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Haptic-based object directed behavior: the role of the parietal operculum.

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Abstract

The aim of this thesis is to provide new insights about the role of the left human parietal operculum (OP) in sensory motor transformations in the context of object-directed behavior. This work is divided in two main parts: an introductory part about the theory underlying the sensory motor integration and the existing literature about the parietal operculum, and an experimental part in which the experiments realized during these three years are described. In **Chapter 1**, the theory underlying the sensory motor transformation in the visual modality and the possible functions of the different front-parietal circuits are described on the basis of the theory proposed in the literature. A specific paragraph is dedicated to ventral premotor cortex (PMv) and its role in visually guided grasping. The **Chapter 2**, is a review of the literature about the cytoarchitecture, the connectivity and the physiology of humans and non-human primates parietal operculum. In **Chapter 3** the literature about the role of OP of primates and humans in sensory motor integration is reviewed together with some literature about studies on lesions. In the experimental part, four transcranial magnetic stimulation (TMS) experiments are described. The last two experiments have been grouped in a single major work and results have been discussed together. In **Chapter 4** the **Experiment I** is described. This experiment aimed to characterize the fronto-parietal network involving the connection between left OP and ipsilateral primary motor cortex (M1). By means of double coil TMS paradigm we were able to assess on five healthy volunteers the functional interaction occurring between these two regions, defining the optimal spot located into the left OP region that was producing the highest inhibition on motor evoked potentials (MEPs) peak-to-peak amplitude. We moreover characterized the timing of their interplay obtaining a 5 ms short latency interaction in support of a plausible direct cortico-cortical connection. In **Chapter 5** the **Experiment II** and **Experiment III** (which is

subdivided in two different experiments named **Experiment IIIa** and **Experiment IIIb**) are described. On the basis of the functional connectivity between OP and ipsilateral M1 at rest proved in Experiment I, the Experiment II aimed to assess whether and how the haptic information about objects' geometry (size) is encoded within the left OP and whether such information is used for guiding object-directed motor actions. Again, by means of double coil TMS paradigm we provided evidences supporting that the excitability of left M1 of twelve healthy volunteers, in presence of a motor plan involving the grasping of an object, was modulated accordingly with the size information stored in ipsilateral OP of such object when such physical feature was previously acquired by haptic exploration. Such modulation didn't occur when the object size was acquired by visual modality. As control we run the same paradigm on other twelve volunteers by conditioning the ventral premotor cortex (PMv) which is well known to play a key role in the visually guided grasping obtaining a clear double dissociation between haptic and visual modality, supporting the hypothesis that haptically guided behavior relies on a distinct network respect to visually guided one. **Experiment III** is divided in two different experiments (**Experiments IIIa** and **Experiments IIIb**). In both of these experiments we aimed to test the effect introduced by high frequency repetitive online TMS (rTMS) during two different tasks both involving only haptic modality. **In Experiment IIIa** a purely discriminative match-to-sample task, in which two cylinders (either equal or different in the diameter feature) were haptically explored in sequence with 2 seconds of delay, was implemented. rTMS was delivered during the delay period and effects on accuracy and sensitivity on discrimination were tested. In such non motor task, no significant effects were obtained on performances of the twelve healthy participants. **In Experiment IIIb** the effects of rTMS applied during different phases of a delayed grasping of a previously haptically explored object on the kinematics of the hand preshaping were assessed. Differently from experiment IIIa, in this motor task we obtained a significant perturbation

of the hand aperture during the preshaping when stimulation was applied between the exploration of the object and the grasping, during the delay phase. Such results might suggest of the involvement of OP in sensory-motor transformations as a putative element of memory when a object-directed action plan is implemented supporting the theory of the existence also for the haptic modality, of a model dual to the one proposed for the visual modality, made of two different streams processing sensory information according with the final use (for action or for perception).

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Glossary

AIP	anterior intraparietal sulcus	Ri	retroinsular area
aIPC	anterior inferior parietal cortex	rTMS	repetitive transcranial magnetic stimulation
aIPS	anterior intraparietal sulcus	SEFs	somatosensory-evoked magnetic fields
aSPC	anterior superior parietal cortex	SI	primary somatosensory cortex
CMA _d	dorsal cingulate motor area	SII	second somatosensory cortex
CMA _r	rostral cingulate motor area	SDT	Signal Detection Theory
condTMS	conditioning TMS stimuli	SMA	supplementary motor area
DTI	diffusion tensor imaging	testTMS	test TMS stimuli
ECD	equivalent current dipole	TMS	transcranial magnetic stimulation
EIP	extensor indicis proprius	TOR	tactile object recognition
EMG	electromyography	V1	primary visual cortex
FEF	frontal eye field	VA	ventroanterior nuclei
fMRI	functional magnetic resonance imaging	VIP	ventral intraparietal
FV	frontal visual area	VL	ventrolateral nucleus
ICMS	Intracortical micro stimulation	VPI	inferior nuclei
ID1	interosseus dorsalis primus	VPL	ventroposterior lateral thalamus
Ig	granular insular cortex	vPM	ventral premotor cortex
IPL	inferior parietal lobule	VS	ventral somatosensory area
IPS	intraparietal sulcus		
ISI	interstimulus interval		
M1	primary motor cortex		
MEG	magnetoencephalography		
MEP	motor evoked potential		
MIP	middle intraparietal sulcus		
MPM	maximum probabilistic map		
PCC	posterior parietal cortex		
PCG	post central gyrus		
PET	emission tomography		
pIPC	posterior inferior parietal cortex		
PMC	premotor cortex		
PM _d	dorsal premotor cortex		
PM _v	ventral premotor cortex		
PR	parietal rostra area		
ProM	proisocortical motor area		
pSPC	posterior superior parietal cortex		
PV	parietal ventral area		
RFs	receptive fields		
RI	retroinsular cortex		

PART I - A THEORETICAL OVERVIEW

Chapter 1

General insights on sensory- motor transformation

The term '*sensorymotor transformation*' is used to describe the process that regulates the computing of sensory stimuli in order to provide valid inputs for the production of motor commands. This process is essential to any biological organism or artificial system that possesses the ability to interact with the surrounding environment. The somatosensory system and the motor system are the two mainstays of such process and their interaction has been widely investigated, though several aspects about mechanisms underlying their interplay are still undiscovered. The somatosensory system is a crucial player involved in several aspects of motor behavior. It provides proprioceptive information such as the reciprocal position of different body parts and it allows the characterization of different kind of stimuli such as touch, pain or difference of temperature and the localization of their provenance. Thanks to the somatosensory system it is possible to acquire information about the physical characteristics of external objects and use it for further potential processes of object recognition or in following object directed actions (Pouget and Snyder 2000) (Dijkerman and de Haan 2007). In a seminal work, which will be taken as the main reference and reported for this introductory chapter, Rizzolatti and colleagues (Rizzolatti, Luppino et al. 1998) reviewed the organization of the cortical motor system describing in non-human primates the possible functions of several specialized parietal-motor circuits involved in the

transformation of sensory information in actions. In such paper, authors reviewed the “old fashioned” idea of the cortical motor organization based on two different regions - the primary motor cortex (M1) embedding the whole area 4 and most of the lateral area 6, and the supplementary motor area (SMA) located on the cortical medial surface – providing clear and valuable details, nowadays available thanks to modern anatomical and functional techniques, about the cortical motor organization and the complex networks underlying the process of sensory motor integration. They proposed a main subdivision of the motor cortex in four areas, namely primary motor cortex (M1), ventral premotor cortex (inferior area 6), dorsal premotor cortex (superior area 6) and presupplementary/supplementary motor area (pre SMA/SMA) (mesial area 6), which are subdivided as well in “*a mosaic of anatomically and functionally distinct areas*”. In such vision, M1 corresponds to the region F1 (F means “frontal”), the ventral premotor cortex is composed by F4, F5ab and F5c, dorsal premotor encloses F2 and F7 and at last preSMA and SMA comprises F3 and F6. Such multiplicity of areas is engaged in distinct and specialized networks with the different regions embedded into the parietal lobe (using the terminology introduced in (Pandya and Seltzer 1982) PE, PEc, PEpc, PEci, PF, PGm, ventral intraparietal (VIP), anterior intraparietal (AIP) medial intraparietal (MIP). In the paper authors highlighted that there is a sort of privileged connections between pairs of regions. This means that there are predominant connections between frontal and parietal areas (this latter named as “predominant input”) that reflects similar functional properties. Of course the connections are not univocal: parietal regions in turn connect with other different motor areas (such connections are named in the paper “additional input”). However such predominant networks represent a sort of segregated-functional circuits which are considered as functional units of the cortical motor system engaged in the sensory-motor transformation process. Such fronto-parietal connections

are following described grouped according to the four motor fields mentioned in the paper.

Primary motor cortex field connection:

F1 ↔ PE

F1 is the motor region involved in the decomposition of complex actions computed in other motor regions in elementary movements. PE is a parietal region whose neurons are involved in processing of proprioceptive information such as multiple joint positions. This connection probably allows the primary motor cortex to codify for the body position and control movement of limbs.

Ventral premotor cortex field connections:

F4 ↔ VIP

In F4 recording studies show that oro-facial movements are represented laterally while arm and axial movements are mapped medially. Additionally, some neurons responded to reaching movements body directed. In this motor region bimodal (visuo-haptic) and unimodal (haptic) neurons coexist almost in the same proportion and have complementary receptive fields (RFs) (that means if the RFs for tactile neurons are mainly located on the face and upper part of the body, for the visual neurons they are located in the peripersonal space). Moreover, this region shows to encode the space in body-parts centered coordinates. Area VIP receives input coming from regions involved in processing vision of motion, or selective for direction or speed of visual stimuli. However, beside the presence of visual neurons, as well as in F4 there's the presence of also bimodal (visual-haptic) neurons and RFs are still complementary. Rizzolatti and colleagues depicted such connection as involved in the encoding of the peripersonal space and in the computation of object location finalized to the movement towards them.

F5ab ↔ AIP

F5 neurons actively discharge during specific goal directed actions, especially if performed with the hand or directed to the mouth. In such frontal field there are represented distal arm movements grasping (with different kind of hand prehension), holding, tearing and manipulation. Neurons belonging to AIP are shown to codify for grasping of object of different shapes and sizes but not for their position. Moreover, there's three different kinds of neurons existing in such parietal region: motor-dominants (active in both light and darkness), visuo-motor (addressed to encode the type of grip related to the intrinsic characteristic of the object), and visuo-dominants (dedicated to fixation of objects). Such network has been described as responsible for the transformation of the intrinsic characteristics of an object into suitable and appropriate hand movements.

F5c ↔ PF

In this frontal field there are specific neurons with motor properties equal to those of F5ab but visual properties significantly different. Such neurons discharge (many of them selectively) with the observation of another individual performing an action such as grasping, manipulation and object placement and they show congruency in firing between observed and executed action and motor requirements for their trigger are quite restricted. Such neurons exist also in PF, however authors stated that their properties have not been studied in details yet. The authors highlighted the importance of that neurons (called mirror neurons) and the fact that their existence show an internal representation of action for the motor cortex which is used in the context of action imitation and recognition.

Dorsal premotor cortex field connections:

F2 ↔ PEc/PEip

In the dimple region of F2, which is the one involved in this connection, movements are somatotopically represented (legs movements are dorsal while arm movements are ventral). Neurons' electrical activity shows to be correlated with movements onset or even in advanced, supporting their probable involvement in motor preparation. Regarding the response to sensory stimuli, they show to answer to proprioceptive stimulation. In relation to area PEc, there's not much evidence in literature about its functional role. However, authors hypothesized its involvement with somatosensory stimuli for motor organization. More evidences exist in literature for PEip which show to respond to somatosensory stimuli, arm movement or limb projection in certain directions. It has been showed the presence of bimodal neurons responding to tactile and visual stimulation. Researchers have found interestingly that RFs of visual neurons are not fixed in their extension but expand according to specific motor action executed. Those two parietal regions have been depicted as involved in somatosensory control of movement and such connection with the frontal F2 has been hypothesized as be engaged in the control of limb movements on the basis of somatosensory information.

F2 ↔ MIP

In ventro-rostra F2, besides responding to proprioceptive stimuli, neurons show to be responsive also to tactile and visual stimulation. Such response is reported also for MIP neurons. Despite the poorness of evidences regarding the functional activation of such parietal field, similarly to the connections F2 ↔ PEc/PEip, also this connection is engaged in the same functional processing of control of limb movements with the probably involvement of the visual information.

F7 ↔ PGm

Electrical discharge of F7 neurons occurs in relation to arm movements or even before their onset. Their response has been showed also as consequence of visual stimuli. Moreover, behavioral paradigms showed F7 neurons as involved in the spatial localization of external stimuli for reaching movements; such neurons appear to assume an important role in the conditional stimulus-response association tasks and in conditional movement selection. Authors report that the functional role of area PGm is largely unknown; however, its neurons present electrical activity during eye and/or arm movements.

