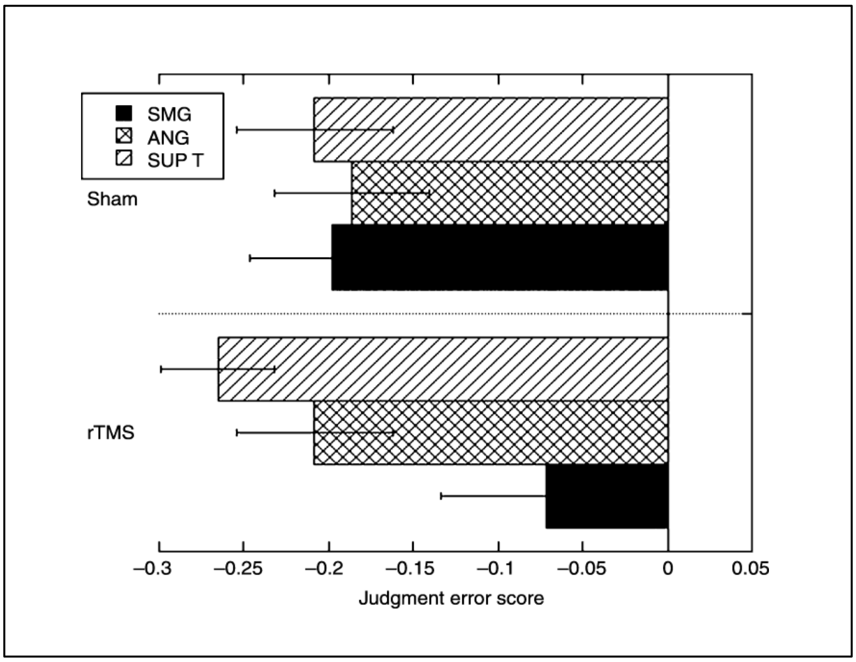


Fig.6 Effects of rTMS and sham condition over SMG (supramarginal gyrus), ANG (angular gyrus) and STG (superior temporal gyrus). rTMS on SMG was able to significantly reduce the leftward bias, however there was no such effect over the ANG and STG as compared to sham rTMS (from Olivieri &Vallar, 2009).

A more recent study of Ricci and colleagues (2012) has contributed to add evidence to the role played by rPPC in visuo-spatial perception. They used interleaved TMS/fMRI technique in three healthy volunteers to investigate the role of the parietal cortex in a line perception judgment task.

Using a single-pulse TMS protocol, they stimulated, in the experimental condition, the right parietal spot (rPPC) and in the control condition, the vertex.

At a behavioral level, participants were slower during the vertex condition, independently of TMS stimulation and tended to choose preferentially the left segment (i.e. response bias). For what concerns rPPC, subjects showed a tendency to underestimate the left segment (i.e. neglect like bias) during TMS as compared to a baseline. TMS was also able to reduce the activity of the right fronto-parietal network in two participants at a single-case level.

All these studies have successfully used different TMS protocols to induce perceptual biases in visuo-spatial tasks similar to those present in spatial neglect. Also, these effects have been mostly reported for right cortical stimulation (Fierro et al., 2000). Within this framework, one of the most frequently used research paradigms in this line of research is the Landmark task, a line bisection judgment task.

1.2 Landmark task

A long debated question in literature on neglect syndrome concerns the nature of the bisection error found in patients.

It has been proposed by Heilman and Valestein (1979) that the symptoms shown in NSU can be explained due to a spatially misdirected response; this phenomenon was called “direction hypokinesia” and attributed to an underactivation of right hemisphere premotor system. This “action bias” (Harvey et al., 1995b) lead the patients to be less prone to initiate and carry out movements towards the contralesional egocentric hemispace, independently of the limbs involved (Bisiach et al., 1990).

However, another line of thought has advanced an alternative explanation about the rightward deviation in neglect: patients could misperceive the left side of the horizontal lines as being shorter. This view tends to place the error on a perceptual level (Harvey et al., 1995b; 1995b; Milner & Harvey, 1995; Milner et al., 1992; 1993).

This perceptual/premotor dichotomy of unilateral neglect has received support in different studies; also these impairments should not be though as mutually exclusive and can coexist in the same patients. This dissociation is hypothetically useful to separate neglect phenomena that are perceptual from those which are action-related and to study how these two factors can be presented in different degree in every single patient (Harvey et al., 1995a).

In order to differentiate between perceptual and premotor deficits, Bisiach and colleagues (1990) tested neglect patients with a standard bisection task and a non-standard one, in which they had to move indirectly the pointer to sign the midpoint. If directional hypokinesia was present, a leftward displacement of the subjective midpoint was predicted in the non-standard bisection task as compared to the standard one. The results confirmed their predictions, although they also found that “perceptual” and “premotor” error coexisted to a certain degree in the majority of their patients.

As regard to the perceptual type of errors, i.e. how the patients tend to bisect to the right of the true midpoint, different explanations were advanced: first Halligan and Marshall (1991) proposed a subjective distortion of space in neglect which is pushed to the right and uniformly contracted, but keeping Euclidean properties. This model successively was generalized to take into account line bisections behavior shown by neglect patients and to predict the error as a function of line location.

An alternative interpretation was proposed by Milner and colleagues (1993) who suggested a distortion of the subjective space on non-Euclidian parameters: this would progressively more compress leftward. Indeed, this misrepresentation could be found in different manifestations of neglect patients who present a distortion of space along the horizontal dimension: to be judged as having the same size, the rightward object needs to be larger/longer than the leftward object. For example, other than a rightward bias observed in line bisection tasks, wherein neglect patients usually bisect the line by shifting the real center toward the right (Heilman & Valenstein, 1979; Bisiach et al., 1983), when asked to point both ends (end point task) of a horizontal line previously seen, typically they tend to put the right endpoint leftward (Bisiach et al., 1994). More evidence of this abnormal representation of space can be inferred from size matching: neglect patients underestimate the size of an object placed in the contralesional side of space (i.e. the neglected space) compared to the right one (Milner et al., 1995, 1998; Milner and Harvey, 1995).

Given the importance to better understand the difficulties of these patients, a simple paradigm that allows testing Milner’s hypothesis and distinguishing between the “perceptual” and the “premotor” factors is the Landmark task (Milner et al., 1992, 1993; Bisiach et al., 1998).

Landmark task is a line bisection judgment task wherein the subject is asked to decide which segment of a pre-bisected line, the right or the left one, is the shortest/longest; the relative length of the two segments varied across the trials. Two versions are available, one with a verbal response and the other with a manual response (Milner et al., 1992, 1993; Bisiach et al., 1998). In the first type (LANDMARK-V), one segment of each line is colored in red, while the other is presented in black; subjects have to name the color of the part chosen. In the LANDMARK-M both segments are black and divided by a small vertical bar (the landmark); the response here is made by pointing at the segment (fig.7).

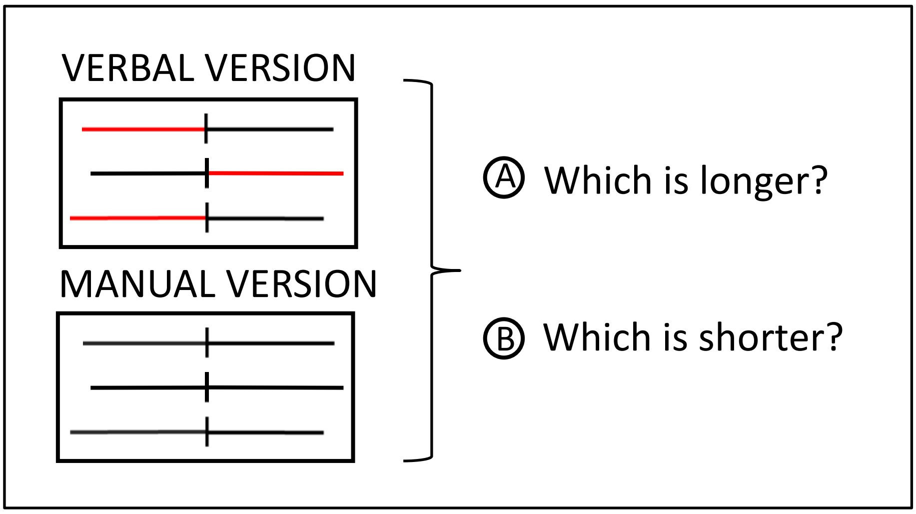


Fig.7 Verbal (LANDMARK-V) and manual (LANDMARK-M) version of the Landmark task.

When the lines are correctly transected, accordingly to the uniform-compression theory and to the directional hypokinesia, the patients should see the subjective midpoint in the same position as the landmark. If, instead, the hypothesis advance by Milner and colleagues (1992; 1993) holds true, neglect patients would perceive the left half of the line as shorter. Also this distortion should change as a function of line location: it would be more pronounced in the neglected hemispace.

Furthermore, the Landmark task is useful for predicting what kinds of response are linked with a more prominent perceptual or premotor nature of errors: patients

with perceptual neglect would made leftward response, while those with premotor neglect rightward response.
A lot of studies have confirmed the guessing made by Milner et al. (1992; 1993) hypothesis: the Landmark task has proved to be able to classify neglect patients and to show the distortion of space, perceiving the left side of line as shorter (Milner at al., 1992; Harvey et al., 1995a; 1995b). In addition to this, it was found a gradient of distortion becoming more important in left hemispace (Milner et al., 1993).

1.2.1 Perceptual and response biases

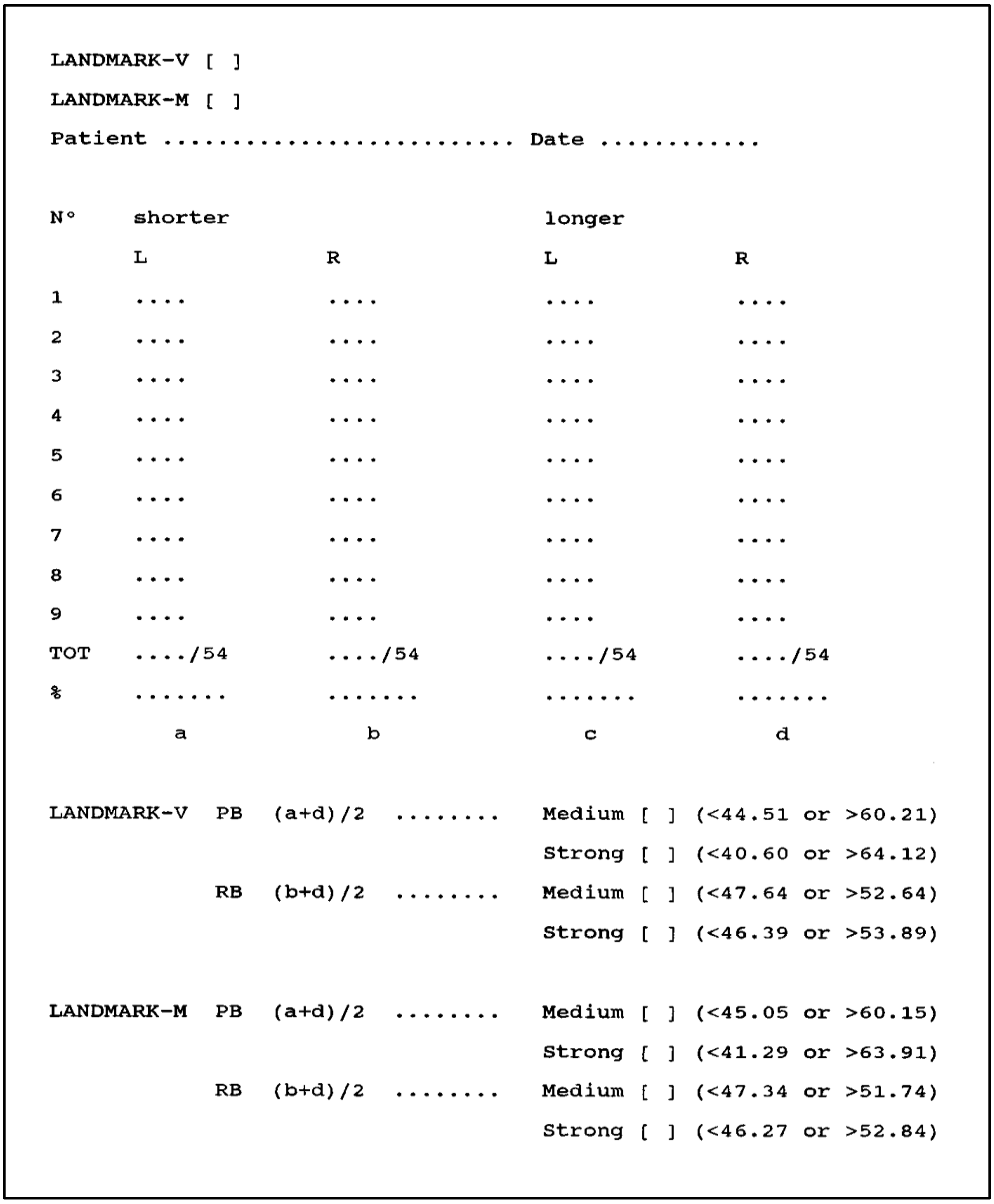
The Landmark task has become a suitable tool for assessment of neglect patients in order to analyze the disorders underlining this syndrome. This task is not only used in the assessment of patients, but also with healthy subjects due to its ability to explore space representation.
The dichotomy of perceptual and premotor difficulties shown by this task should not be thought only as a pathological impairment, rather as a distinction between input and output related factors present also in healthy people.
Indeed, the Landmark task has proved to be useful for research purposes, because it can disentangle perceptual bias (PB) from response bias (RB). These factors have often led to a problematic evaluation of the size of space perception distortion in neglect patients and healthy subjects (Bisiach et al., 1998; Capitani et al., 2000; Toraldo et al., 2014).

Perceptual bias can be defined as the consistent bias in the two opposite tasks of the Landmark that is the constant error across conditions. The response bias, instead, is the tendency to report the segment from the same side independently of the task request, i.e. the degree of response consistency between conditions.
With left neglect condition, PB should be recognizable from a misjudgment of left segment as ‘shorter’ and of right one as ‘longer’; RB, instead, would lead to a predominance of right segmented responses independently of the task request.

These two biases have been also operationalized into indices: the sum of ‘left shorter’ responses and the ‘right longer’ response gives the PB index, the sum of ‘right shorter’ response and the ‘right longer’ response the RB index. In neglect patients usually high value of PB and RB would be found; the converse would be the case of right neglect (Bisiach et al., 1998; Capitani et al., 2000; Toraldo et al., 2014).

In a work by Bisiach and colleagues (1998), the two versions of the Milner Landmark task (LANDMARK-V and LANDMARK-M) underwent through testing with neglect patients in order to: combine the ‘shorter’ and ‘longer’ instructions in the same task, classify PB and RB and understand how they related to each other. The results obtained from patients were confronted with data of healthy control subjects.

The authors have made use of the PB and RB indices in order to tear apart perceptual and premotor nature shown by the patients and to classify them based on the prevalence of the origin of their errors. In figure 8 it is shown the scoring of the computation of PB and RB on either task: the columns ‘a’ and ‘b’ correspond to the trials wherein participants were asked to judge which segment was the shorter, ‘c’ and ‘d’ which was the longer; furthermore columns ‘a’ and ‘c’ stand for ‘left’ response, ‘b’ and ‘d’ for ‘right’ response.



LANDMARK-V [ ]

LANDMARK-M [ ]

Patient .... Date ....

N°	shorter		longer	
	L	R	L	R
1	....	....	....	....
2	....	....	....	....
3	....	....	....	....
4	....	....	....	....
5	....	....	....	....
6	....	....	....	....
7	....	....	....	....
8	....	....	....	....
9	....	....	....	....
TOT	..../54	..../54	..../54	..../54
%	....	....	....	....
	a	b	c	d

LANDMARK-V	PB	(a+d)/2	....	Medium [ ]	(<44.51 or >60.21)
				Strong [ ]	(<40.60 or >64.12)
	RB	(b+d)/2	....	Medium [ ]	(<47.64 or >52.64)
				Strong [ ]	(<46.39 or >53.89)
LANDMARK-M	PB	(a+d)/2	....	Medium [ ]	(<45.05 or >60.15)
				Strong [ ]	(<41.29 or >63.91)
	RB	(b+d)/2	....	Medium [ ]	(<47.34 or >51.74)
				Strong [ ]	(<46.27 or >52.84)

Fig.8 Scoring and computation of PB and RB indices on LANDMARK-V and LANDMARK-M tasks (from Bisiach et al., 1998).

As shown in figure 8, for what concerns PB, high value of (a + d)/2 would have meant a misjudgment of left segment in left neglect patients; compatibly high value of RB would have signified the choice of right segments in either condition (shorter and longer tasks), calculated from (b + d)/2.

The results of the two Landmark tasks, the manual and verbal version, have demonstrated no general difference in sensitivity between the two of them.

Furthermore, the majority of the patients have behaved accordingly to the hypnotized direction.

The authors have also found a dissociation regarding the sensitivity of the two tests: the LANDMARK-V seemed to be more prone to detect PB than the LANDMARK-M and vice versa.

The perceptual bias encountered in those patients has been framed into the anisometry space representation theory (Milner et al., 1993; Bisiach et al., 1994, 1996): on the horizontal dimension the percepts appear shorter on the contralesional side of egocentric space due to distortion of space representation along the horizontal dimension.

Another important aspect emerged from this experiment concerns the possible range of PB and RB scores: there is a limited spectrum of combinations of PB and RB (fig.9). Their values can range above or below 50, number that means no bias, with the highest amount of 100.

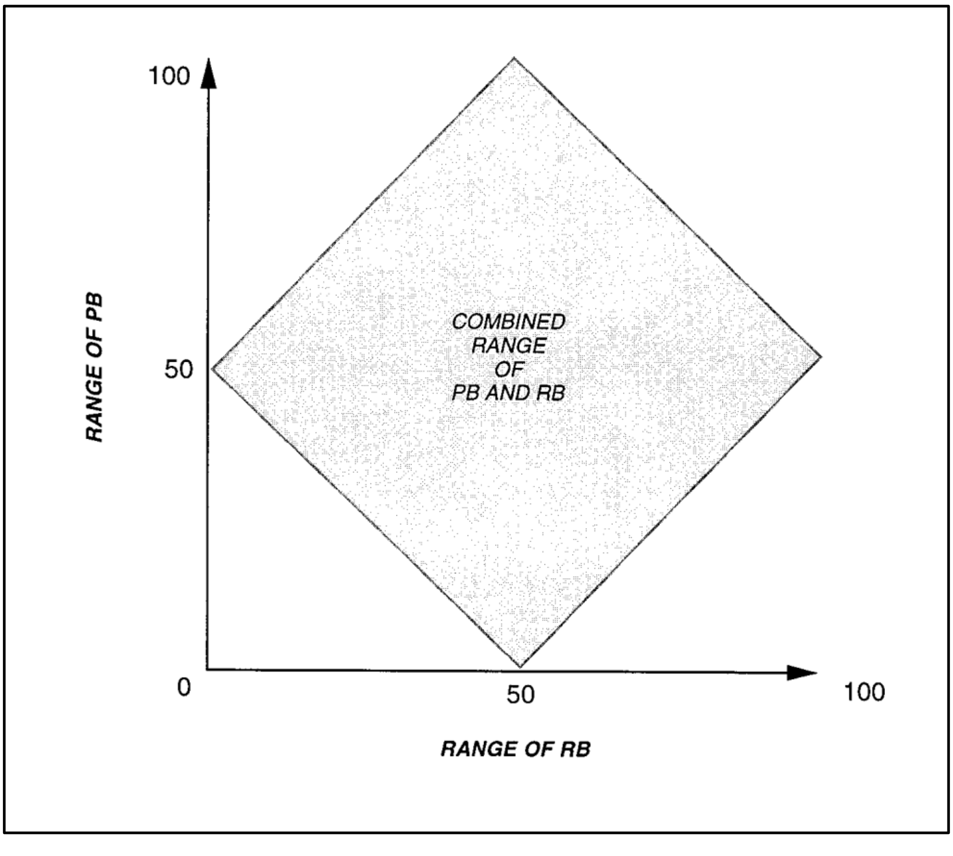


Fig.9 Possible range of PB and RB (from Bisiach et al., 1998).

In conclusion, the Landmark task has proved to be particularly useful for research purposes because it gives the possibility to disentangle between perceptual (underestimation of the contralesional side) and response biases (the tendency to report preferentially the ipsilesional segment), factors that often led to a problematic evaluation of the size of space perception distortion in neglect patients (Bisiach et al., 1998; Toraldo et al., 2014).

1.2.2 Neural correlates of the Landmark task

The Landmark task is so widely used not only for its ability to disentangle between perceptual and response biases or for the assessment of visuo-spatial neglect, but also because its neural correlates are well known.

One of the first attempts to elucidate the neural mechanisms underlying the Landmark task has been made by Fink and colleagues (2000; 2001), by means of functional magnetic resonance (fMRI). They asked healthy subjects to judge whether a vertical bar was placed correctly to the veridical center of horizontal and vertical lines. This task was compared to a visual detection control task.
Their results were consistent with lesion studies: the Landmark task increased the activity in the superior and inferior parietal lobes bilaterally, but prevalently in the right hemisphere and in the prefrontal cortex. The authors suggested that the inferior parietal cortex was involved in visuo-spatial judgments and this activation was strictly related to the Landmark task (fig.10).