PreSMA/SMA field connections:

F3 ↔ PEci

Similarly to area F1, neurons belonging to this field appear to discharge in response to active movements but differently from area F1, representation of movements are somatotopically organized in a mixed order, and multijoints movements are observed as consequence of micro stimulation. Its involvement in control of posture and postural adjustments has also been proposed. F3 neurons show to respond additionally in association to visual and somatosensory stimuli. Area PEci is also named as supplementary somatosensory area due to the fact that a complete somatotopic map is embedded in such field. Authors' hypothesis is that such circuit is involved in a global control of the motor activity.

F6 ↔ prefrontal lobe

This peculiar frontal field is considered as a region addressed too control motor processing rather than involved in sensorymotor transformation. Its activation is not

trivial; it shows to occur in correspondence of complex movements, visual presentation of stimuli and increase when the stimulus is moved toward the subject and the discharge is modulated according whether an object can or cannot be grasped. Authors propose F6 as a region controlling a potential action encoded in lateral parieto-frontal networks. Their hypothesis comes from the idea that even if neurons encoding a specific action are activated, the real onset of the action is conditioned by external factors (e.g. motivation). In their vision, F6 whose activity is conditioned by external contingencies acts as a controller for the onset of such action fulfilling a role in sequence organization of movements.

PMv and visually guided object directed behavior

Among all the fronto-parietal circuits described above, we want to concentrate on one specific connection which is crucial for the visually-guided object directed behavior. Such connection is the AIP-F5ab. Classically, the sensory modality through which we mainly interact with the surrounding environment is the visual modality. By means of vision we acquire information about physical characteristics and features of objects (e.g. size, shape, position) which are then processed and used to produce a motor action towards them. For example, in the case of grasping, an important prerequisite for producing an efficient movement is that the adopted hand preshaping is congruent and compatible with the geometrical properties of the target object (Jeannerod, Arbib et al. 1995). Thus it is necessary that such information are acquired by vision, processed in the circuit and used by the motor cortex. The connection between AIP-F5ab is suggested to be involved in transforming the size and the shape of the objects (which are coded in AIP) into an appropriate motor schema (which is computed in F5ab (Rizzolatti and Matelli 2003). The most likely human homologue for such circuit is the

connection between an area located at the junction between the anterior IPS and the inferior postcentral sulcus (Binkofski, Buccino et al. 1999) (Frey, Vinton et al. 2005) and the ventral part of the premotor cortex (including part of area 6a alpha and area BA 44 (Binkofski and Buccino 2006) (which has been classically considered to be a speech area but it is also related to hand actions (Rizzolatti, Fogassi et al. 2002)).

A specific review of the literature related to the premotor cortex is out of the purpose of this thesis; however, it is important to characterize its role into visually guided grasping. Neurons belonging to this region have been shown to be activated during manipulation and grasping. In (Binkofski, Buccino et al. 1999) for example, strong activation of PMv were obtained in subjects manipulating complex objects respect to simple objects (sphere) or in complex object manipulation followed by a naming task on the explored objects. In (Ehrsson, Fagergren et al. 2000) by means of an fMRI study, authors shown engagement of bilateral ventral premotor cortex, the rostral cingulate motor area (CMAr), the supramarginal cortex, the ventral lateral prefrontal cortex, and the right intraparietal cortex in subjects performing a precision grip respect to respect to when they used a palmar power grasp during a similar manipulative task. It was also shown in monkeys that such region contains neurons selective for specific different types of grasp which appear to be represented by different overall levels of activity within the F5-M1 circuit. In fact, in monkey grasping different objects, different pattern of modulation in neural electrical activity of PMv (F5) and M1 were recorded by suggesting, as written in the papers that *“F5 grasping-related neurons play a role in translating visual information about the physical properties of an object into the motor commands that are appropriate for grasping, and which are elaborated within M1 for delivery to the appropriate spinal machinery controlling hand and digit muscles”*. The possible involvement of PMv in grasping through its corticospinal projections has been evaluated in (Shimazu, Maier et

al. 2004). In their study on macaque monkeys, they found that a conditioning electrical stimulation delivered over F5 could produce a significant modulation of motor outputs from M1 while by little or no detectable corticospinal output were obtained by stimulation of F5 alone. Details about the causality that characterize the relationship between bilateral PMv and its interaction with M1 in the context of hand and finger control during grasping have been provided in (Davare, Andres et al. 2006). By means of rTMS delivered either bilaterally or single unilaterally on subjects performing a simple grip-lift task with the right hand, authors showed the causal involvement of PMv in the grasping component of the movement. They in fact observed an incorrect positioning of finger on the object and an modified recruitment of hand muscles when the stimulation was delivered on PMv. In a following study (Davare, Montague et al. 2009), by means of double coil TMS, authors provided further details about the functional interplay between these regions during grasping by finding a short latency interactions at 6-8 ms. Furthermore, by observing effects of conditioning TMS over PMv on different muscles involved either in visually guided precision grasp or in whole hand grasp, they demonstrated that during a grasp preparation, interactions between PMv and M1 are modulated depending on the object to be grasped.

Taken together such results support the importance of PMv in visually based object directed behavior and its relevance in transformation of visually acquired information about object shape in motor action. In this thesis we aimed to investigate whether OP could be involved in sensorymotor transformation in an analogue manner in the context of haptic modality.

In the next chapter...

This first chapter is meant to report the modern vision of the neural basis underlying the sensory motor transformations by highlighting the role of a specific cortical region in the visual-based object directed behavior. In the next chapter, some of the literature about the cytoarchitecture, the anatomical connectivity and the physiology of the parietal operculum in primates and humans will be reviewed.

Chapter 2

The parietal operculum – cytoarchitecture, connectivity and physiology

Anatomy of OP in non human primates

In monkeys different cytoarchitectonic maps propose several parcellations of the dorsal Sylvian operculum and adjacent areas of cerebral cortex. According to the map produced by Brodmann in 1905 (Brodmann 1905) by examining the brain of an old-world monkey of the genus *Cercopithecus* (Figure 1A), the cortices of the inferior parietal lobule (IPL) and of OP are all grouped in the same cytoarchitectonic area, namely area 8. A slightly more detailed subdivision of the cortex lying below the intraparietal sulcus (IPS) was provided by the Vogts (Vogt and Vogt 1919) who identified three different parcellations, named in rostro-caudal order: area 2, area 7b and area 7a (Figure 1B). More recently Roberts and Akert, (Roberts and Akert 1963) studied the insular and opercular cortex and their thalamic projections in the *Rhesus* macaque and produced the map reported in Figure 2A. Data were derived from histological analysis and topographic rectilinear reconstruction of a series of coronal sections of the monkey brain processed with dyes. In their work authors defined second somatosensory cortex (SII) as region entirely limited within the OP area identifying its rostral limit at the end point of intersection between the projection of the central sulcus and the Sylvian fissure and its caudal limit coinciding with the end of the lateral sulcus. Regarding cytoarchitecture of cortical layers, authors described SII cortical patterns similar to postcentral granular cortex with however some differences. Similarly to postcentral regions, layer II and V presented a high density of cell population, the latter composed mainly of small medium sized cells. However, layer IV which was clearly visible, didn't appear to be as

developed as classical post central areas and layer VI resulted to be divided into inner and outer zone less sharply separated from the adjacent white matter than the same layer in postcentral cortex.

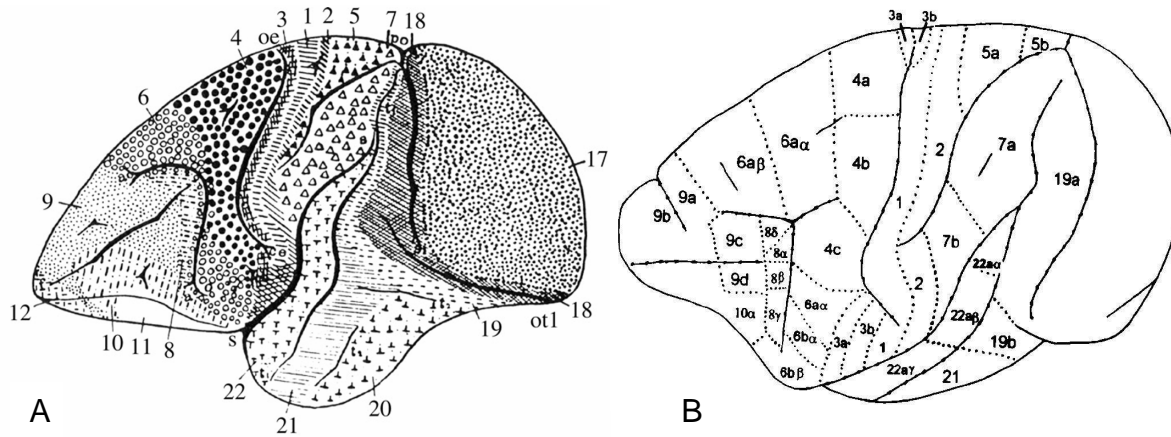


Figure 1. A: Brodmann cytoarchitectonic map of *cercopithecus* (1905). B: Vogt and Vogt architectonic map (from (Cipolloni and Pandya 1999))

A further subdivision of parieto-opercular cortex has been provided by Pandya and Seltzer (Pandya and Seltzer 1982). With their research, by means of autoradiographic and ablation degeneration techniques on *Rhesus monkey*, they were able to divide the inferior parietal lobule in four different architectonic regions named PF, PFG, PG and OPt. Furthermore, they discerned two additional zones within OP with distinct architectonic features which they named PFop, PGop. Their maps are reported in Figure 2B and Figure 2C. As it is showed in the picture, the more rostral region lying below the intraparietal sulcus is PF followed caudally by PFG and at last by PG. Ventrally to area PF and lied along the Sylvian fissure is the area PFop which is followed by area PGop which remains ventral to area PFG and PG. Considering area PFop and PGop, authors identified PFop as the location for SII (referring to parcellation proposed by Jones and Burton (Jones and Burton 1976) while PGop was defined as a ventral extension of the

cortex (SI) and it extends rostro-caudally along the upper bank of the Sylvian fissure sharing boundaries with retroinsular cortex (RI), the region PF and the posterior part of the insula (I_g). Their results were obtained by the observation of series of slices of the primates brains processed with dyes. Beside animal brains, also one human brain was included in the study and it was used for comparisons of results related to insula anatomical structure alone and not for surrounding regions. In relation to results regarding cytoarchitecture of anterior parietal regions, authors characterized the cortex of area SI (area 1 and 2) as granular cortex according with its densely cellular layer IV and moderately well-developed granularity in layer II. They moreover found distinct demarcation between layer V and VI. Regarding SII cortical architecture, authors

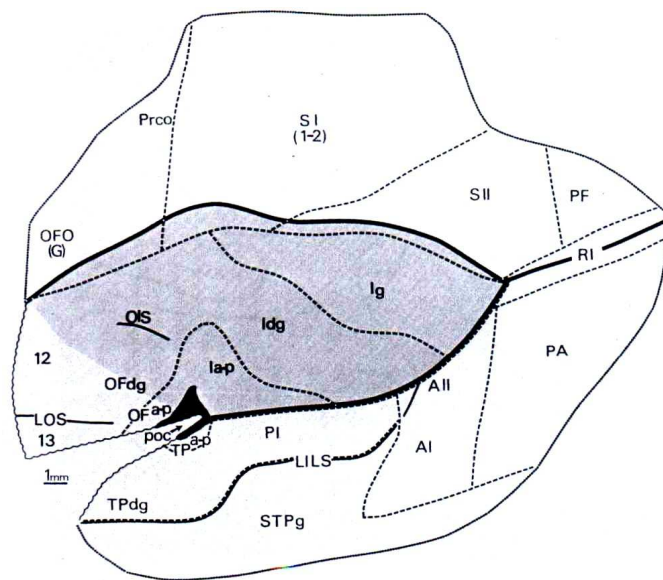


Figure 3. Architectonic map of the insula and surrounding regions. Abbreviations: AI, first auditory area; All, second auditory area; CS, central sulcus; I, insula; I_{a-p}, insula agranular periallocortical; I_{dg} insula dysgranular, I_g, insula granular; OF, orbitofrontal cortex; OF a-p, orbitofrontal cortex agranular periallocortical; OFdg orbitofrontal cortex dysgranular; OFg, orbitofrontal cortex granular; OFO, opercular cortex; PA, postauditory cortex; PF, anterior inferior parietal cortex; PI parainsular cortex; POC, prepiriform olfactory cortex; Prco, precentral operculum; RI, retroinsular cortex; SI, primary somatosensory cortex; SII second somatosensory cortex; STPg, supertemporal cortex granular; TPdg, temporo-polar cortex dysgranular (Mesulam and Mufson 1982).

highlighted difficulties in differentiate it from SI. Both of these regions shown sublamination in layer III based on a variation on cells size. However authors found that, respect to SI structure, layer II in SII tended to be thicker, layer III appeared to have denser cellularity and separation form layer V and layer VI seemed be less clear.