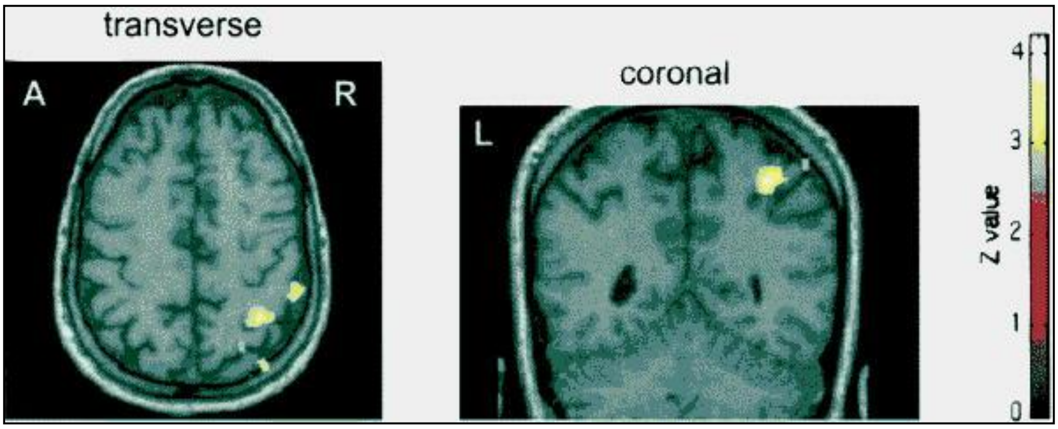


Fig.10 Increased activity in right intraparietal sulcus and right parietal convexity during the Landmark task (from Fink et al., 2000).

The same group later investigated the cognitive strategy underlying Landmark task (2002). Two possible strategies were hypothesized: the stimulus could be analyzed as composed by two different objects, i.e. segments, so the participants would compare the length of them in order to solve the task ("line length comparison task") or it could be perceived as one single object, that means that they would have judge if the transection mark was place in the center ("line center judgment task"). If two distinct strategies could be adopted, this would also implicate differential neural correlates.

It was found that both approaches increased the activity in inferior parietal lobes bilaterally and right temporo-occipital cortex. Line center judgments activated differentially left posterior parietal cortex (and also a tendency non-statistically significant in homologous right area), while length comparison strategy lingual gyrus bilaterally and anterior cingulate cortex (fig.11).

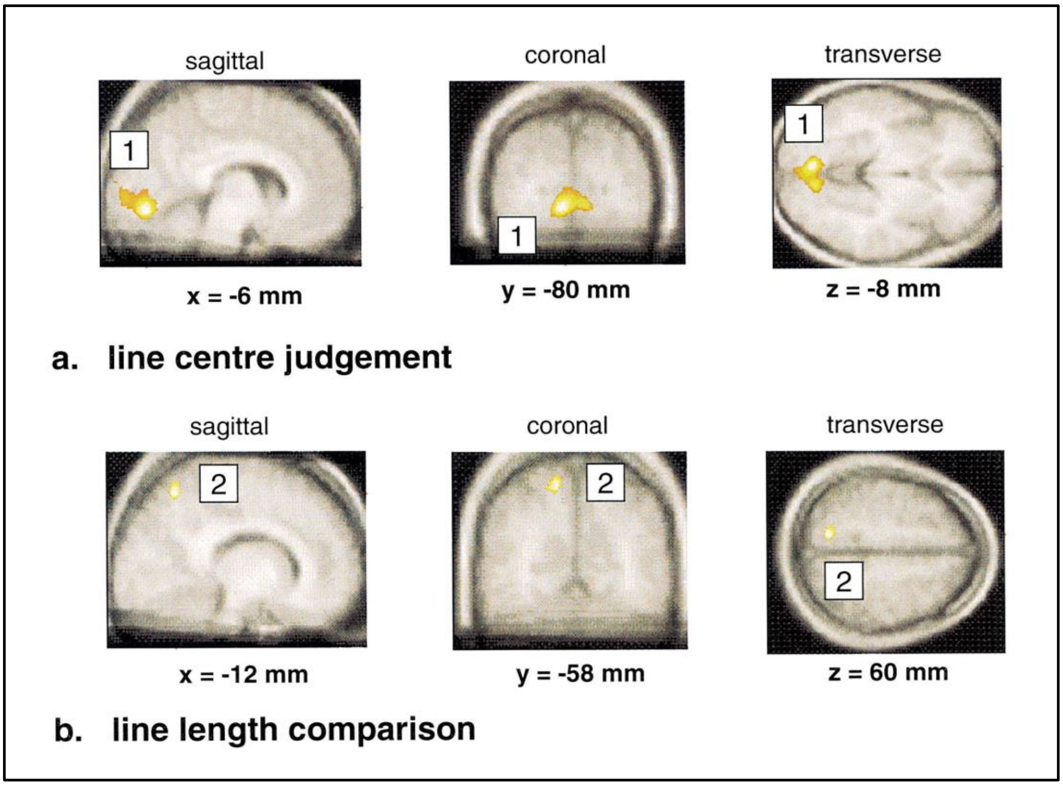


Fig.11 Increased activity in “line centre judgement” and in “line length comparison” tasks. In the first task (a) the authors have identified the activation of lingual gyrus bilaterally and in the second one (b) oh left superior posterior parietal cortex (from Fink et al., 2002).

Other authors have arrived to the same conclusions of Fink et al. (2001; 2002); Çiçek and colleagues (2009) used fMRI to assess brain activity related to line length perception judgments.

Their results supported the engagement of the intraparietal sulcus (IPS) and the lateral peristriate cortex (fig12), but failed to find activation in prefrontal cortex during the Landmark task like previously reported by Fink et al. (2000; 2001; 2002).

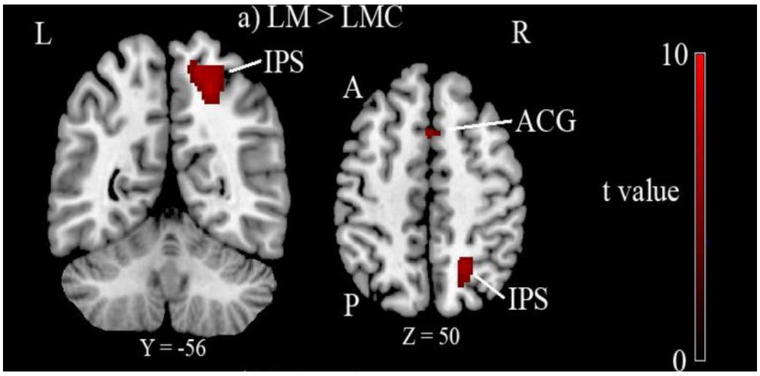


Fig.12 Landmark task activated IPS, in the right hemisphere, anterior cingulate gyrus and lateral peristriate cortex (from Çiçek et al., 2009).

Vossel and colleagues (2010) questioned the relationship between motor/intentional and perceptual deficits distinguished by means of Landmark task, while performing a lesion mapping study. They used the manual version (LANDMARK-M) to test if perceptual bias would be related to parietal lesions, while response/motor bias to frontal, subcortical and parietal damages. Sixty-eight right hemispheric stroke patients underwent neglect assessment, comprehensive of manual version of the Landmark task.

Perceptual bias correlated with inferior parietal, middle occipital and frontal brain damages, whether response bias to caudate nucleus. In the end they failed to find a relation between frontal lesion and response bias, while the involvement of inferior-parietal and parieto-occipital cortex was in accordance with the previous study.

A possible explanation for these different findings for the RB was advanced by the authors themselves: the malfunctioning of distant cortical brain areas (i.e. diaschisis) would have led to an interruption of the subcortical-frontal circuit, rather than a local effect of these only subcortical structures.

In summary, all the studies have successfully found an activation of parietal cortex, mainly in the right hemisphere. Notably, the inferior parietal lobe and the intraparietal sulcus seem to be strictly related with the Landmark task (Fink et al., 2000; 2001; 2002; Çiçek et al., 2009; Vossel et al., 2010), while there is no accordance relatively to the involvement of prefrontal cortex (Vossel et al., 2010).

In conclusion, the combined use of TMS and the landmark task has been able to shed some light on the role of rPPC in the space representation in the healthy brain and to allow a comparison between perceptual bias in normal subjects and neglect patients.

1.3 Hunting procedure

In almost all the studies that have investigated the role of the PPC in visuo-spatial perception, the site to be stimulated with TMS was either chosen with MRI based stereotaxy or by the 10-20 EEG system.

These methods present both strengths and weaknesses; for what concerns the MRI scans, even if they would lead to a great anatomical accuracy, it is not always possible to have them, even more if the subjects are healthy participants.

Instead the 10-20 EEG system, although is a cheap and rather rapid method, it does not take into account that the neural network subdued to visuo-spatial perception is rather wide and differs between individuals, i.e. each subject could present a different site in the PPC that, if stimulated, would produce the greatest impact on visuo-spatial perception.

Over the course of time it has become clear that in the field of research about the contribution of PPC in visuo-spatial perception a new approach to localize the parietal hotspot was necessary.

The first attempt to introduce a new technique was made by Ashbridge and colleagues (1997). In a context of a study on the temporal aspect of a visual search task, the authors mapped out the region of the scalp at which TMS was able to induce the biggest effect at a behavioral level and give an estimation of the stimulation time.

The idea of the motor ‘hotspot’ as a location landmark was transposed in the visual cortex; i.e. instead of using electrode coordinates, they used a “win-stay/lose-shift” paradigm to select the parietal site. The stimulation was applied over a grid (a square of 3x3 cm) of nine points centered over the P4 electrode: the procedure was repeated until a deficit, in terms of slower reaction times (RT), was obtained.

This procedure was resumed by Oliver et al. (2009): their aim was to localize the target of stimulation functionally rather than anatomically and to propose a new TMS 'hunting procedure'. In order to overcome the problem that space representation is supported by a widely distributed network and to obtain a greater impact on visuo-spatial perception processing, they tried to conceive a new form of the "hunting procedure" initially proposed by Ashbridge and colleagues (1997). They also put to test the validity and the reproducibility of this method in the identification of the site in the right intraparietal sulcus (IPS).

The authors assessed the effect of TMS over a number of different sites while the subjects were performing a visuo-spatial task. The hotspot was chosen in terms of behavioral impact, i.e. the site that produced the biggest change as compared to a baseline level.

Short train of TMS (10 Hz for 0.5 sec) were delivered over a 9-point grid centered over P4 following a spiral-shaped path, while the participants had to detect a small gap in the far left or right of a horizontal line.

In their first experiment, using a "miss-stay/hit-shift" protocol and moving of 0.5 cm away from P4, they localized the parietal spot long the anterior intraparietal sulcus, posterior to its junction with the sensory cortex.

The stimulation successfully impaired the sensitivity for the gap when delivered over the parietal hotspot. The spatial specificity of the hunting procedure was also confirmed by finding the same impairment moving the center of the grid over the putative site.

Recently Salatino and colleagues (2014) proposed a new hunting procedure to identify the PPC site that best modulated the performance on a Landmark task. Single-pulse TMS was delivered 150 ms after the stimulus onset on the right and left parietal cortex.

Using the same 3x3 cm grid of Ashbridge et al. (1997) and Oliver et al. (2009) centered over P3 and P4, the authors delivered 10 pulses of TMS for each of the 9 points (named S1 to S9) at an intensity of 115% of the resting motor threshold (rMT) while participants has to report the shortest segment of a prebisected horizontal line.

In line with the previous studies on the modulation of visuo-spatial perception in the PPC (Fierro et al., 2000; 2001; Ricci et al., 2012), a neglect-like perceptual bias was detected when the TMS was applied over right PPC. This effect was found on two sites located posterior and dorsoposterior to P4 (fig.13).

Neuroimaging results revealed that the brain structure underlying the site where TMS was able to induce rightward biases corresponded to right angular gyrus (AG).

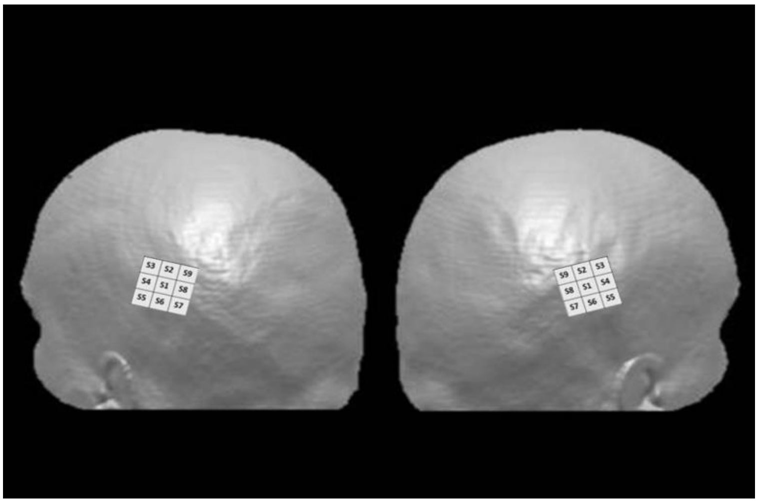


Fig.13 Position of the hunting grid for TMS stimulation over left and right parietal cortices. The grid was centered over P3 in the left hemisphere and P4 in the right one (from Salatino et al., 2014).

In summary, the hunting procedure seems to offer in healthy subjects an economical and rather easy approach to find the optimal parietal spot, that, if stimulated by TMS, would induce the highest behavioral change as compared to a baseline level (i.e. without stimulation). Another advantage offered by this protocol is the possibility to investigate different contributions to specific visuo-spatial functions of distinct portions of the parietal cortex.

2 RATIONALE

Given this background, the general aim of this study is to investigate the contribution of rPPC in visuo-spatial representation in the healthy brain, in terms of activation and timing of engagement, by observing the modification of perceptual bias induce by TMS stimulation in a Landmark task.

By combining EEG and TMS, the purpose of my PhD project is to study the behavioral (i.e. modulation of perceptual bias) and neurophysiological (i.e. brain activity changes) effects of single-pulse TMS over rPPC. To our knowledge, no studies have ever used combined TMS-EEG in order to investigate the contribution of the rPPC to visuo-spatial processing.

Also by using this combined methodology, we aim to shed some light on the effects of TMS in neural activity modulation: whether TMS increases neural noise in the stimulated area (Ruzzoli et al., 2010), has a general effect of suppression (Harris et al., 2008) or its behavioral effects depend on the state of the stimulated neurons (Silvanto & Pascual-Leone, 2008).

We decided to made use of both TMS and EEG for two orders of reason:

- To overcome the limitations of TMS and to better understand its effects on neural activity. Sometimes it is ambiguous how the stimulation during the same task can results both in a facilitatory or inhibitory effect at a behavioral level. Also, it is possible that the result is not entirely due to the stimulated area, but to the activation of a cerebral network. Another important issue is the intensity of the stimulation; it could be possible that no effect is found because the stimulation was not strong enough to affect a cerebral cortex (Miniussi & Thut, 2010).
- EEG has a high temporal resolution, but it cannot add any casual relation information (only correlation between brain activities can be inferred).

Combining TMS and EEG can therefore help to overcome these limitations: merging the correlation analysis and time window information provided by EEG with the casual relation between brain areas and behavioral outcome inferred by TMS stimulation can help us to better clarify the contribution of the right PPC to visuo-spatial perception.

3 MATERIALS AND METHODS

3.1 Subjects

Forty healthy subjects, 23 females and 17 males, mean age 22,95 (DS = 3,12), with normal or corrected to normal vision and no history of neurological or psychiatric illness have been recruited and asked to sign an informed consent form before taking part to the study. All the participants have been screened against the criteria of a safety use of TMS (Rossi et al., 2009) and for the right hand dominance with Edinburgh Handedness Inventory (Oldfield 1971). Furthermore all the subjects were naïve to the aim of the experiment and to the Landmark task.
All experimental procedures were approved by the local ethical committees and performed in accordance with the declaration of Helsinki (2013).

3.2 Visual stimuli

Visual stimuli (fig.14) consisted of white 0.09° of visual angle thick and 20.26° of visual angle long horizontal line, presented on a 17’’ LCD monitor (LG L1753HM). Three types of lines have been presented:

1. Symmetrically bisected by a 0.09° thick and 0.095° high vertical bar;
2. Asymmetrically bisected lines to the right (right segment 9.47°, left segment 10.79°), i.e. left elongated;
3. Asymmetrically bisected lines to the left (right segment 10.79°, left segment 9.47°), i.e. right elongated.

The stimuli were generated with E-Prime software (Psychology Software Tools, Pittsburgh, PA).

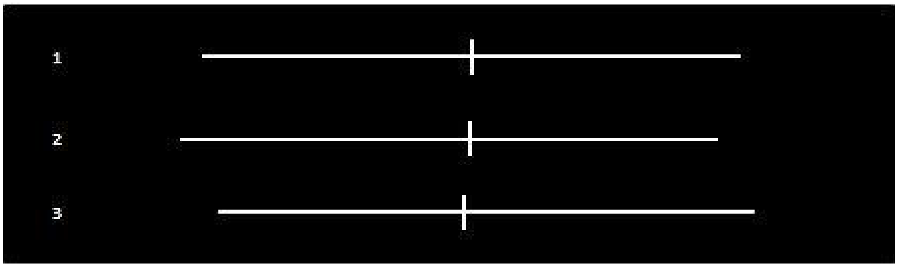


Fig.14 Visual stimuli used in the study: symmetrically bisected (1), asymmetrically bisected to the right (2, left elongated), asymmetrically bisected to the left (3, right elongated).

3.3 Experimental procedure

Figure 15 illustrates the three main sessions of the experimental design. Before starting the study, all subjects were asked to bisect five 20 cm long and 1.5 mm thick black horizontal lines presented on five separate A4 white sheets aligned to the sagittal midline of their trunk. We decided to test them with a paper-and-pencil line bisection task in order to rule out any possible perceptual problems they might have.

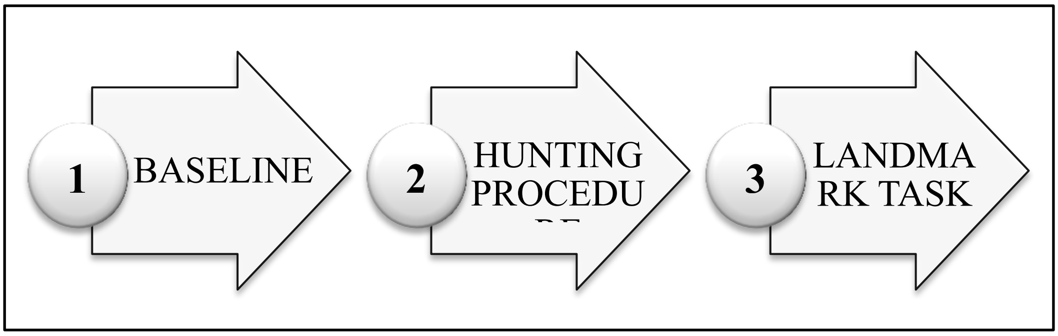


Fig.15 Main session of the experimental design: (1) we assessed the baseline PB that is the bias shown by the participants without the TMS, (2) then performed an hunting procedure in order to find the best parietal spot to be stimulated in the experiment for each subject and finally (3) administered the computerized version of the Landmark task, while recording the EEG.

First participants underwent a screening session, to get them familiar with the instruction and the task. In this part, wherein no stimulation was delivered, subjects were asked to judge which segment of a pre-bisected line was the shortest in half of the trials, and which one was the longest in the other half by pressing the “b” key for the “left” responses and the “n” key for the “right” one (Landmark task). These phase included 2 blocks of 10 lines equally bisected and 10 asymmetrically bisected (40 lines in total).

Right after applying the cap with electrodes TMS compatible for EEG recording, we measured in the right hemisphere the resting motor threshold (rMT), i.e. the lowest stimulus intensity of TMS able to elicit visible twitch in the abductor pollicis brevis muscle of the right hand in at least 5 of 10 consecutive stimulations of the motor hotspot.

Afterwards the participants were seated in front of the monitor with the eyes at 57 cm from its center with their head stabilized on a chinrest. The baseline perceptual bias (i.e. the bias showed without any TMS stimulation) was assessed prior to any TMS stimulation: ten trials with equally bisected lines were presented; in half of the trials they have to judge which segment was the shortest, in the other one the longest. The instructions were randomize between participants.

3.3.1 Hunting procedure

After having assessed the baseline perceptual bias we administered the hunting procedure (Salatino et al., 2014). We used a 3x3 cm grid of 9 points, named from S1 to S9, centrally located over P6 according to 10-20 International EEG system, while performing the line bisection judgments task (Landmark task); the grid was placed over a custom made 4 cm thick block of plywood centered over the same electrode position (fig.16), in order to make possible, in the subsequent session, to record EEG signal from the electrode right underneath the coil.

Ten single-pulses for each of the 9 points (for a total of 90 pulses) were delivered, at an intensity of 115% of the subject’s resting motor threshold, 150 ms after the visual stimulus onset, as in previous TMS studies showing induction of neglect-like biases on the Landmark task (Fierro et al., 2001; Ricci et al., 2012). The coil was placed tangentially to the scalp, with the handle pointing backward and 45° downward from the parasagittal line.

Prior to starting the procedure, we recorded the 9 points of the grid for each subject in the neuronavigation system, by means of the wireless markers.

In this part of the experiment all the visual stimuli of the Landmark task were equally bisected lines, as we were interested in finding the spot that, if stimulated, would induced the biggest change in the perceptual bias as compared to the baseline one.