Summary or cytoarchitecture of primates

Discussed cytoarchitectonic maps differ from each others for both numbers and names of fields identified within the OP region. Also definition of boundaries for SII field seems to vary across maps, however generally this region is defined as an area extending along the upper bank of the lateral sulcus, and caudally limited by SI. Its rostral limit appears to be close to the final part of the Sylvian fissure. Concerning its laminar architecture, results mainly show SII region characterized by granular cortex with layer II and layer IV which appear to have higher density of cell respect to other layers.

Anatomy of OP in humans

Classical cytoarchitectonic maps

In humans according with Brodmann cytoarchitectonic map, the OP comprises areas 39, 40, 43, 3, 1 and 2. However, different nomenclature and locations have been identified in others classical cytoarchitectonic subdivisions such as Vogt and Vogt (1919) (Vogt and Vogt 1919) and von Economo and Koskinas (1925) (von Economo and Koskinas 1925). Considering Brodmann map at first, areas 43 and 40 lying below the IPL extend in sequence rostro-caudally from the central sulcus into the OP. The area 43 occupies the postcentral gyrus and the precentral gyrus between the ventrolateral extreme of the central sulcus and the depth of the lateral sulcus at the insula. Its borders are defined from the anterior and the posterior central sulcus. The area 40 shares its rostral border with the area 43 and it is bounded approximately by the intraparietal sulcus, the inferior postcentral sulcus and the lateral sulcus. Due to their topography, BA 43 and BA 40 are generally considered good candidates for human SII. On the basis of their myeloarchitectonic studies Vogt and Vogt (Vogt and Vogt 1919) defined two regions named area 72 and area 88 which can be considered the putative location for SII. Area 72 is a small subcentral region and differently from area BA 43, its extension hardly reaches the anterior part of the subcentral cortex rostral to the central sulcus. The area 88 lies caudally to the area 72 along the inferior supermarginal gyrus. It is larger than the area 72 but it remains in the anterior part of the inferior parietal lobule. Other two additionally areas lying in the depth of Sylvian fissure were defined by Vogt and Vogt (Vogt and Vogt 1919). Named area 73 (rostrally) and area 74 (caudally) they don't have any candidate homologous in Brodmann map. A further cytoarchitectonic parcellation of the cortex proposed by von Economo and Koskinas (von Economo and Koskinas 1925)

identified two regions named PFD and PFop. The former is a subcentral region which might correspond roughly to the location of BA 43. The latter, located caudally to PFD, might be the candidate for area BA 40 and taken together, these regions could be considered the location for SII in von Economo and Koskinas (von Economo and Koskinas 1925) map. Classical maps discussed above are reported in Figure 4.

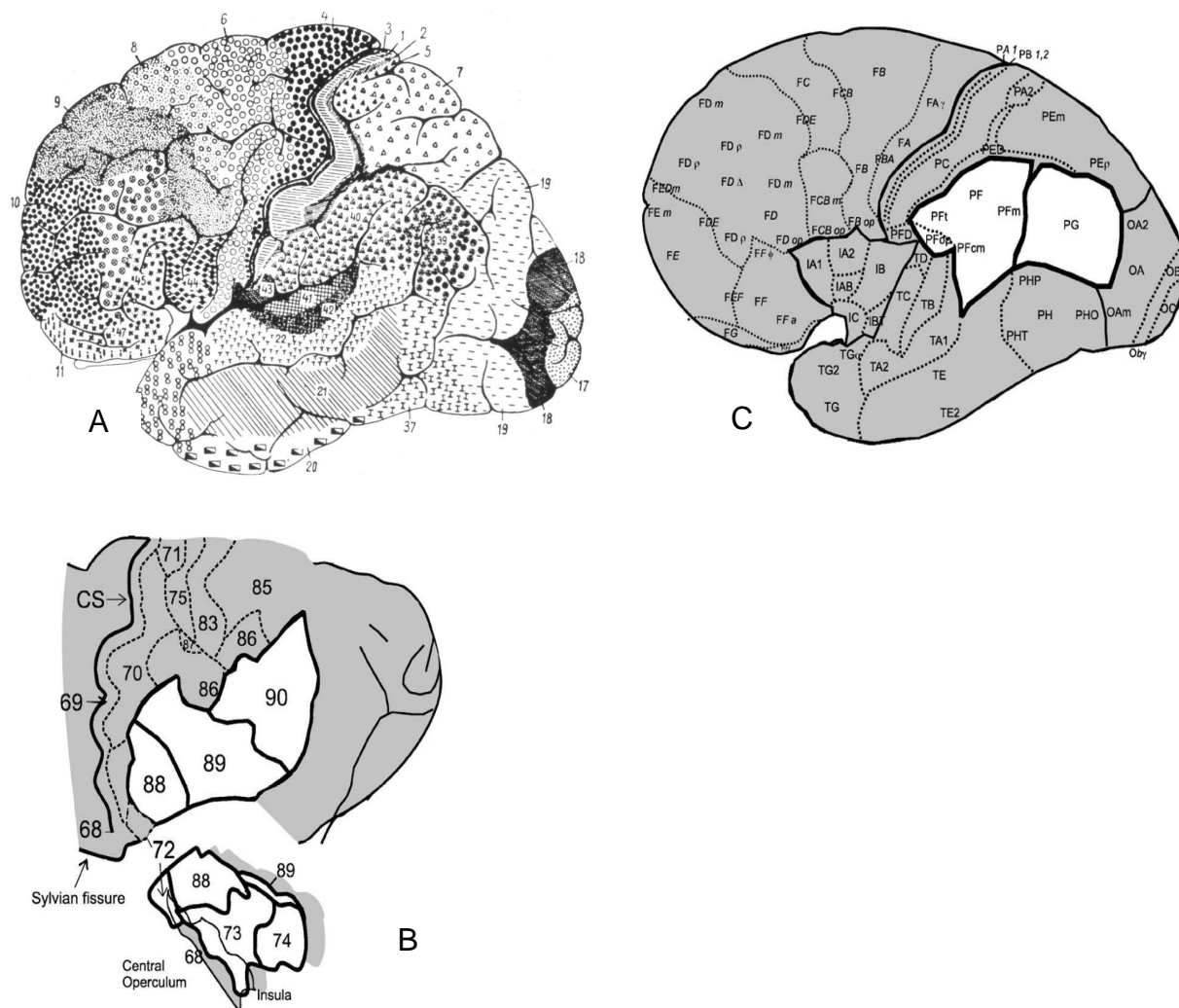


Figure 4. Classical cytoarchitectonic maps. A: Brodmann map (Brodmann 1905). B: par of Vogt and Vogt parcellation of parietal operculum (Vogt and Vogt 1919). C: von Economo and Koskinas map (von Economo and Koskinas 1925). Adapted from (Caspers, Geyer et al. 2006)

Recent cytoarchitectonic atlases

Recently, Eickhoff and colleagues proposed a new parcellation of the human OP based on a quantitative statistically testable approach. In their paper (Eickhoff, Schleicher et al. 2006) they motivated efforts done for this new cytoarchitectonic map with the fact that in the classical maps the definition of borders is observer dependent because the parcellation rely purely on visual inspection of histological sections. They moreover raised issues about the small size of samples used for the studies and about the problem of biological variability between examined brains. They thus used a method for the observer-independent detection of borders for parcellation of the OP region on ten post mortem human brains. In their work, after the isolation of the OP region within the pre-prepared histological sections, they selected several so called “profiles” which were very thin slices/sections of cortex taken in the direction perpendicular to the cortical layers. For each profile they quantified a 10 features vector representing the volume fraction of cell bodies. At last, they compared blocks of profiles evaluating the mutual distance and, assuming that profiles sampled from different areas resulted in high distance, they were able to differentiate regions of cortex and thus, to produce a new parcellation of the OP cortex. They distinguished four cytoarchitectonically different regions which they named from OP1 to OP4. Area OP1 (caudally) and area OP4 (rostrally) are located more superficially on the OP region than OP2 (caudally) OP3 (rostrally) which lie in the folded cortex in the depth of the Sylvian fissure. Figure 5 shows an image of their OP parcellation. In general they found a common border between OP4 and primary somatosensory cortex, and a shared border between OP1 and the inferior parietal cortex. The cortex caudal to OP2 was occupied by a Ri area while ventrally both OP2 and OP3 shared the border with the Ig cortex. In their work, authors proposed these new four OP regions as the human putative homologous for non human primates

specific regions. They observed that based on its topography, OP4 may correspond to the parietal ventral area (PV) (Krubitzer, Clarey et al. 1995). In fact, similarly to PV, OP4 was identified as located superficially within the Sylvian fissure close to the central sulcus and bordering the primary somatosensory cortex. Area OP1 which lied caudally to OP4 next to the inferior parietal cortex, was proposed as the analogue areas for non human primates SII (Krubitzer, Clarey et al. 1995). Similarly to SII and PV, also OP1 and OP4 shared a common border along medial to lateral direction. Area OP3 which was located deeper in the lateral sulcus than OP1 and OP4 and bordering with both of those areas, may topologically correspond to non human primates VS (Cusick, Wall et al. 1989). The association between OP2 with its corresponding primate area was more complex.

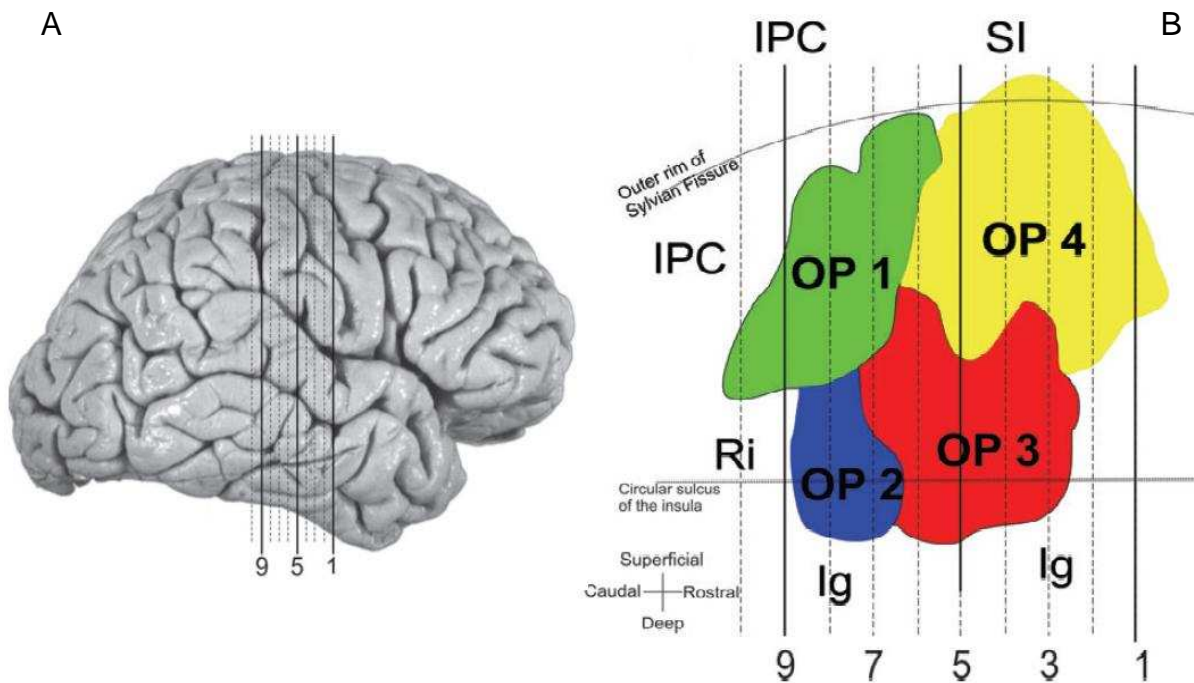


Figure 5. The location of areas OP 1-4 in according with (Eickhoff, Schleicher et al. 2006). A: slices considered for the analysis. B: flat map of OP parcellation (Eickhoff, Schleicher et al. 2006)

Authors reported that no distinct areas have yet been described in non human primates SII cortex corresponding to topographically to OP2. Moreover in primates, this region is not considered a somatosensory cortical field and neurons respond to complex stimuli including input coming from other sensory modalities like auditory and vestibular system (Robinson and Burton 1980). In a following work of Eickhoff and colleagues (Eickhoff, Weiss et al. 2006) OP2 was defined not a somatosensory cortical field and it was proposed as a good candidate for the parieto insular vestibular cortex (PIVC) in non human primates. With respect to analogies with human classical architectonic maps, OP4 and OP3 may correspond to Brodmann BA 43 even though, BA 43 cover the whole subcentral gyrus while OP4 and OP3 remain slightly rostrally. Area BA40, extends from the intraparietal sulcus to the insular cortex and covers OP region. Its border to BA43 coincides with the location of the border shared between OP4 and OP1. Thus OP1 seems to be comprised in Brodmann's definition of BA 40. According to von Economo and Koskinas parcellation (von Economo and Koskinas 1925), their cytoarchitectonic characterization of PFD region is close to the one of OP4 area. Region named PFop can be considered a good candidate for OP1 region. No subparcellation of the OP corresponding to OP2 and OP3 were described in von Economo and Koskinas maps (von Economo and Koskinas 1925), but possible homologous for these two regions located in the depth of the Sylvian fissure can be found in area 73 and 74 of Vogt and Vogt (Vogt and Vogt 1919) myeloarchitectonic map which is considered by Eickhoff and colleagues the most similar to their parcellation. In this last classical map, beside area 73 and 74, it is possible to identify other two regions named area 72 and area 88 which are located superficially and extent onto the free surface of the OP recalling the subdivision found by Eickhoff and colleagues.

Connectivity of OP in non-humans primates

Cortico-cortical connectivity of the upper bank of the Sylvian fissure has been widely studied in non human primates by means of injection of tracers producing detailed data about cortical projections of the OP. In the work of Pandya and Seltzer (Pandya and Seltzer 1982), connections of the posterior parietal cortex in *Rhesus monkey* were investigated by means of autoradiographic and ablation-degeneration techniques highlighting strong connections between the OP and the post central gyrus and the dorsal areas of the inferior parietal lobule. In their study they considered different regions of the post central gyrus (BA 3,1,2) and the posterior parietal cortex (superior parietal lobule: PE, PEc; inferior parietal lobule: PF, PFG, PG; parietal operculum: PFop, PGop). By observing labels originated from tracers or by evaluating terminal degeneration they were able to identify anatomical pathways connecting these cortical regions. Regarding IPL and OP connectivity, ablation performed in correspondence of BA 3,1 and 2 and in a portion of area PF produced a terminal degeneration in the area immediately caudal to the lesion PF and PFG, and more ventrally in PFop and PGop. Also isotope injection in correspondence of PF and PFG, besides highlighting connections in regions immediately close sites of injections, produced terminal labeling over the PFop (SII). Additionally, their study showed further afferents opercular projection originating in more caudal regions of the parietal lobe such as PEc and PG. In the same year Mesulam and Mufson published their research about afferents and efferents connections of the insula in *Rhesus monkeys* providing evidences for existing connections with OP region (Mesulam and Mufson 1982; Mufson and Mesulam 1982). Retrograde tracers injections into three different parts of insula (anterior, midanterior and posterior insula) revealed that afferents originate from different regions such as frontal lobe, prepiriform olfactory cortex, cingulated gyrus, parietal lobe and temporal lobe. Regarding the parietal lobe

projections, labels produced by injections in the posterior insula have revealed the origin of those projections in regions such as SI, SII, area PF and area 5. In the same way, anterograde tracers injected in posterior insula to investigate efferent projections produced labeling located in region SII, PF and area 5. Considering the obtained results on efferents and afferents, authors highlighted reciprocity of connections between posterior insula and the OP cortical areas. In another study conducted by Krubitzer and Kaas (Krubitzer and Kaas 1990) on *marmoset monkeys*, beside confirming OP cortical connectivity with already discussed parietal sites such as BA 3, 1, 2, authors found the existence of anatomical interconnections with others parietal areas such as the one immediately rostral to SII (the PV area). They moreover investigated connectivity with frontal regions such as motor cortex and oculomotor fields. Injections were placed into SI (BA 3b) and SII, which were identified with microelectrode mapping techniques. Concerning results obtained from injection in SII, significant patches of labels in area 3b were obtained, supporting the evidence of strong interconnections. Labeling patches resulted also in midportion of areas 3a and 1, extending into adjoining parts of area 2. Moreover, labels were obtained also in PV region and in sites immediately rostral to PV. In relation to connectivity with frontal lobe, the appearance of various scattered foci in frontal cortex nearby the central sulcus demonstrated interconnections also with M1. Projections to SMA were also obtained in two animals. In this study, the SII injections also resulted in labels in correspondence of others frontal regions in the frontal eye field (FEF) and the frontal visual area (FV) fields. Moreover, SII showed to have interconnections with limbic cortex medial to SMA. Authors investigated also interhemispheric connections revealing callosal connections between SII and contralateral PV and SII. Additional foci due to transcallosal pathways were found in areas 1, 3b, and M1. Results related to connectivity with the frontal lobe are quite consistent with those obtained in a following study (Stepniewska, Preuss et al. 1993) conducted on owl

monkeys. In their work, Stepniewska and colleagues injected tracers in primary motor cortex of the animal after identification of the injection site by means of intracortical microstimulation. They were then able to provide evidences for somatotopically distributed connections with motor areas such as dorsal premotor cortex (PMd), ventral premotor cortex (PMv), and SMA, but more interestingly, they confirmed the existence of an anatomical projection with somatosensory regions BA 3a,b, 1, 2, and SII and PV. They moreover highlighted connections with posterior parietal cortex, and cingulate cortex. Authors were furthermore able to differentiate connectivity between caudal M1 (M1c) and rostral M1 (M1r) which shown to produce different labeling. The former appeared to be connected primarily with somatosensory areas, while the latter with both non-primary motor cortex and somatosensory cortex. An exhaustive paper published by Cipolloni and Pandya (Cipolloni and Pandya 1999) investigated in *Rhesus monkeys* by using antero and retrograde tracer technique, the cortico cortical connectivity for fronto-parietal opercular areas considering the entire upper bank of the Sylvian fissure. In their work they subdivided the dorsal Sylvian opercular area into four rostrocaudal sectors. As they described: “*The frontal operculum extends from the beginning of Sylvian fissure to the inferior precentral dimple; the pericentral operculum refers to the cortex at the level of the ventral tip of the central sulcus from the inferior precentral dimple to the rostral tip of the intraparietal sulcus; the parietal opercular region behind the pericentral operculum extends from the end of the pericentral operculum to the caudal end of the Sylvian fissure and is divided into rostral and caudal regions.*”. In relation to parietal projection of the OP region, their results show pathways of connections to midpoint of BA 3 as well as caudal portion of BA 2, 1. Additional projection to area BA 5 and 7 of IPL were highlighted by tracers. Additionally, others connections towards rostral portion of the SII region in the operculum as well as to the rostral insula, the gustatory area, and the vestibular cortex were also shown. According with labels obtained in the frontal lobe,

pathways of connection between the OP and the midportion of area 4, area 44, the ventral portion of area 6, and with proisocortical motor area (ProM) were identified. At last, authors showed this area projecting to the caudo-ventral portion of area 46, to the cingulate motor area and to SMA. With fluorescent retrograde tracers, they were also able to show that the OP receives connections from virtually all of the cortical areas to which it projects. Ipsilateral and interhemispheric cortico-cortical connectivity of regions embedded in OP in marmosets have been investigated also by Qui and colleagues (Qi, Lyon et al. 2002). In their study they focused their attention in discuss PV cortical connectivity. SII and PV were selected as injection sites and they were identified by means of microelectrode mapping methods. Their results identified in area BA 3b the major inputs provider to area PV. Patches obtained from labeling showed anatomical connections with other parietal regions such as BA 3a and 1, SII, the region rostrally to PV (named parietal rostral areas (PR)) and a region folded into the lateral sulcus defined ventral somatosensory cortex (VS) by Cusick and colleague in 1989 (Cusick, Wall et al. 1989). They moreover found noteworthy callosal connections with contralateral PV and, as they wrote, "*probably also with contralateral SII*". In the end, they also investigated thalamocortical connections of area PV, showing that major thalamic connections of PV were with ventroposterior (VP) and ventroposterior inferior nucleus of thalamus (VPI). Finally, Disbrow and colleagues (Disbrow, Litinas et al. 2003) investigated the OP ipsilateral as well as contralateral connectivity in order to identify specific patterns of connections of S2 and PV on four *macaque monkeys*. Neuroanatomical tracers were injected into electrophysiologically defined somatosensory cortical fields such as SII and PV. At first, for both SII and PV, authors demonstrated significant connections immediately close to the injection site. Regarding connections with anterior parietal fields, the injection centered in SII resulted in labels in area BA 3b as well as area 1 at the same mediolateral locations as that in 3b suggesting that connections with area 1

are matched as well. However, label in area 1 was much less dense than in area 3b. A dense patch of label was also highlighted in the field termed area 7b. In one of the four cases, labeling appeared also in orbitofrontal cortex rostral to the arcuate sulcus. Transcallosal connections were with the middle portion of both S2 and PV were identified and labels were also observed in area 7b, and in area 3b. According with labeling obtained after injection in PV region, this area appears connected with area 3b and area 1. Very dense label was also observed with area 7b. Differently from injection in S2, tracers in PV produced a dense patch in PR, just rostral to PV. Interhemispheric connections resulted in transported tracers predominantly in a middle portion of PV. A small patch of label was also observed near the 3b/PV border. At last, other two recent studies investigated the frontoparietal pathways of connections by injections of tracers in motor regions electrophysiologically defined. In the former study (Gharbawie, Stepniewska et al. 2011) by means of long trains of electrical stimulation regions, authors identified in M1, PMC and area 2 of four macaque monkey regions that they defined "grasp zones". Stimulation over such region evoked movement classified from authors as grasping (i.e. flexion of all digits, flexion of digit 2 and opposition with digit 1 and concurrent flexion of all digits and forearm supination of wrist extension). Retrograde tracers were injected in such regions. Regarding connections originated from M1 injection, they mainly found labeled cells in frontal cortex motor regions equally distributed among SMA, dorsal cingulate motor area (CMA_d) and PM_v. Considering labeling obtained outside the frontal cortex, the densest concentration of stained cells were found in anterior parietal cortex, mainly in area 3a, and S2/PV. The same authors published a second study (Gharbawie, Stepniewska et al. 2011) in which they examined the connections of posterior parietal cortex (PCC) with motor and premotor cortex of new world monkeys (squirrel monkeys and owl monkeys). Again, by means of electrical stimulation they identified in M1, PM and PCC different zones such grasp zone, reach

zone, the defense zone by classifying the nature of evoked movements. In such zones they injected retrograde tracers. They found dense connections between reach, defense and grasp zones with hand and forelimb representation in M1. Regarding M1 connections, beside dense inputs received from PCC they obtained connections with somatosensory hand/forelimb representation in area 3a, 3b, 1 and the area S2 /PV. In particular the latter region resulted labeled for connection with the grasp zone of M1. Together, such results are indeed in support of the involvement of OP in cortical networks involved in grasping. At last, a seminal work of (Galea and Dariansmith 1994) by injecting retrograde tracers in the cervical spinal cord of 11 *Macaque monkeys* investigated the corticospinal projections from neurons belonging to the somatosensory cortex. They identified at least nine different somatotopically organized projections.

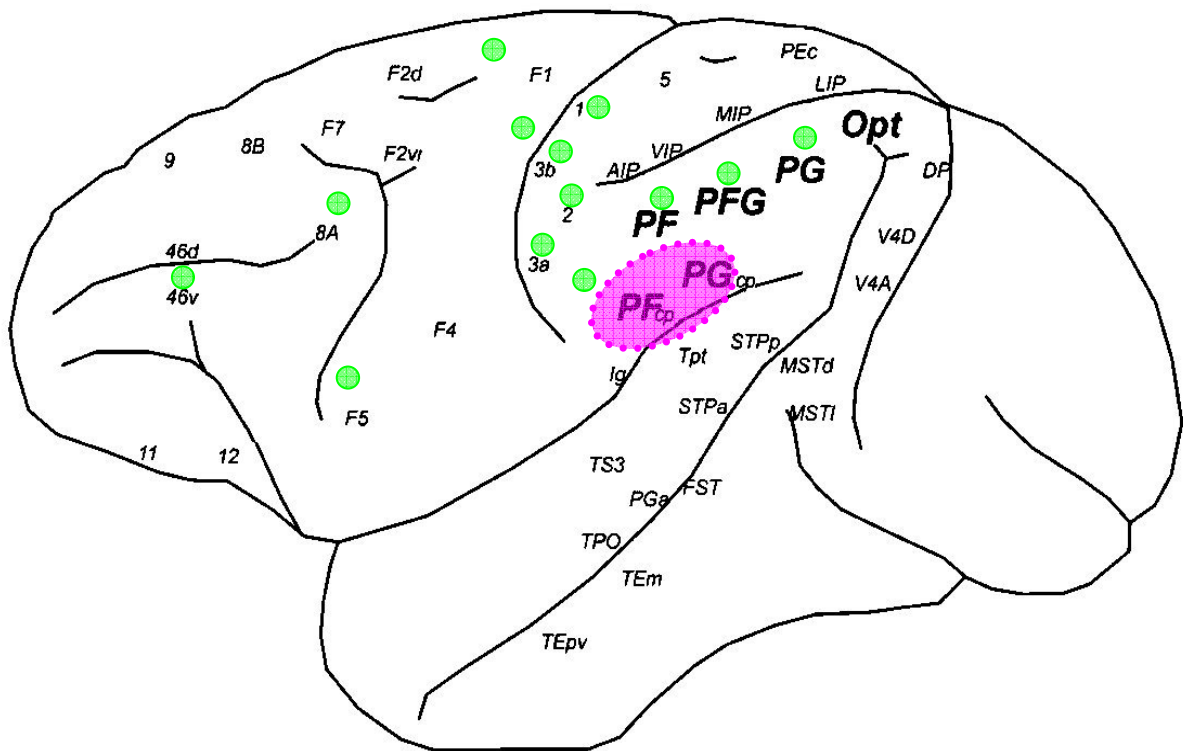


Figure 6. OP connectivity map summarizing anatomical connections. The parcellation scheme is the one proposed in (Pandya and Seltzer 1982) anche the image was taken and adapted form (Caspers, Eickhoff et al. 2011) Pink ellipsoid represent the OP region. Green dots represent point which showed connections with OP. Transcallosal connections to controlateral SII and PV are not shown here as well as thalamic connections and posterior part of ipsilateral insula.