Also, participants could decide the timing at which the procedure was carried out: when the space bar was pressed the subsequent line appeared. We decided to let them chose the pace of stimulus presentation and subsequently the TMS pulse (delivered 150 ms after the stimulus onset) because this was the first time that the stimulation was delivered, so they would get a little bit familiar with this technique.

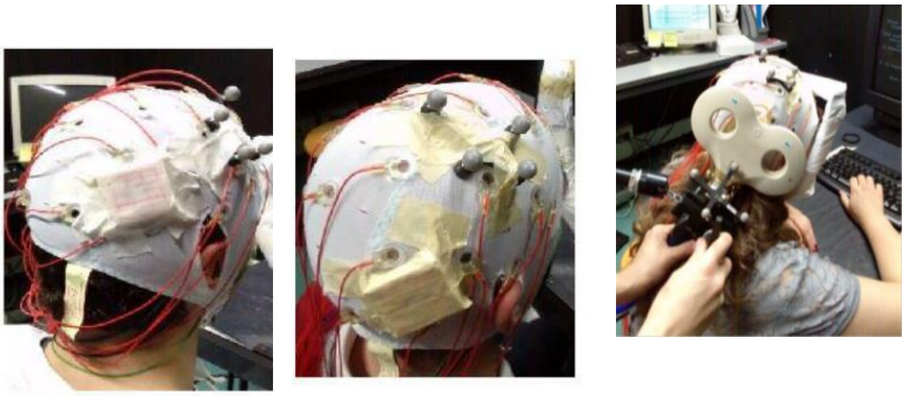


Fig.16 (a) Montage of the grid, custom made 4 cm thick block of polystyrene (both cantered over P6) and wireless marker on a subject’s head. (b) Position of the coil.

3.3.2 Experimental session

In the last part of the study, we administered the Landmark task while recording EEG with (TMS ON condition) and without (TMS OFF condition) the stimulation of the parietal hotspot. In both conditions, two types of stimuli were used: symmetrically and asymmetrically bisected lines (fig.17).

Every condition consisted of 10 blocks of 320 stimuli (32 stimuli per block), for a total of 640 trials for the entire experimental session; in half of the blocks participants were asked to judge which segment was the shortest, in the other half which one was the longest by pressing the same keys of the previous sessions (‘b’ for ‘left’ and ‘n’ for ‘right’).

Both conditions (TMS ON and TMS OFF) and instructions (longest vs shortest) were counterbalanced between subjects.

During the TMS ON condition, in order to stabilize the coil in the correct position and orientation over the parietal hotspot chosen in the hunting procedure, we made use of a mechanical arm (Manfrotto magic arm, Italy, www.manfrotto.com).

In both conditions, at first a central fixation appeared for a random period between 300 and 600 ms, followed by the bisector (landmark). After that, one of the 3 types of lines, two asymmetrically and one symmetrically bisected, compared for 50 ms. The TMS pulse was delivered 150 ms after the visual stimulus onset. Subjects had 3 seconds to give their answer: they were instructed to respond as fast as they could, but no scarifying accuracy for speed. Furthermore, in this part, the timing of the experiment was fixed, i.e. participants could not decide the pace at which the stimuli were presented like in the hunting procedure.

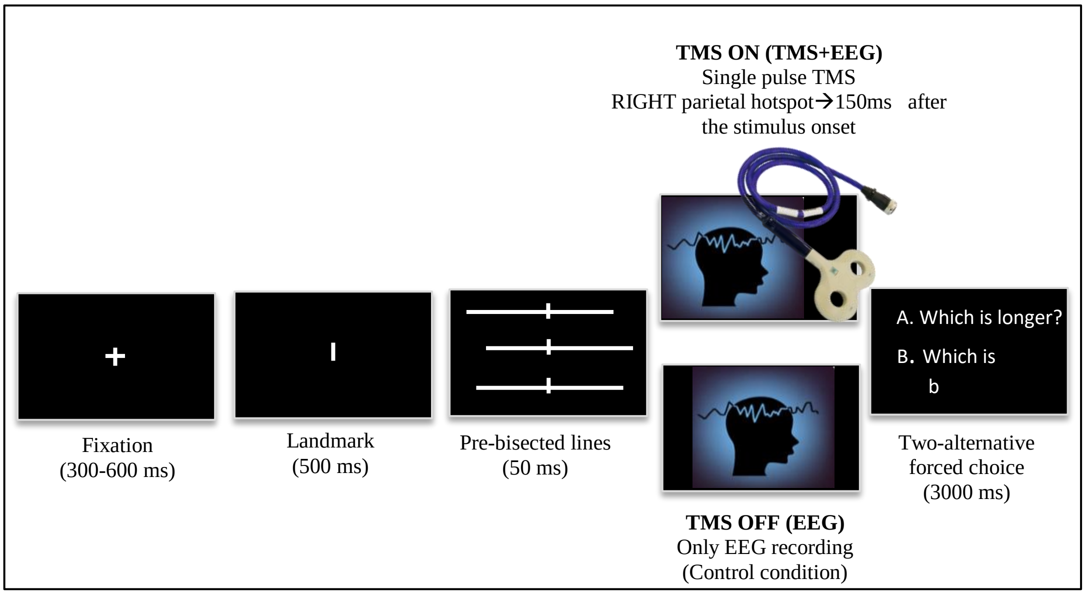


Fig.17 Outline of the experimental procedure.

3.4 Magnetic stimulation

Single-pulses magnetic stimulation (inter-stimulus interval > 4 s) was delivered through a 70 mm figure-of-eight coil connected to a biphasic Magstim Rapid2 system (maximum output 3.5 T) (Magstim Company Limited, Whitland, UK) at an intensity of 115% of the subject’s resting motor threshold (rMT), 150 ms after the visual stimulus onset.

Neuronavigation software (SofTaxic, E.M.S., Bologna, Italy) combined with a 3D optical digitizer (Polaris Vicra, NDI, Waterloo, Canada) was used throughout the experiment to maintain the coil position over the participant's head within a 2 mm accuracy threshold. The handle of the coil pointed backward and 45° downward from the parasagittal line.

A 3 cm x 3 cm grid, cantered over P6 (according to the 10-20 International EEG system) has been used in the experiment. The grid was divided in 9 points, named S1-S9, with S1 at the center and overlapping with the position of the electrode P6, the S7–S8–S9 spots corresponded to the most posterior portion of the grid, and S3–S4–S5 to the most anterior sites.

3.5 EEG recording

TMS-compatible EEG equipment (BrainAmp, BrainProducts, Munich, Germany) was used to record EEG signals (Brain Vision Recorder). The EEG activity was continuously recorded by means of a Fast'nEasy cap with 27 TMS-compatible compatible Ag/AgCl pellet pin electrodes (EasyCapGmbH, Herrsching, Germany) placed according to the 10-20 International System (O1,O2,P7,P3, Pz, P4, P8, CP5, CP1, CP2, CP6, T7, C3, Cz, C4, T8, FC5, FC1, FC2, FC6, F7, F3, Fz, F4, F8, Fp1, Fp2). All scalp channels were online referenced to the right mastoid (RM) and then re-referenced offline to the average of mastoids. The ground electrode was placed in AFz.

Blinks (vertical eyes movements) and horizontal eyes movements were tracked respectively with electrodes placed at the left and right canthi and above and below the right eye. The impedance of all the electrodes was kept below 5kΩ. The EEG was recorded at 5000Hz sampling rate and processed off-line with Brain Vision Analyzer 2 software (Brain Products, Munich, Germany), Matlab (Mathworks, Natick, MA, USA) and Fieldtrip (Oostenveld et al., 2011).

3.6 Behavioral analysis

Behavioral data obtained from all the sessions of the experiment was used to compute perceptual and response bias, processed as following:

- PB (perceptual bias): constant error across condition

PB = [(“left” short + “right” long)/2]*100

- RB (response bias): degree of response consistency between conditions

RB = [(“right” short + “right” long)/2]*100

Participants were subsequently divided in groups depending on the effect of TMS on the PB.

3.7 TMS-EEG data analysis

Continuous EEG signals were epoched starting from 300ms before and ending 800ms after TMS pulse. Due to TMS artefacts affecting the recording of EEG in a period of a few milliseconds after delivery of the magnetic pulse, a “linear interpolation” function was applied in a time range comprised between 1 ms before and 15 ms after the TMS pulse. Then a round of ocular correction ICA was used to correct ocular artefacts (i.e. blinks).

The epochs were filtered offline with a 40Hz high cut-off filter and baseline corrected from -300ms to -150ms (i.e. during the pre-stimulus time period), resulting in 320 trials for each condition. The data was visually inspected to remove all trials contaminated by eye movements and blinking artefacts, involuntary motor acts or excessive noisy EEG. After preprocessing, we segmented again the trials in four conditions: TMS ON equally bisected trials, TMS OFF equally bisected trials, TMS ON asymmetrically bisected trials (collapsing together left and right elongated lines) and TMS OFF asymmetrically bisected trials. Finally we extracted MAT files to proceed with the analysis in Matlab software.

In order to detect the potential changes induced in the neural signal by the stimulation, a mass univariate analysis (Groppe et al. 2011a; 2011b) was performed on the difference between asymmetrical vs symmetrical lines for the TMS ON and the TMS OFF conditions, separately on each group. After observing the EEG data, different time windows, specific for each group, was chosen to carry out the analysis. These a priori constraints would help to maximize the statistical power of the analysis.

4 RESULTS

4.1 Behavioral results

Participants were divided in three different groups based on the behavioral modulation induced by TMS i.e. the changing of PB. They were assigned to their group based on 2.5 standard deviation criterion from the percentage of PB change from the TMS OFF to the TMS ON condition.

In the Neglect bias group (N= 16, mean age=22.89, sd=4.08) we have found an increment of the PB value from the baseline to the site of stimulation in the hunting, but also from the TMS OFF condition to the TMS ON. The opposite pattern has been observed in the Pseudoneglect bias group (N= 14, mean age=23.31, sd=2.50) that is a decrease of the PB. Finally, in the last group, the no bias group (N= 14, mean age=22.29, sd=2.33), there was no modulation of the PB both in the hunting and in the experiment.

In table 1 the means of PB change in the hunting and experimental sessions are reported: for what concern the Neglect like bias group (hereafter named Neglect group) there is an increase of 9.38% of the PB in the hunting procedure and 12.66% in the experimental session from the TMS OFF condition to the TMS ON condition. In Pseudoneglect group there is a 2.14% and 13.57% of PB change respectively in the hunting and in the experiment, in the opposite direction as compared to the previous group. Finally, in the No Bias Group it could be observed almost no changes in the two sessions (2.14% in the hunting and 0.44% in the experimental session).

In figure 18 the trends of the PB change for each group, both in the hunting and in the experimental session, are represented.