The most prominent projections were to frontal cortex (area 4, area 6, SMA, postarcuate cortex and the cingulate cortex). Beside projection to frontal cortex, the highlighted ipsilateral and contralateral projections from S2. They were able thus to show different separate population of corticospinal neurons and each of which with continuous access to all spinal motoneuron populations, stating the importance of cortical and spinal connections and highlighting their involvement in action coordination. A map summarizing main connection of the OP cortical fields is reported in Figure 6. The parcellation scheme was chosen according with the one proposed in (Pandya and Seltzer 1982).

Connectivity of OP in humans

Literature about anatomical connectivity of the human OP is quite poor. And it is therefore necessary to refer to the one available for monkey studies. Despite the little presence of documentation related to this topic, a non-invasive anatomical investigation published by Eickhoff and colleagues (Eickhoff, Jbabdi et al. 2010) explored possible connection pathways between the OP and different brain regions. Through diffusion tensor imaging DTI probabilistic tractography technique they produced different distributions of possible connections between the subregions OP1 and OP4 (see (Eickhoff, Jbabdi et al. 2010)) and parietal areas, frontal regions and thalamus. Diffusion weighted images were acquired along 60 independent directions at 1.5 Tesla for seventeen healthy subjects. As seed region for DTI probabilistic tractography they considered the maximum probabilistic map (MPM) of area OP4 and OP1. For each subject, these maps identified the most likely anatomical area to which each voxel belonged after coregistration to a standard template. Same approach was adopted to create target mask to reach with probabilistic tractography. Target regions were chosen

in frontal lobe (Broca, PMC) and primary motor cortex (M1)), parietal lobe (post central gyrus (PCG), anterior intraparietal sulcus (aIPS), anterior superior parietal cortex (aSPC), posterior superior parietal cortex (pSPC), anterior inferior parietal cortex (aIPC) and posterior inferior parietal cortex (pIPC)) and thalamus (ventroposterior lateral/inferior nuclei VPL/VPI and ventrolateral nucleus (VL) and ventroanterior nuclei (VA)). For each subject they drew 5000 samples from the connectivity distribution and computed the mean probability of connection for each seed-target combination. IPC and PCG appeared as the most connected regions to OP1 and OP4. In particular, while aIPC showed significantly higher connection probabilities with OP1 than with OP4, PCG turned out to be more densely connected with OP4. Moreover, a strong level of connectivity was also computed between OP1 and VPL/VPI as well as a significant number of transcallosal connections. Area OP4 showed instead significant higher probabilities of connections with Broca region, PMC and M1. No significant difference between both OP areas with respect to their possible connections with aIPS, pIPC VI/VA and SPC. In comparison OP connectivity found in monkey studies which shown SII and PV areas with dense reciprocal connections to PCG and IPC (Krubitzer and Kaas 1990) (Disbrow, Litinas et al. 2003), Eickhoff work confirms these pathways of connectivity OP1 (SII) and OP4 (PV) in humans. In support of human direct connectivity of OP with M1 there are also neurophysiological evidences provided in (Cattaneo and Barchiesi 2011). In this work authors mapped by means of double-coil transcranial magnetic stimulation (TMS) the functional interaction between left M1 and several loci belonging to different ipsilateral cortical regions such as posterior dorsal frontal cortex, ventral frontal cortex, anterior dorsal frontal cortex, OP, inferior parietal cortex and superior parietal cortex. Concerning OP, authors provided a novel finding related to its functional connectivity to M1 showing how the conditioning stimulation applied over OP introduced a significant inhibition of the motor evoked potentials (MEP) originated by the test stimuli

delivered over ipsilateral M1 at a very short latency (4-7 ms) from conditioning stimuli. Such results doesn't provide direct evidences of anatomical connections between the two regions, however, the resulting short-latency interaction supports the plausible existence of direct pathways of connections between OP and M1.

Physiology of OP in non human primates

As already discussed, the OP embeds the area SII. Basically, neurons in this area fire accordingly to different tactile stimulations of skin receptors. They moreover show activation during processes of texture discrimination and their response is also obtained with noxious and visual stimuli. Furthermore, neuronal firing within SII region has been recorded also in presence of thermal and proprioceptive inputs. Hereafter some literature about physiological properties of SII and characterization of its receptive fields defined in non human primates by means of electrophysiological intracortical recordings and stimulation will be illustrated.

Recodring studies

In 1995 Krubitzer and colleagues (Krubitzer, Clarey et al. 1995) proposed their definition of somatosensory areas in the lateral sulcus of *macaque monkeys*. By recording electrical response of neurons in SII region they were able to detect two complete representations of body part embedded into OP. Light cutaneous stimulations delivered with probes, brushes, or fingers and joints manipulations were applied to the animal. Signals recorded from about 1000 sites were used to determine the somatotopic organization of the region. Results shown that, in a number of distantly located regions of the lateral sulcus, the same body parts were represented bringing authors to identify

two major symmetrically organized body maps lying in correspondence of the OP. The field adjacent to the lateral boundary of area 1 and 2 was designated SII while the field immediately rostral bordering lateral boundary of area of area 3b and 1 was defined PV. The mediolateral sequence of organization was similar for both fields. Figure 7 shows a summary map of the somatotopic organization of the OP for *macaque* and for *marmoset* (according with (Krubitzer and Kaas 1990)). As it is possible to see in the figure, oral structures and face are represented most superficially on the lateral sulcus or medially in the flattened cortex (inner part of the sulcus). The forelimb representation lies laterally to the one of the face or deeper in the intact brain. Hindlimbs are mapped in the deepest portion of the upper bank of the Sylvian sulcus. The trunk representation in SII is located caudal to limbs mapping while in PV it lies rostrally. In a following paper, written by Fitzgerald and colleagues (Fitzgerald, Lane et al. 2006) authors investigated response properties of neurons belonging to SII region produced by cutaneous and proprioceptive stimulation of the hand of *macaque monkeys*. They moreover characterized sizes and shapes of receptive fields (RFs) and response dependency from orientation of the tactile stimulus. Cutaneous stimulation consisted in skin brushing or tapping of finger pads. Proprioceptive inputs were identified in different kinds of manipulation (passive extension/flexion /torsion of hand joints, maintaining the joints in different conformation and observations of active movements of manipulation of objects). Responses dependency from orientation was tested using a direction-adjustable bar. They identified three separate functional fields (anterior, central and posterior) within the OP region responding to stimuli. Central field showed to be more reactive to cutaneous stimuli and it appeared to be medial to lateral somatotopically organized with digits represented in an orderly manner. Anterior and posterior fields responded more weakly to cutaneous stimulation and for these regions authors were not able to define a distal to proximal

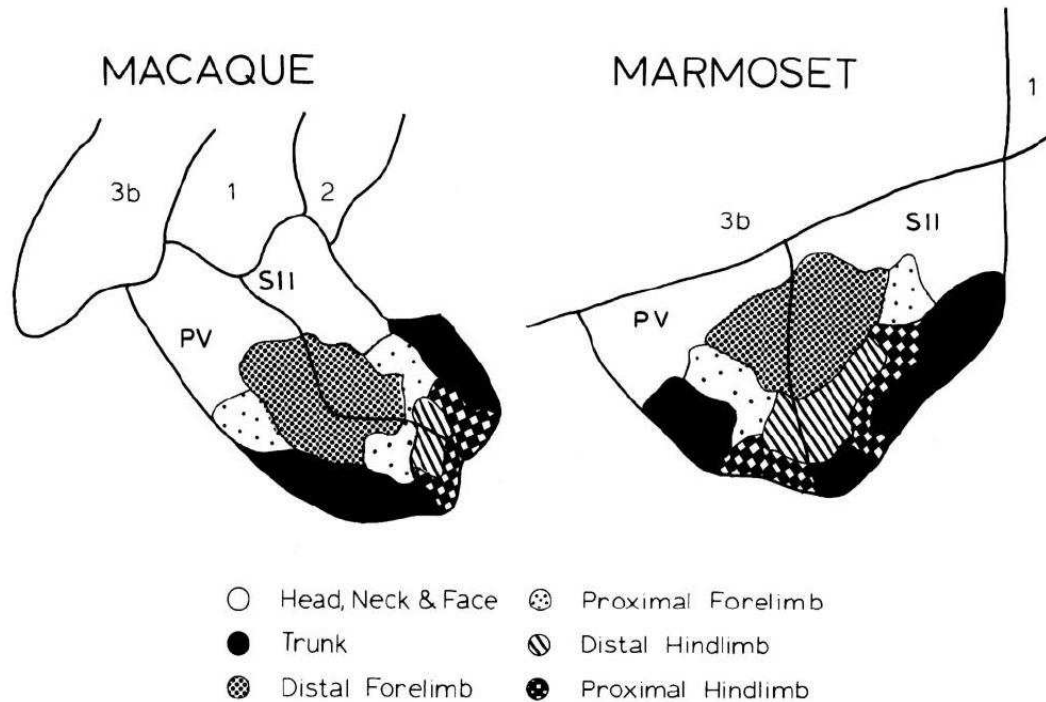


Figure 7. Summary map of the somatotopic organization of SII and PV in macaque monkeys (left). For comparison also the somatotopic organization of marmoset has been proposed (right) (Krubitzer and Kaas 1990). The two fields SII and PV appear to have a symmetric organization and they share a common boundary at the representation of the digits, toes and portion of the face.

organized digit representation. They were generally responsive to passive flexion or extension of digits and to grasping movements performed by the animal. In relation to RFs properties authors saw that RFs size appeared to vary significantly along anterior-posterior axis while the RF shape (in terms of contiguity of surface) did not seem to vary substantially along anterior posterior axis. Firing rate showed different features for central field during cutaneous stimulation respect to anterior and posterior. Respect to bar orientation, authors found that neuron response belonging to anterior and central fields was more commonly modulated respect to those of neurons belonging to posterior field. Despite different responses, authors did not define these three fields as separate cortical regions. According with pre-existent studies (e.g. (Krubitzer, Clarey et al. 1995))

they hypothesized their anterior field may correspond to area PV and that combined central-posterior fields might be the equivalent for SII.

Stimulation studies

Intracortical micro stimulation (ICMS) of SII in non human primates provided interesting results about motor effects yielded by the stimulation. In mid 80's, Mori and colleagues (Mori, Babb et al. 1985) applied ICMS on brain of three *Macaca fascicularis*. Electrodes for both stimulation and recording were placed in the region bounded by the Sylvian fissure, the intraparietal sulcus, and the caudal border of the postcentral gyrus. Authors obtained seven sites located deep in the Sylvian sulcus which yielded effects such as wrist extension, eye blinking, knee extension, ear retraction, foot extension, and elbow extension. Each of these effects was evoked singularly in only one site and they appeared only contralaterally. Tactile stimulation was then applied to body parts which previously showed motor effects. Neuronal firing recording revealed that the most effective stimuli were related to the type of movement evoked from the cortex. This means that touch of the skin around the eye produced firing of neurons that, if stimulated electrically caused eye blink. The same result was obtained for touch of the knee skin and firing of those neurons which previously caused knee extension. In another paper (Burish et al., 2008) frontoparietal cortex of marmosets was extensively stimulated with ICMS involving motor, premotor and somatosensory areas. Results coming from ICMS were correlated with architectonic features of stimulated cortex obtained by means of anatomical techniques. Location of electrodes implant was estimated based on bone landmarks and cortical surface vasculature. Movements evoked during experimental session were broadly classified into four body regions: facial, forelimb, trunk and hind limb movements. Concerning results related to the OP region, authors reported that the

majority of stimulation sites in PV and SII were unresponsive. However they found high threshold face movements evoked from both areas including the lower lip, eyelid and nose and in one case they obtained elbow movement evoked from presumably area SII.

Non invasive neuroimaging study

In a recent non invasive neuroimaging study (Nelissen and Vanduffel 2011) by means of fMRI authors provided interesting results about grasping-related brain response in macaque monkeys. Two rhesus monkeys were tested in two different task both performed in the dark in order to ensure that fMRI activations were due only to the motor aspects of the task and not to the visualization of the object involved. Authors compared activations during two different motor tasks: a reach and grasp task and a reach-only task. As results, evaluating grasping versus reaching, they obtained in the controlateral hemisphere a significant difference in activation in portions of premotor F5, a larger activity in the hand motor area F1, in region S1, in area AIP, in the rostral lower bank of IPS, and in area PFG. Moreover, they showed several activations in regions corresponding to SII and PV and also in area VS. Such regions belonging to OP cortex were found activated also in the ipsilateral hemisphere. They justified the activity of somatosensory regions as a tactile feedback necessary for grasping and control of finger force. They however were not able to attribute the differential fMRI response in somatosensory cortex during the two tasks to either the hand preshaping or the haptic contact of with the object due to the fact that they didn't record detailed hand kinematics.

Physiology of OP in humans

In human OP, its physiological properties and receptive fields have been studied with different techniques. At first, electrophysiological intracortical recording and stimulation studies have been conducted on epilepsy patients in early 50's. Later, with the advent of non invasive techniques the activation of human OP has been largely investigated with neuroimaging studies which supported electrophysiological outcomes and provided further details about its somatotopic arrangements.

Recording and stimulation studies

Effects of cortical stimulation of the OP region on epilepsy patients were firstly investigated by Penfield and colleagues (Penfield and Boldrey 1937) (Penfield and Rasmussen 1950). In their study they collected data from over 400 patients providing important details about first and second sensorymotor cortex and supporting evidences for location of SII in the parietal operculum and its somatotopic mapping. A more recent study involving both cortical stimulation and direct recording have been conducted by Woolsey and colleagues (Woolsey, Erickson et al. 1979). In this study cortical stimulation was applied on 20 neurosurgical patients whose disorders required wide exposure of the Rolandic region in order to arouse paresthesia and to induce movements due to excitation of postcentral and precentral cortex. In relation to the cortical recording, elicitation of potentials was done by using a tapping device which applied a mechanical stimulation over the body surface. Results of this study, beside providing evidences for somatotopic mapping of body parts for both primary motor cortex and primary sensory cortex, allowed authors to identify a second somatic sensory area located within the OP. Excellent evoked potentials were in fact yielded in correspondence of one recording site belonging to the OP by the mechanical stimulation

of both hands. Moreover, cortical stimulation applied in a point immediately medial produced plantar flexion contralateral foot. Authors highlighted consistency of these result with SII somatotopy obtained for primates.

Non invasive neuroimaging studies

Non invasive neuroimaging studies allow the investigation, in our case, of OP region in healthy humans avoiding researchers to include in results potential effects due to disease. In one positron emission tomography (PET) study published by Burton and colleagues (Burton, MacLeod et al. 1997), authors assessed the number and the distribution of cortical regions activated by rubbing textured surfaces across fingertips. Surface contact was applied directly on the skin or through an imposed tool. Different peaks of activation in correspondence to hand region of SI was obtained according with skin modes stimulation. In relation to the OP activation, authors found that two modes of stimulation yielded different peaks as well. They in fact observed that peak activated during skin mode stimulation lied rostrally respect to peak activate during tool mode stimulation. These findings brought authors to hypothesize that, as was found in monkey studies, also human S2 could be subdivided in two major fields one of those reacts stronger to tactile stimulation. Some years later Disbrow and colleagues (Disbrow, Roberts et al. 2000) by means of functional magnetic resonance imaging (fMRI) provided detailed evidences supporting such subdivision of cortex within the OP. By applying tactile stimulation over different body parts (rubbing with large sponge right palm and back of the hand, foot, cheek and half mouth, shoulder and leg) authors provided evidence for four somatosensory fields. Two of them, identified as SII and PV, showed a mirror symmetric representation of the body surface. Their statistical analysis on the Talairach coordinates of center of mass of body part representations indicated a

medial to lateral progression from foot to hand to face. Bordering these body part representations they observed activation in response to stimulation over shoulder and hips. The other two fields identified by authors, named rostral region (RL) and caudal region (C) showed not consistent activation across subjects in response to somatosensory stimuli. The representation of activation obtained for OP cortex is shown in Figure 8A. The somatotopic arrangement for SII and PV and the one obtained for macaque monkey (according with (Krubitzer, Clarey et al. 1995)) are reported in Figure 8B. In another multimodal neuroimaging study Del Gratta and colleagues (Del Gratta, Della Penna et al. 2002), the response of OP cortex as consequence of nerve stimulation was showed. By means of comparison between fMRI activation and equivalent current dipole (ECD) localization provided by magnetoencephalography (MEG), authors produced a rough but clear somatotopy of SII region. Electrical rectangular pulses were delivered to medial nerve at wrist or to tibial nerve at the medial malleolus during both fMRI and MEG acquisition. Their fMRI results showed a consistent segregation of activated voxels within the OP region due to stimulation of upper and lower limb nerves highlighting a topographical organization of SII with the lower limb located more caudally and medially then the upper limb. Authors found confirmation of fMRI results in MEG recording. ECD localization showed the median nerve area located more anteriorly and more inferiorly respect to the tibial nerve one confirming the somatotopic organization of SII. Authors found a slight mismatch between ECD and fMRI activations which they ascribed to coregistration errors between MEG and structural MRI data. Beside response to tactile stimulation and nerve stimulation, SII activation can be elicited as a consequence of different somatosensory stimuli. This has been demonstrated in a fMRI investigation conducted by Mazzola and colleagues (Mazzola, Faillenot et al. 2012) In this study authors investigated contralateral and ipsilateral parieto-insular cortical response to five different types of stimuli applied on left

hand of subjects. On the base of cytoarchitectonic subdivision proposed by Eickhoff (Eickhoff, Schleicher et al. 2006) authors aimed to investigate whether these proposed subdivisions (named from OP1 to OP4) could embed distinct aspect of discriminative somatosensory functions. Stimuli employed for all subjects on the same body area were tactile stimulation, passive movements, innocuous cold stimuli, non noxious warm stimuli and heat pain. Results related to activation of controlateral and ipsilateral OP and insula are showed in Figure 9.

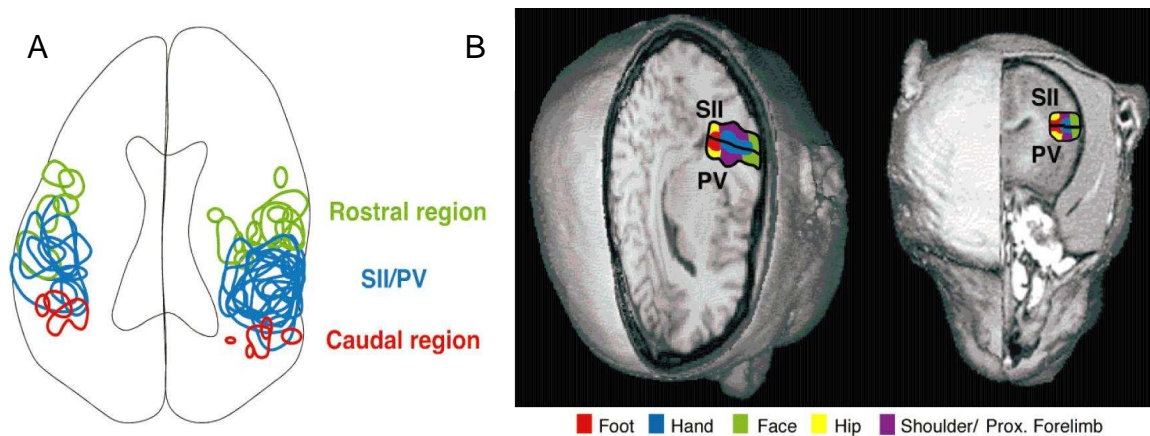


Figure 8. A: Reconstruction of an axial slice through the lateral sulcus with the activity patterns from all 16 cases collapsed onto a single drawing. All of the blue encircled areas denote the SII/PV region from all of the cases, the green denotes the rostral field (RL), and the red denotes the caudal field (C). B: Summary of the somatotopic organization of the second somatosensory area (SII) and the parietal ventral area (PV) on the upper bank and parietal operculum of the lateral sulcus of human (left) and macaque monkey (right) (as described in (Krubitzer, Clarey et al. 1995)).

In this work authors showed that all the different types of stimulation tested activated at least one region in SII and insula cortex and moreover that different stimuli corresponded to different patterns of activation. In relation to OP areas, controlateral OP1 showed activation for all types of stimulation suggesting its unspecific role in processing different somatosensory inputs. Both brush and passive movements activated controlateral and ipsilateral OP1 reminding to the similarities between the two

stimuli in terms of stimulated peripheral fibers. OP2, activated with cooling stimuli, was proposed by author as a possible sensory cortex for cold sensations. OP3 revealed its activation during brushing and pain stimuli and according to authors, this result was consistent with literature. At last, a bilateral widespread and intense activation of OP1 and OP4 was obtained after painful stimulation which leded authors to argue in favor of an anterior specificity OP region to pain stimuli.

Summary of physiology of OP

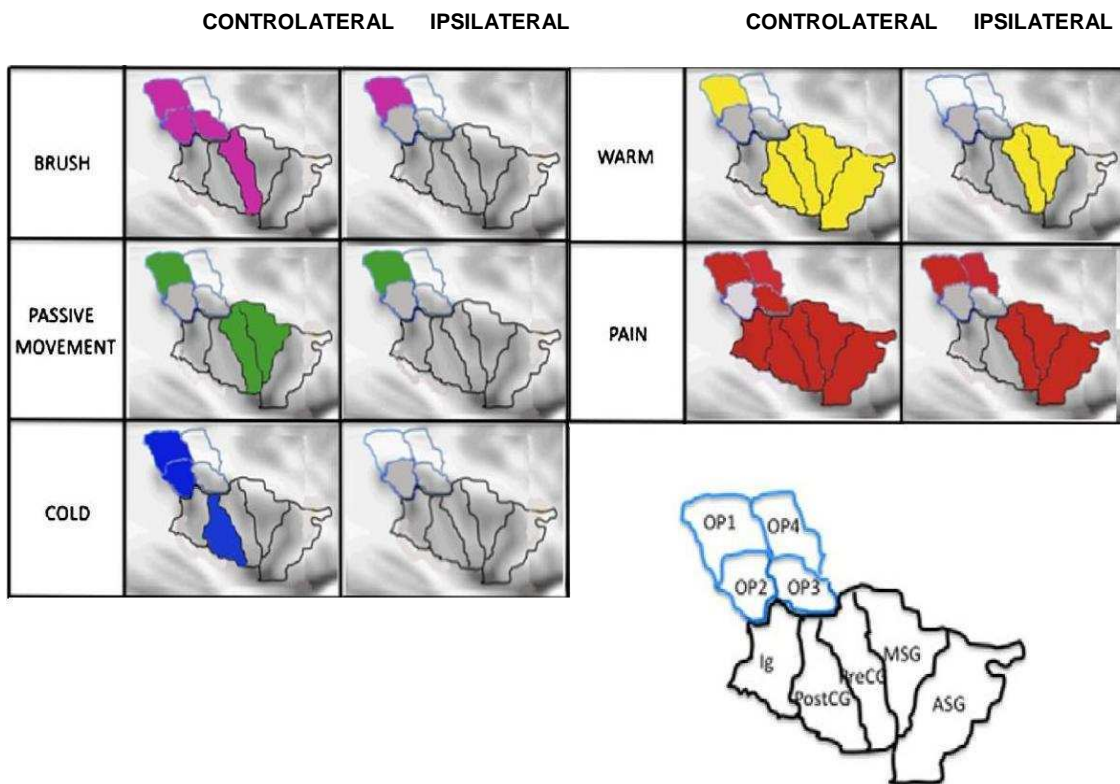


Figure 9. Group activation maps after stimulation of the left hand. Left: controlateral activation of SII/insula. Right: Ipsilateral activation of SII/insula. (Mazzola, Faillenot et al. 2012). On the low-right: the new mask representing the regions of interests used in the analysis. Parietal operculum is composed by OP1, OP2, OP3 and OP4 (Eickhoff, Schleicher et al. 2006). Anterior insular cortex is composed by anterior short gyrus (ASG), middle short gyrus (MSG) and precentral insular gyrus (PreCG). Posterior insula includes Ig1, Ig2, Id1 and the post-central gyrus (PostCG) according to the probabilistic maps based on cytoarchitectonic maps included in (Eickhoff, Stephan et al. 2005).

According with results obtained for non human primates and humans, the OP is generally considered the site for the second somatosensory cortex. Despite slight differences presented in literature related to the number, the extension and the borders of fields composing this somatosensory area, a shared outcome is represented by its activation due to tactile stimulation. This feature allowed researchers to investigate its somatotopic organization leading them to find high analogies between non human primates and humans mapping. Moreover, non invasive neuroimaging studies allowed researchers to study SII response to different kind of stimulation, showing its activation also after noxious input, nerve stimulation and joints manipulation. Results have been investigated with different techniques which showed consistency in terms of location of cortical activation.

In the next chapter...

This second chapter focused on the literature related to the anatomy and and the physiology of the parietal operculum in both primates and humans. In the next chapter, some of the literature about the involvement of such region in sensorymotor transformation in feedback model and in feed-forward models will be reviewed together with some studies on tactile agnosia.