	HUNTING		**EXPERIMENT**	
Groups	**Baseline**	**Hotspot**	**TMS OFF**	**TMS ON**
Neglect	42.81	52.19	35.90	48.56
Pseudoneglect	51.43	49.29	55.13	41.56
No Bias	55.00	52.86	44.12	44.56

Tab.1 Mean subjects' PB% values in baseline condition and during the stimulation of the hotspot (hunting) and in the TMS OFF and TMS ON condition of the experimental session.

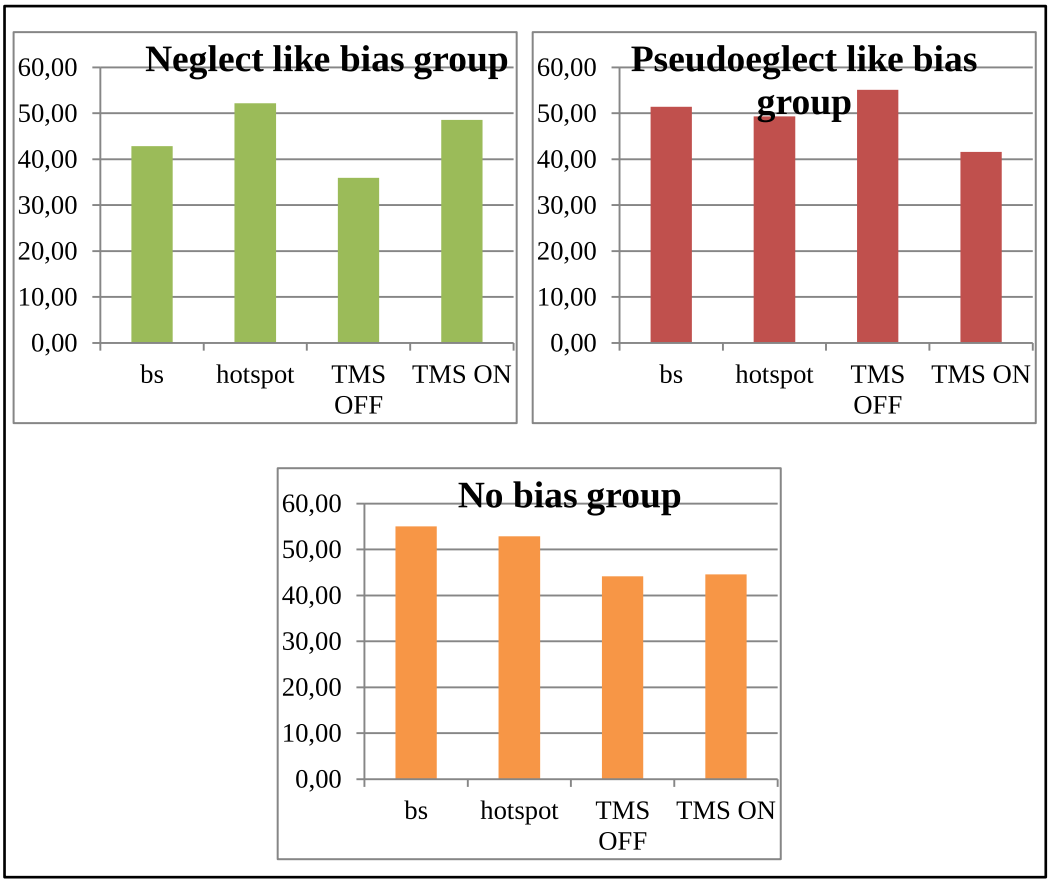


Fig.18 Mean average of PB change in baseline condition and during the stimulation of the hotspot (hunting) and in the TMS OFF and TMS ON condition of the experimental session for each group.

4.2 TMS-EEG results

In order to detect the potential changes induced in the neural signal by the stimulation, a mass univariate analysis (Groppe et al. 2011a; 2011b) was performed on the difference between asymmetrical vs symmetrical lines for the TMS ON and the TMS OFF conditions, separately on each group.

The mass univariate analysis consists of large number of univariate t-tests to compare ERPs/TEPs at a number of time points and scalp locations. We used FDR (false discovery rate) correction for our analysis that is based on FDP (false discovery proportion), i.e. the proportion of rejected null hypotheses that are mistaken. Instead of controlling for the possibility that every single t values are bogus, it controls for the total proportion of fake effects (Groppe et al. 2011a; 2011b).

After observing the EEG data, different time windows, specific for each group, was chosen to carry out the analysis. These a priori constraints would help to maximize the statistical power of the analysis. In table 2 the exact time of the three windows for each group are specified.

The lower and the upper bound of the first time window were respectively 101 ms and 135 ms.

In figure 19 are plotted the results for each group in this time window: on the y axis there are the electrodes, on the x axis the time; so in each line is represented the significance of the t-test for that electrode in that time window. When a box is gray, it means that the effect is not significant.

For the Neglect like bias group there are no significant effects in this time window. For the Pseudoneglect group ($t_{(13)}$=3.94, p<0.01, two-tailed) and the No Bias group ($t_{(13)}$=3.51, p<0.01, two-tailed), the difference wave in the TMS OFF is significant in two electrodes: O2 and P8. This effect has been found in the TMS ON condition only for the last of these two groups ($t_{(13)}$=3.90, p<0.01, two-tailed).

By the observation of the EEG data (fig.18b, black squares) a larger early component (with and without TMS) for asymmetrically bisected lines, as compared to the symmetrical ones can be seen.

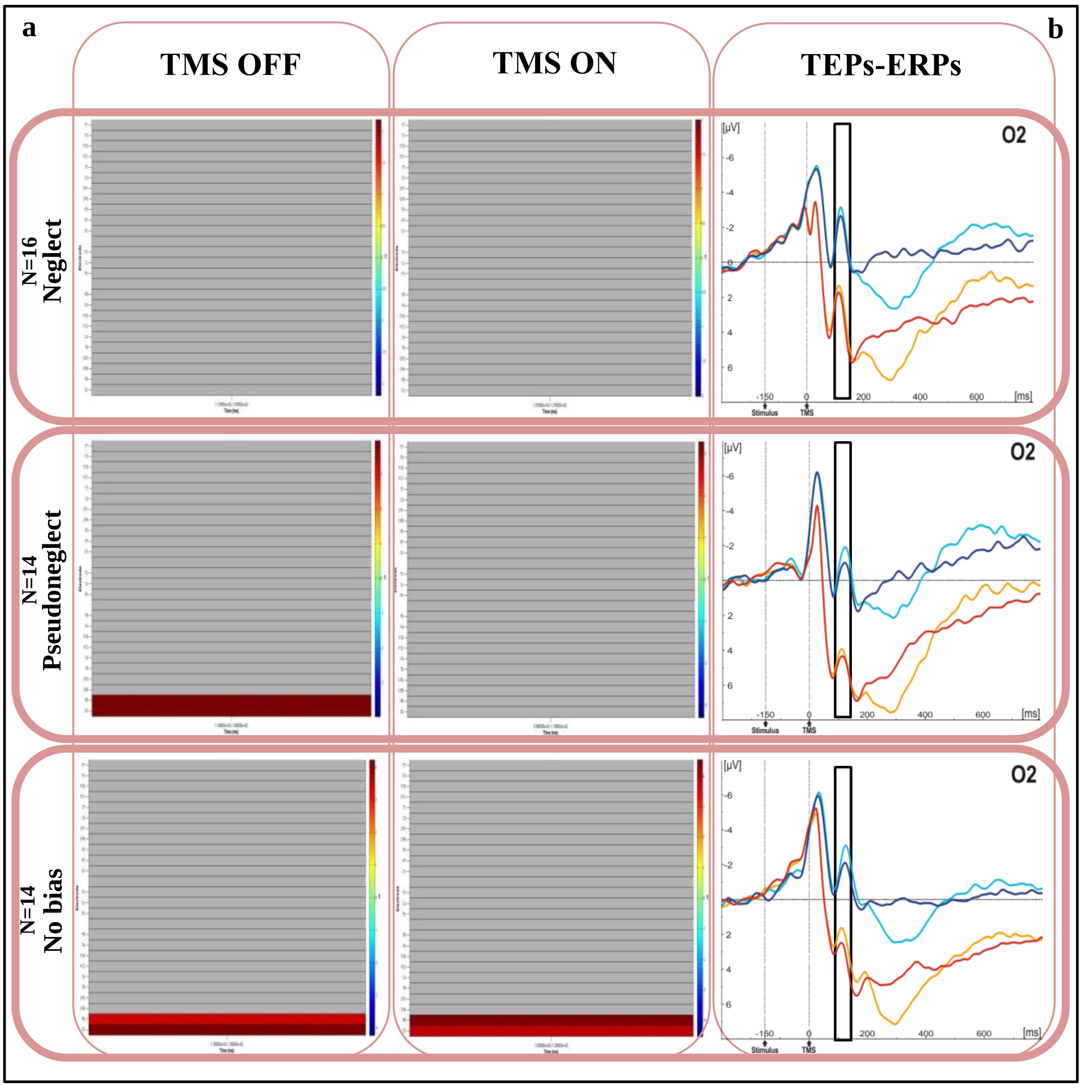


Fig.19 (a) Mass univariate analysis on the difference between asymmetrical vs symmetrical lines for the TMS ON and the TMS OFF conditions, separately on each group. In the plots on the y axis there are the electrodes, on the x axis the time; so in each line is represented the significance of the t-test for that electrode in that time window. When a box is gray, it means that the effect is not significant. (b) ERPs and TEPs recorded on the electrode O2 for each group: blue waves represent the ERPs for symmetrically bisected lines, light blue the ERPs for asymmetrically bisected lines, red the TEPs for symmetrically bisected lines and yellow he TEPs for asymmetrically bisected lines.

The second time window, in which we performed the analysis, ranged from 153 ms to 177 ms.

For the two bias groups, no effect was found for both conditions. Instead for the No bias group the difference was significant in the TMS ON condition for O1, O2, Pz, P3 and P8 electrodes ($t_{(13)}=3.57$ $p<0.01$, two-tailed).

Furthermore, only for this group of participants, we have found a significant interaction effect ($t_{(13)}=-2.41$, $p<0.01$) of the TMS conditions on the types of stimuli on the electrodes in the left hemisphere (fig.20).