Chapter 3

The parietal operculum in sensory-motor integration: a literature review

The above mentioned studies deal with the issue of how the brain recognizes passively presented complex tactile stimuli. In daily life in ecological settings, haptic knowledge of the world relies on active exploration, therefore movement and haptic recognition and manipulation are intimately linked. Information provided by sensory modalities are processed within the brain in order to produce a suitable object directed action. These sensory inputs are the framework that allows the motor system to plan, coordinate, and execute the motor programs responsible for voluntary and purposeful movements. Motor control relies on several processes that are generally classified into feed-forward (or open loop) circuits and feed-back (or closed-loop) circuits. Prediction and correction are two concepts related to these two models. For example during a voluntary grasping action, in a feed-back model, sensory signals are monitored and compared with a desired state, represented by a reference signal. The difference, or error signal, is used to adjust the output acting directly on the limb. In a feed forward mode, perturbations are detected with various sensory modes and anticipatory movement strategies based on experience are applied. Sensory information can be used therefore in two ways, for feedback and for feed-forward. In particular, considering the aspect of connectivity already discussed, these somatosensory information which are processed in motor control might reach M1 also via a cortical loop connecting it with the OP regions (in particular PV and SII).

OP and sensory-motor integration

Human and monkey studies discussed above (e.g. (Krubitzer, Clarey et al. 1995), (Disbrow, Roberts et al. 2000)) related to somatotopic organization of the OP allowed inferences about possible participation of this region in sensorymotor integration and in tasks involving hand motor control. Beside these studies, there are also several studies which specifically investigate the OP cortical activation and neural circuitry involved in hand-object interaction and effects on OP cortex regions of motor task execution. In one fMRI study conducted by Binkofski and colleagues (Binkofski, Buccino et al. 1999) subjects were required to perform a continuous manipulation and tactile exploration of three-dimensional either recognizable or not recognizable complex objects requiring thus continuous finger movements and a constant change in finger configurations. Different conditions such as manipulation of complex object versus rest, or versus manipulation of a sphere were tested. Authors found an activation of SII and adjacent areas in all tasks of their experiment observing a particularly strong activation in the condition in which complex object manipulation was compared to sphere manipulation. Authors proposed SII as a component involved in the control of exploratory manipulation and in description of the objects in terms of their intrinsic physical properties highlighting its functional role in capturing information from the external world. In a similar paper the same authors (Binkofski, Buccino et al. 1999) compared the activation of SI and SII in complex and meaningless object manipulation. Since they observed a high statistical significance of SII activation during manipulation but they didn't observe a parallel activation in SI they hypothesized that such activity in SII was probably not due merely increased tactile stimulation. They proposed a couple of explanation for this activation: the first one following the theory of Murray and Mishkin (1984) which depicts SII as a player in tactile object discrimination; the other hypothesis is supporting connections between SII and

ventral PMC and the possibility of information exchange between the two region finalized to direct and control of finger in order to adapting them online to stimuli configuration. In another study Lin and colleagues investigated SII involvement in muscle contraction and motor task modulation (Lin, Simoes et al. 2000). They in fact explored the effect of motor activity from different body parts on somatosensory responses to left median nerve stimulation. Authors recorded somatosensory-evoked magnetic fields (SEFs) during five different conditions such as rest without voluntary contraction, contraction of the masseter muscles, contraction of the left deltoid muscles, contraction of the left thenar muscles, and contraction of the left tibialis anterior muscles. Authors obtained that responses from both the right (controlateral) and ipsilateral second somatosensory cortices (SII) were significantly enhanced during contraction of the left thenar muscles. Responses from the left SII were significantly enhanced also during contraction of the left deltoid muscles, but they were decreased during contraction of the masseter and left tibialis anterior muscles. Their results indicated that motor activity from various body parts differentially influenced SII activity and that the modulation of SII activity seemed to depend on the topographical proximity of the contracting muscles to the stimulated body part. In their discussion authors argued that the dependence of SII activation on motor activity at different body parts implied spatial tuning which could be helpful for monitoring and correcting sensorymotor performance. In another fMRI study (Milner, Franklin et al. 2007) the OP is hypothesized as a region of integration of proprioceptive and tactile information aimed to control of movements. In this work the neural mechanisms that contribute to the ability to manipulate an object with complex dynamics were studied by asking participants to perform two different tasks: the formers was a complex object manipulation consisting in balancing in an unstable equilibrium position a weighted flexible ruler, the second task required to squeeze a soft foam balls. Besides obtaining a strong activation of cerebellum during the complex object manipulation, they obtained a

significant bilateral activation of both SII region and insula. They discussed the role of SII as a region involved in somatosensory integration finalized to produce a coherent image of the object for cognitive action. Considering together that afferent of SII are cutaneous and proprioceptive types and that such region project also to M1, authors presented SII as a feedback provider for M1 due to its putative involvement into integration of proprioceptive and tactile information.

OP in feedback mode - visually guided movements

However despite the growing literature on the OP involvement in sensory motor integration, the issue of definition whether OP sensory inputs are involved in motor control such as grasping actions as feedback signals in closed loop mode or if they provide inputs for anticipatory strategies in feed forward mode is still open. If we have a look at studies that did not explicitly address this issue but that more generally investigated brain activations during grasp, we can clearly see that systematically the OP region is involved. In a PET study Grafton and colleagues (Grafton, Fagg et al. 1996) investigated healthy subjects performing three visually guided motor paradigms consisting in grasping, pointing and a control condition. In the grasp task, subjects reached and grasped cylindrical targets repetitively; in the point task, subjects were instructed to point over the top of the cylindrical targets with their right hand using a natural pointing hand position with the index finger extended and all others flexed in a comfortable fist. As in the grasp task, they returned the hand to a resting position. In the control task, subjects were told to leave the right hand at rest on the chest and to simply look at each target as it was illuminated. Different cortical regions such as primary sensory and motor cortex, premotor cortex, mesial frontal cortex, and cingulate sulcus showed their activation in proposed tasks in individual analysis. In the group analysis

authors found two adjacent sites showing significant task effects in the OP. Authors in fact reported that the main difference between the grasp and the point tasks was the marked activity in the OP during grasping, especially in the site containing the putative SII. As primitive interpretations of obtained results authors stated that the increased SII activity could be merely due to greater simple sensory input because the subjects were touching the objects in the grasp task and not in the point task. However, considering that the primary sensory cortex showed no difference in activity between the two tasks they speculated that the OP might be also involved in higher level processing of object shape based on tactile information in prehension. One year later Faillenot and colleagues (Faillenot, Toni et al. 1997) published another PET study aimed to identify the functional anatomy of the mechanisms involved in visually guided prehension and in object recognition. In the study subjects were required to perform different tasks such pointing, grasping and matching. The first required to point with the right index finger towards the center of the different objects (whose shape was not referred to usual objects) and come back to the starting position. The second task consisted in grasping with precision grip the object with the right hand and placing it on the table. A third task require the participant to observe the objects and press a mouse button each time the shapes of two consecutive objects were identical, irrespective of size and orientation. Authors obtained activation of left inferior postcentral sulcus (BA 2/40) and extended towards the area SII in both the pointing and the grasping tasks by comparing them with activity during matching task. They moreover observed that the ventral part of the postcentral sulcus was activated by the grasping task only. They moreover verified that coordinates of this ventral part corresponded to those of the area found by (Grafton, Fagg et al. 1996) in the lateral OP involved in the grasping task but not in the pointing task. In one fMRI study (Frey, Vinton et al. 2005), again OP activation was shown during grasping. With their research Frey and colleagues investigated the role of human

putative aIPS in visually guided grasping assuming it as a crucial component in using visual shape to determine opposition space of the hands. Stimulus shapes grasped by subjects consisted of all 12 objects and used in psychophysical testing (Blake, Brady et al. 1992). Subjects performed serially three conditions in a fixed order such as point with right index, grasp with right thumb and index, and rest. By comparing activation due to grasping versus the one due to pointing, authors identified two significant sites of activation in the hemisphere contralateral to the performing right hand. The first site was described as area BA40, corresponding to area PF or PDE. The second activation was located in the left lateral sulcus extending into OP. Results of this study provided once again evidences for supporting involvement of human OP in grasping, by showing its activation in association with available haptic information concerning the objects' shapes. All these studies are conducted in the visual modality and therefore OP activation is related to a feed-back control of action during the grasp phase. Interestingly if we compare grasping studies with the few studies on reaching (e.g. (Culham, Danckert et al. 2003) we see that OP is not active. In fact in their fMRI study Culham and colleagues obtained activation in human AIP when object size and shape computations were required to preshape the hand during grasping, while this region was not active during reaching which did not require preshaping.

OP in feed forward mode - working memory

On the contrary, the neuroimaging literature on the use of tactile information for predictive movements is not abundant. Useful insights related to this issue can be found in studies which investigate in general the working memory defined as the short term maintenance and manipulation of information for performing upcoming tasks and the neural substrate underling this processes.

Non-human primates

In monkeys for example, bilateral removal of SII area showed impairment in tactile learning and retention of somatosensory inputs. In the study of Ridley and Ettlinger (Ridley and Ettlinger 1978) monkeys were required to perform a series of different tasks such as object tactile discrimination, object's weight discrimination, unfamiliar objects tactile discrimination, intermanual transfer of unfamiliar tactile discrimination and retention of information acquired during tactile discrimination. Behavioral results were compared to those obtained for a control group of monkey whose did not receive ablation. Mainly, authors showed that lesions of SII caused significantly reduction of performance of animals with lesions during tactile discrimination tasks, during tasks involving inter-manual transfer and showed some evidence of impairment on another unfamiliar tactile learning task. Interestingly, SII with lesions animals were not impaired on tests of weight discrimination and generalization, highlighting that discrimination impairment was confined to touch and did not extend to proprioception. Authors argued that impairments occurred during discrimination tasks were related more to learning than to perception. They in fact claimed that the poor retention of tactile tasks learnt post-operatively could not be ascribed to poor perception given that, in tasks involving prizes according to successful discrimination of some surfaces feature, there was a strong preference in animal response to the unrewarded stimulus. According to authors' opinion, this indicates that all animals could immediately perceive a difference between stimuli and SII was involved in processes higher than haptic perception. In a more recent study, temporary memory for haptically perceived objects in a delayed matching to sample task was investigated by recording neural activity in parietal cortex. In the work of Koch and Fuster (Koch and Fuster 1989) the monkey with vision occluded was first presented with a sample tactile stimulus and, after a short delay (seconds or minutes),

two or more comparison stimuli, one of which was identical to the sample. To obtain a reward, the animal had to choose the stimulus that matches the sample. Reach, grasp and tactile exploration were the actions required to accomplish the task. Authors focused their research on neurons activity in parietal areas BA 2, 5 and 7 (which in the lower part embeds also the OP region). Basically, they found that during the sample manipulation activity there was a gradient of decreasing responsiveness from area 1 to area 7 likely due to decreasing density of specific thalamic inputs and increasing complexity of processing that occurs in moving caudally through the parietal lobe. They however found remaining strongly activated cells in area 7 which authors identified as cells responsive to active touch and somatomotor activity. During delay activity, authors found both sustained inhibition for some cells, and delayed activation for others. The latter were subdivided in non-differentially delayed activated cells and stimuli-specific activated cells. Inhibition was hypothesized as a mechanism for decreasing background noise to enhance the neural responses of an anticipated perceptual event (choice discrimination). Activated cells were proposed as participants in the short term retention of the attributes of the sample object. Non-differentially delay-activated cells were hypothesized as involved in the retention of stimulus features that are common to all trials, while the differentially-activated cells may play a role in the temporary memory of specific haptic attributes. In particular authors explained that activated cells firing during the first half of the delay period, showed a topographic gradient in activation in anterior parietal region like activation occurring during sample manipulation. They hypothesized that these delayed activated cells supported the haptic discrimination that occurs during the sample manipulation period highlighting the role of anterior parietal cortex as neural substrate retentions of haptically acquired somatosensory inputs designed for action. In another study the electrical activity of single neurons belonging to SII cortex was recorded during a decision making task involving a consequent motor action (Romo, Hernandez et al.

2002). Two vibrotactile stimulations at different frequency ($v_1 > v_2$ or $v_1 < v_2$) were presented to the skin of the distal segment of the right hand of four monkeys (*Macaca mulatta*) separated by a period of about 3 seconds. Monkeys were trained to use their left hand to indicate at the end of each trial which of the two stimuli had the higher vibration frequency by pressing one of the two push button located either on the monkey's left side or in front of it. They interestingly that while during the first stimulation, the firing rate of SII neurons encoded the stimulus frequency, during the second stimulation their response were a function of both past and current stimuli. Moreover, by analyzing responses during error trials they found that a bunch of hundred milliseconds after the presentation of the second stimulus SII response was correlated to monkey's choice of action. They thus interpreted these evidences as a putative role of OP in processing past and present sensory information for decision-making.