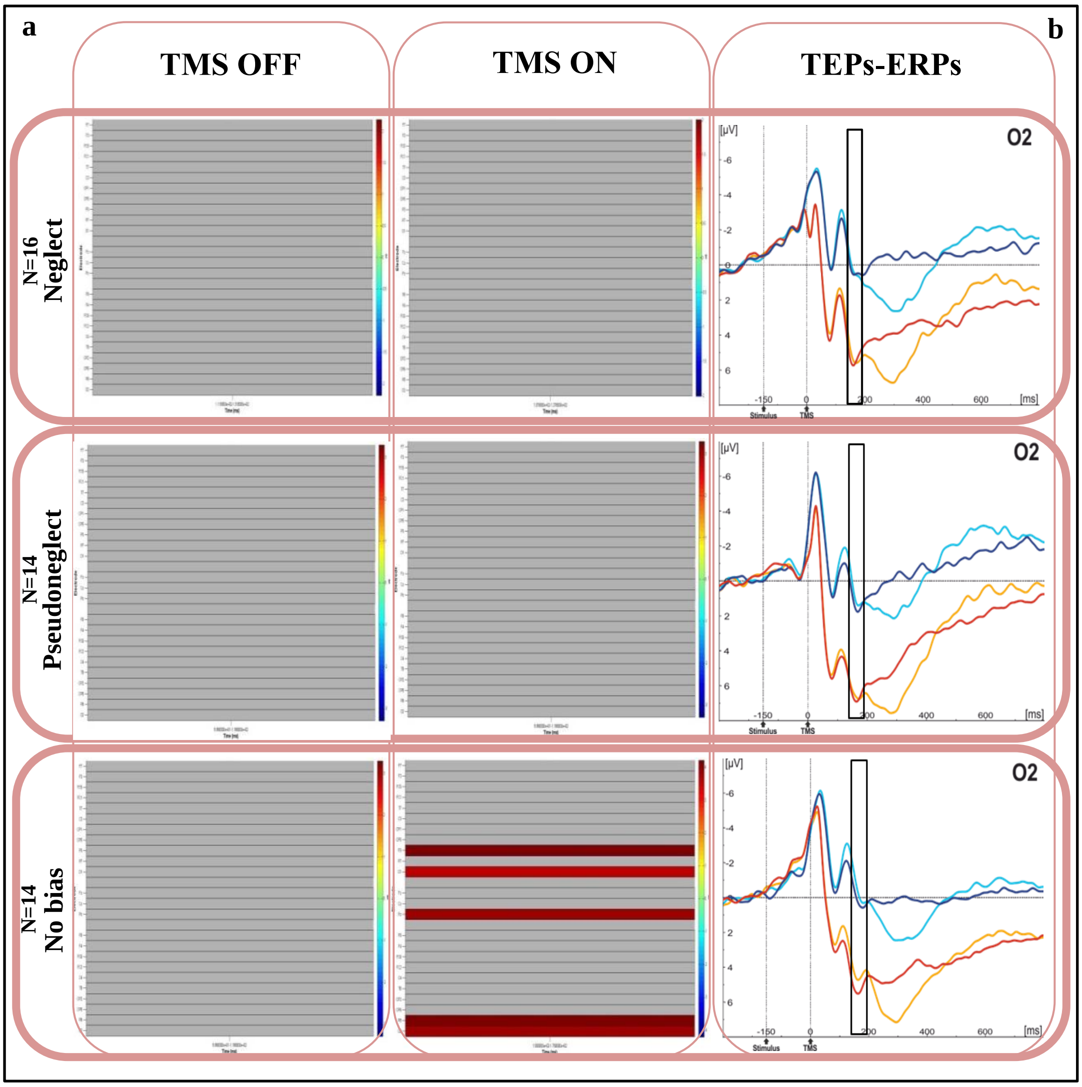


Fig.20 (a) Mass univariate analysis on the difference between asymmetrical vs symmetrical lines for the TMS ON and the TMS OFF conditions, separately on each group in the second time window (153-177 ms). (b) ERPs and TEPs recorded on the electrode O2 for each group: blue waves represent the ERPs for symmetrically bisected lines, light blue the ERPs for asymmetrically bisected lines, red the TEPs for symmetrically bisected lines and yellow he TEPs for asymmetrically bisected lines.

Finally, in the last time window (200-430 ms), in all the groups, the difference waves were significant in almost all our electrodes in the TMS OFF (Neglect group: $t_{(15)}=3.77$, $p<0.01$, two-tailed; Pseudoneglect group: $t_{(13)}=3.98$, $p<0.01$, two-tailed; No Bias group: $t_{(13)}=2.24$, $p<0.01$, two-tailed) and TMS ON (Neglect group: $t_{(15)}=3.77$, $p<0.01$, two-tailed; Pseudoneglect group: $t_{(13)}=2.31$, $p<0.01$, two-tailed; No Bias group: $t_{(13)}=2.26$, $p<0.01$, two-tailed). By looking at the EEG (fig.21), we could see a larger P3 like component produced by asymmetrically bisected lines (yellow and light blue wave) as compared to the one produced by equally bisected lines (in red and blue).

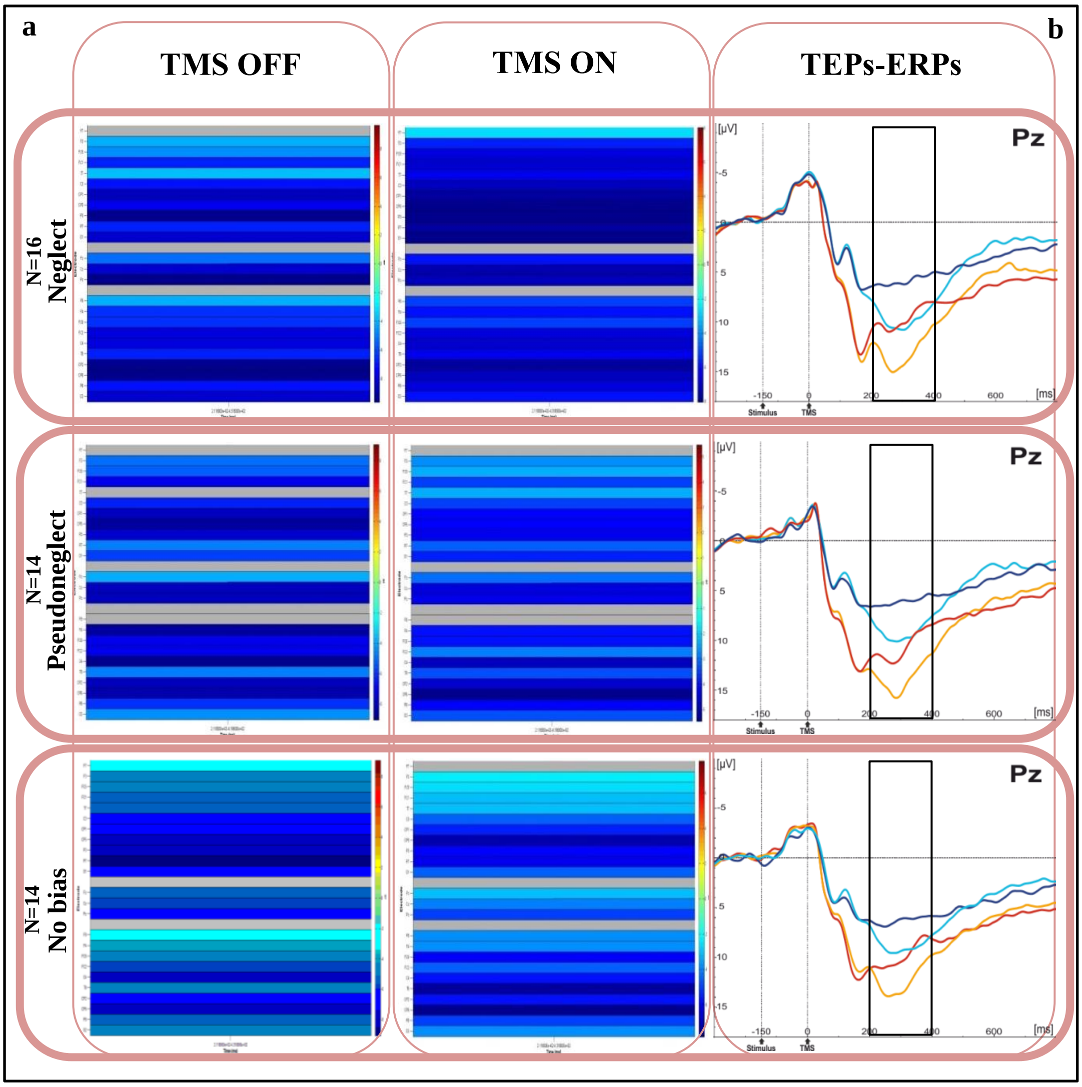


Fig.21 (a) Mass univariate analysis on the difference between asymmetrical vs symmetrical lines for the TMS ON and the TMS OFF conditions, separately on each group in the second time window (200-430 ms). (b) ERPs and TEPs recorded on the electrode Pz for each group: blue waves represent the ERPs for symmetrically bisected lines, light blue the ERPs for asymmetrically bisected lines, red the TEPs for symmetrically bisected lines and yellow he TEPs for asymmetrically bisected lines.

5 DISCUSSION

The first aim of this study was to investigate the contribution of PPC in visuo-spatial representation in the healthy brain, in terms of activation and timing of engagement, by observing the modification of perceptual bias induce by TMS stimulation in a Landmark task, by means of TMS-EEG combined methodology.

In accordance with previous data (Fierro et al., 2000; 2001; Brighina et al., 2002), our experiment has heightened how the rPPC is involved in the estimation of magnitude of line length.

This result contributes also to the assessment of the Landmark task as a useful method to investigate the high-order perception function in healthy subjects. Our task was composed by symmetrically bisected lines, that were used to calculate the perceptual and response biases, and by asymmetrically bisected lines, that were used as control trials for participants' accuracy while they were doing the experiment. It is presumable that they would have showed a nearly ceiling result with these stimuli, and also it allowed us to control if they were really performing the task.

Secondly, the Landmark task has proved to be a sensible method to tear apart perceptual and response bias (Bisiach et al., 1998; Capitani et al., 2000; Toraldo et al., 2014). This advantage is really helpful to control how the TMS modulates separately the perceptual and the "motor" bias, the impact of the stimulation and virtually how the biases are related to each other.

Another proof of this different effect on these two biases and the capacity of the Landmark task to separate them derives from the absence of a modification of the RB before and after the TMS. In table 2 are reported the values of RB both in the hunting and in the experimental condition: there is no great modification in each group both in the hunting procedure (from the baseline without the TMS to the parietal hotspot) and the experiment itself.

	HUNTING		**EXPERIMENT**	
Groups	**Baseline**	**Hotspot**	**TMS OFF**	**TMS ON**
Neglect	45.00	46.14	44.35	42.09
Pseudoneglect	44.69	48.75	44.00	45.72
No Bias	40.00	42.14	38.04	39.76

Tab.2 Mean subjects' RB% values in baseline condition and during the stimulation of the hotspot (hunting) and in the TMS OFF and TMS ON condition of the experimental session. When the value of RB is around 50 ±5 there is no response bias.

We have also found that single-pulse TMS over the right parietal cortex can interfere with visuo-spatial perception in a Landmark task when delivered 150 ms after the visual stimulus onset. This confirms the previous findings reported by Fierro and colleagues (2001): the time of interference of TMS pulse suggests that this is the time for the information to travel from extrastriate to parietal cortex.

Before staring the experiment we took into account the possible range of PB values and we predict three possible type of behavioural modulation induced by the TMS. The first one is the neglect like bias modulation: in this situation, when TMS is applied over the parietal hotspot, at a behavioural level, there is an increase in the value of perceptual bias. Conversely, the second type of modulation, pseudoneglect like bias, consists of a decrease of PB values. The last possible condition occurs when TMS has no modulation effect that means there is no changing in PB value before and after the TMS.

Indeed, we have found different behavioral modulations induced by TMS. So we divided our participants in three groups, depending on their modulation of PB (i.e. their behavioral performance). In the Neglect like bias group we could have seen an increment of the PB value from the baseline to the site of stimulation in the hunting, but also from the TMS OFF condition to the TMS ON. The opposite pattern has been observed in the Pseudoneglect like bias group that is a decrease of the PB. Finally in the last group, the No Bias group, there is no modulation of the PB both in the hunting and in the experiment.

Generally, we could conclude that the condition with the TMS, one in the hunting procedure and the other one in the experiment (TMS ON condition), have the same type of modulation of PB. In other words, if there were an increase in the PB values after the stimulation in the hunting, the same pattern was found also in the experiment.

Our behavioural results are somehow in contrast with previous research (Fierro et al., 2000; Bjoertomt et al., 2002; Ellison et al., 2004; Fierro et al., 2006), because, in addition to the participants whose perceptual biases don't seem to be modulate by the TMS (No Bias group), we have also found a group (Pseudoneglect like bias group) that show the opposite pattern of PB change as compared to the neglect induced behavior.

In a recent study (2015), Learmonth and colleagues have used different visuo-spatial tasks to test their stability in measuring the perceptual bias; besides finding that every task employed was reliable over time and not correlated with the others, they revealed that the perceptual bias is a multicomponent entity and probably different functions of the same network contributed to this phenomenon. It might, thus, be possible that differences in this system could be accounted for the peculiar modulation in PB that we have found.

In literature (Benwell et al., 2014) it has also been shown that left bias (pseudoneglect) was associated with strong right parieto-occipital responses. Maybe different participants have different "level of activation" of this network, and consequently the effect of the stimulation varies accordingly to the state of the neurons (Silvanto & Pascual-Leone, 2008).

Given these behavioral differences, we were wondering if we could find the same pattern also at a neurophysiological level.

In the first time window considered (101-135 ms) we have found a significant effects in the same electrodes (O2, P8) in the TMS OFF condition in two groups (Pseudoneglect and No Bias), but not in the Neglect group. This effect is also present, in the same sites, in the TMS ON condition only for the No Bias group.

One possible explanation for these results is that when the TMS could modulate the PB, at a neural level the difference between the symmetrical and asymmetrical bisected lines was reduced (fig.20). Indeed, in the two biases group in which the percentage of PB change is higher, the difference between the TEPs for the asymmetrical bisected line (yellow ones) and symmetrically bisected lines is little. Instead, when the TMS could not modulate the PB, we can see a larger difference between the TEPs of the two types of stimuli.

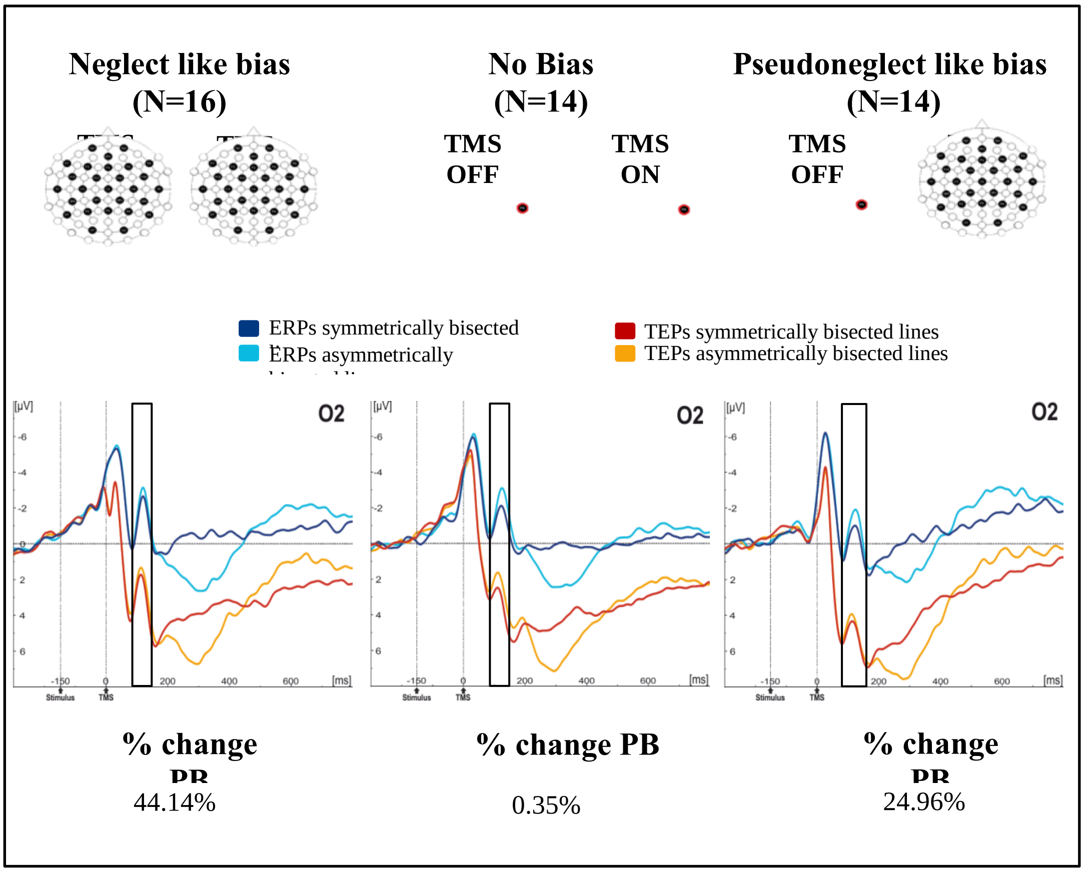


Fig.20 ERPs and TEPs of the three groups of participants in the first time window (101-135 ms). For each group it is indicated the percentage of PB changes: the Neglect and Pseudoneglect like bias groups show a higher values as compared to the No Bias group.

In the second time window (153-177 ms), for the two bias groups, no effect was found in both TMS ON and OFF conditions. Instead, for the No bias group the difference was significant in the TMS ON condition for O1, O2, Pz, P3 and P8 electrodes. Probably this effect is due to previous difference between asymmetrically and symmetrically bisected lines found in the first time window. Another hint in this direction can be inferred from the interaction effect for the left hemisphere electrodes significant only for these participants: this could be interpreted as another evidence that this group is different from the others. interestingly, we have found this interaction effect on the left hemisphere and this could posit to the fact that more linguistic or cognitive components were at place to make the No bias group solve the task.

Finally in the last time window (200-430 ms) in all the groups, the difference waves are significant in almost all our electrodes. By looking at the EEG (fig.21), we can see a larger P3 like component produced by asymmetrically bisected lines (yellow and light blue wave) as compared to the one produced by equally bisected lines (in red and blue). We have hypothesized that this might be interpreted as a "certainty index" with which the participants could have given their answers, i.e. the participants were more sure about their answers with the asymmetrically stimuli compared to the other type.

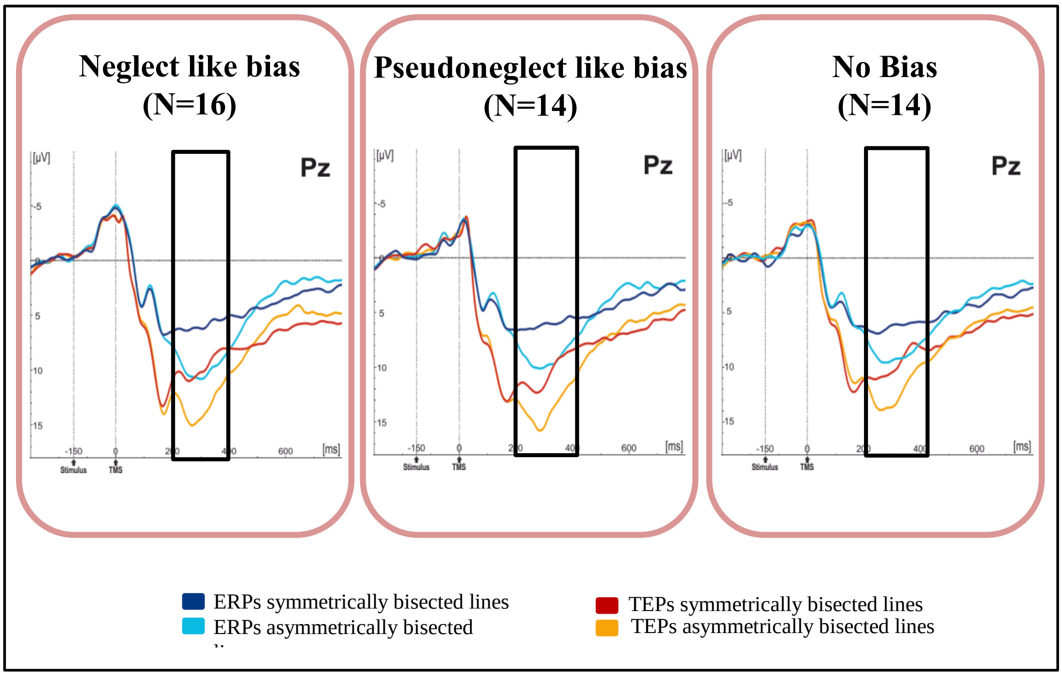


Fig.21 ERPs and TEPs of the three groups of participants in the last time window (200-430 ms). The black squares indicate the P3 like component for each group.

In conclusion the present data thus show that rPPC is involved in magnitude estimation of line length.

TMS could not modulate the PB in a group of participants (No bias), probably due to preexisting differences among individuals, as our results in the early time window in the TMS OFF condition would suggest. One possibility is that the effects of the TMS are determined not only by the properties of the stimulus or by the TMS itself, but also by the state of the cortex during the task execution (Silvanto & Pascual-Leone, 2008).

In the future we are planning to test differences between groups. This further step would help us clarify if, at a neural level, the No Bias is significantly different from the Neglect and Pseudoneglect like bias group.

We will also try to better understand the pre-existing differences found in our participants. What we could have seen until now is that the TMS cannot modulate the perceptual bias in all subjects. There are some differences in the neural signal that make some people more likely to have a bias modulation, like our Neglect like bias group that have the largest percentage of PB change as compared to the other two groups.

Finally, we also want to better analyze the effects of the TMS stimulation on brain activity: i.e. whether there are different effects at different latencies or the TMS have different impact on different participants.

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