Humans

In humans, the role of the OP in sensory motor integration, tactile learning and working memory has been investigated with neuroimaging studies. In 2001 Jäncke and colleagues (Jäncke, Kleinschmidt et al. 2001) studied the role of the parietal cortex in linking tactile perception of objects and the manual construction. In the experiment, as control task subjects were required to manipulate a plasticine cube. Activation were investigated for different condition tasks such as: exploring a 3D object in order to haptically infer its shape, imagining the construction of the previously palpated object and finally constructing the object from an amorphous lump of plasticine. With the comparison between the manual modeling in absence of vision and the imagination of the construction authors aimed to dissociate the conceptual component of object construction and perception from the concomitant low level sensorymotor processing

involved in both construction and exploration. Outcomes produced in their work showed similar bilateral activation of region such as anterior and posterior intraparietal sulcus (IPS and AIP) during object exploration and modeling conditions; during imagery, a left-sided network was activated including posterior IPS and ventral premotor cortex vPMC similarly to modeling and exploring conditions. Respect to tactile exploration, authors showed that manual modeling revealed additional activation of supplementary motor area SMA. In relation to activation of SII, in both exploration and modeling respect to imaging task Jäncke and colleagues found that the OP region resulted to be significantly activated even though they did not discuss such activation. The major conclusions of authors was that manual construction on the basis of haptically acquired features such as shape and size involves the retention and the adequate processing of such information and it requires different functions such as production of mental representation of the object and its transformations into appropriate motor trajectories together with to fine tuned bimanual interactions and sensorymotor dexterity. Activation of OP showed in the discussed study highlights active involvement of OP cortex in such processes. In another fMRI study, published by Reed and colleagues (Reed, Shoham et al. 2004) investigated whether the somatosensory system has a specialized neural pathway for object recognition under conditions of naturalistic tactile object recognition (TOR). In the experiment, TOR of real objects was compared versus a rest condition and in a second step TOR of real object was compared to TOR of “non sense” objects. Subjects were instructed execute a “grasp, rub, rub” motor sequence and covertly name the object in case of the real object. Authors found that, accounting for activation of SI and M1 due to motor and somatosensory stimulation, other three networks of stimulation were involved: a TOR network for higher level somatosensory representation, a somatomotor attention circuit and a multimodal ventro-temporal object recognition stream. According with results obtained for TOR network, bilateral activation of inferior

somatosensory association areas including SII were observed. In the somatomotor attention network, authors showed the involvement of frontal regions such as prefrontal cortex, SMA, premotor cortex, frontal eye fields. In relation to the ventro-temporal object recognition stream, they obtained activation of lateral occipital cortex and medial temporal lobe. By focusing on SII activation, authors identified this region belonging to ventrolateral somatosensory pathway which was previously proposed by (Mishkin 1979) as key route for tactile object processing in the integration of features. Authors, observing that significant activation of inferior somatosensory association areas such SII was still existing when sensory motor components were accounted for, concluded that such cortical region might have a key role in non perceptual aspects of TOR such as for example, tactile learning and sensory motor integration of information gained from exploration aimed to generate coherent image of an object.

Lesions studies in humans

Lesion analyses are one of the main neuroscientific tools to infer structure-function relationships. They rely on the combined analysis of a defined neurological deficit and the underlying critical brain area. In our case, studies related to parietal lesions and sensory syndromes and impairments in motor behavior provide useful findings about the involvement of this cortical region.

Among different sensory syndromes there are:

- Elementary sensory deficits
- Astereognosis
- Deficit of sense of position

The above syndromes can be caused by lesions of the peripheral nervous system or of the ascending sensory pathways. A fourth syndrome is tactile agnosia (or deficit in

TOR). Tactile agnosia is an exquisitely cortical syndrome defined as a modality-specific disorder characterized by impaired ability to recognize objects tactually in the absence of more basic somesthetic dysfunction. Following results a couple of studies on patients presenting tactile agnosia due to parietal lesions will be briefly reported. In the first study conducted by Reed and Caselli (Reed and Caselli 1994), authors investigated the issue of finding the faulty step in the perceptual-mnemonic pathway in a tactile agnostic person. Their patient presented a small left inferior parietal cortical infarction and after neurological examination, impaired right hand TOR was confirmed while intellect, language and motor skills (in particular hand movement) were considered normal. Unilaterality of tactile agnosia, the ability to acquire sensory data, to combine sensory stimuli, to manipulate and associate sensory perceived stimuli, and episodic object discrimination were tested. As control for assessing right hand performance, authors used the patient's unimpaired left hand. At first they hypothesized that an inaccurate acquisition of sensory data imputed either to a disordered search strategy or to interference of competing stimuli could represent the faulty step in the process. However, this hypothesis was discarded due to the fact that they observed that somatosensory input was sufficiently decoded and multidimensional processing of object structure was also preserved. Authors investigated then whether the problem could be due to mismatch between stored mental representation of the object and processing of rudimentary sensory percept. They however verified that the patient had no difficulty in accessing somatosensory and visual knowledge. The third aspect investigated was if the recognition level failure occurred at a deeper taxonomic level but authors found no evidence of impaired association between the percept and episodic memory. Authors proposed that in the case under examination, tactile agnosia was a high-level perceptual disorder resulting from a failure to integrate accurately acquired perceptive features into a haptic mental image due to lesions to inferior parietal cortex. In the last study

discussed in this review, again evidences about involvement of inferior parietal cortex including SII in TOR are provided. In the work of Platz (Platz 1996) perceptual, motor and cognitive functions were studied in a man with impaired tactile object-recognition (TOR) in his left hand due to a meningioma into the middle and the lower third of the right parietal cortex which was surgically removed. The author identified the patient as a case of tactile agnosia since its primary motor and somatosensory functions were not impaired, and its abilities for discrimination of various tactile aspects and its cognitive skills were preserved but he was not able to appreciate the object's nature or significance when it was placed in his left hand and he was unable to name or to describe or demonstrate the use of these objects. In the study, sensibility and perceptual modalities were tested with delivering stimuli such as light touch, pain, temperature variation. Sense of position, ability to discriminate two points and discrimination of weight, texture and size 2D and 3D form and TOR were also tested. Moreover, also motor ability was tested: maximal fist closure, maximum speed of finger movements and fine grip of object with vision occluded. The patient showed preserved perceptual functions and ability to discriminate object characteristics such as shape, size, weight and texture. He was moreover able to use these skills during tactile selection and tactile-visual matching. The author reported that the patient, despite its preserved skills, was not able to realize object's nature or significance when it was placed in his hand, neither to name it, describe it, and to demonstrate the use. The author observed that patient's exploratory movements were abnormal if compared to non-agnostic hand and often the object was lost during manipulation. Since motor impairment was excluded, the author hypothesized that these motor findings might imply sensory motor integration related to object manipulation. Also in this work it has been highlighted the role of lower part of inferior parietal lobule which embeds OP cortical region in sensory-motor integration and behavior control.

Summarizing the parietal operculum

- Cytoarchitecture of the OP in non human primates showed distinctive features compared to its neighboring regions, namely the primary somatic cortices and the inferior parietal lobule, thus confirming the specific identity of the opercular region.
- Connectivity studies provided evidence for major anatomical connections with the primary sensory cortex, inferior parietal lobule, premotor cortex and primary motor cortex.
- In humans, the classical cytoarchitectonic maps showed different levels of parcellation of IPL. All of them however identified an area of granular cortex within IPL that is referred to as secondary somatosensory area (SII).
- A modern cytoarchitectonic map provided a detailed subdivision of human OP in four regions (OP1-OP4). Their probabilistic connectivity with other parts of the cortex was investigated with non invasive neuroimaging techniques. Significant connections were found with the primary somatosensory area, the inferior parietal lobule, the premotor cortex and the primary motor cortex.
- Physiological properties of OP have been illustrated by means of single-cell recordings and intracortical microstimulation (ICMS) in non human primates. In humans most studies employ non-invasive techniques, though in some cases it was possible to record intracortical responses from SII in pre-surgical epileptic patients. Neural activation of the OP was observed for different types and orientation of tactile stimulation. For both non-human primates and humans, OP showed to embed two somatotopic maps, sharing one border along medio-lateral direction.
- The role of the OP in motor control was reviewed. Beside its involvement in tactual perception and processing of haptically acquired features of manipulated objects, its

role in the use of tactile information for predictive movements was highlighted in works on visually guided grasping.

- Finally some evidence indicates a role of the OP in tactile learning and tactile memory, retention and recall of haptically acquired information and mental representation of objects.

In the next chapter...

This third chapter focused on the literature related to the involvement of OP in sensorymotor transformations. In the next chapter, after stating the aim of this thesis, a general introduction on the experimental designs and stimulation paradigms will follow. At last, the first experiment, focused on the investigation and characterization functional connectivity between the left parietal operculum and the ipsilateral primary motor cortex will be described and discussed.

PART II – AIM OF THE STUDY

Given the theoretical aspects discussed in the first chapter in which the new vision of the organization of the cortical motor system has been reported describing the possible functions of the different fronto-parietal circuits in the fundamental process of sensory-motor transformations, and given all the anatomical, functional and connectivity aspects of the primates and human parietal operculum discussed in the second and third chapter, the aim of this work is to investigate the role of such parietal region on the context of haptic-based object directed behavior. Such context is essential for most of the everyday life actions. The concert of movements that a human being performs when approaching an object appears to be quite natural and immediate, however, such perfect and linear symphony of actions is the result of a complex interaction of different cortical regions. In fact, there are specific regions that acquire information about different aspects such as the surrounding space, the geometry of the objects, their position in the space, the position of the human body. Such information must be converted then in a suitable signal and at last, it must be exchanged with other specific cortical regions at appropriate timing. Thus, providing insights about underlying processes involved in different aspects of such transformation (e.g. cortical circuits involved, timing of communication between active players, characterization of the encoding of sensory information, involvement in working memory aspects) turns out to be extremely important. The role of the cortical region under investigation, often underplayed, is slowly arousing attention of researchers due to its complex, multimodal and still not completely defined behavior in the context of sensory-motor transformation. In order to provide insights related to different aspects of the role of parietal operculum in sensory-motor transformation in the context of haptic-based object directed behavior, four TMS

experiments have been conducted by using different paradigms of stimulation and experimental tasks. Methods, results and their discussion will follow in the experimental session of this thesis.

PART III - EXPERIMENTAL WORK

Introduction to studies and stimulation paradigms

The aim of this work is to investigate the role of OP on the context of haptic-based object directed behavior. In order to provide insights related to different aspects of this issue, four TMS experiments have been conducted by using different paradigms of stimulation and experimental tasks

Experiment I

This experiment aimed to test cortico-cortical connectivity between left OP and ipsilateral M1. This experimental hypothesis is supported by anatomical studies conducted on non-human primates that provide evidences of direct anatomical connections between the two regions justifying the hypothesis of their direct functional interaction. In this experiment a dual coil TMS paradigm was used in a no task-design and peak to peak amplitude of conditioned MEPs were compared to unconditioned ones in order to asses any possible effect introduced by the conditioning stimulation over OP.

Experiment II

On the basis of results obtained with Experiment I supporting the functional connectivity between OP and primary motor regions, the aim of the Experiment II was to assess the possible involvement of OP in a reach to grasp task when the size of the graspable object was previously explored in different sensory modalities. Correlation between conditioned MEPs of two muscles (acting as antagonists in case of the type of grasp

required) and size of the object were compared with unconditioned for both exploration modalities and for different conditioning sites.

Experiment III

Experiment III is composed by two experiments hereafter called Experiment IIIa and Experiment IIIb.

Experiment IIIa

Given outcomes achieved with experiment 2, suggesting OP as a putative site for working memory in presence of a motor plan which requires grasping an object of a given geometry, a further step has been made in order to provide additional insights related to this functional aspect of OP. In the Experiment IIIa by using an on-line rTMS paradigm, the effects of repetitive stimulation were investigated by comparing the performance of participants receiving the stimulation over OP respect to different control conditions executing a purely discriminative match to sample task without motor planning.

Experiment IIIb

Similarly to the Experiment IIIa, the on-line rTMS paradigm was used also in this experiment. The aim is to study whether the stimulation over OP, by exploiting virtual lesion effect of rTMS, produces any sort of variations of the hand preshaping in a task requiring a motor plan such as the voluntary delayed grasping of previously haptically explored size-variable objects. The effect of the stimulation site and the temporal phase in which it was delivered was evaluated on a specific metric representing the hand preshaping.

Dual coil TMS paradigm

Dual coil transcranial magnetic stimulation (TMS), which is also referred to as twin-coil TMS or bifocal TMS, is a powerful method to probe the functional link between cortical areas and to study the time course of such interaction ((Civardi, Cantello et al. 2001; Koch, Del Olmo et al. 2007; O'Shea, Sebastian et al. 2007; Davare, Montague et al. 2009) by applying simultaneously two distinct coils to the participant's head. Generally, a suprathreshold stimulus (testTMS) is applied over primary motor cortex in order to elicit a motor evoked potential which is a measurable entity. Before testTMS, a subthreshold conditioning stimulus (condTMS) is delivered in correspondence of a specific cortical region which is hypothesized to functionally interact with M1. The interstimulus interval (ISI) that produced significant variation of motor evoked potential is indicative of direct cortico-cortical connections. In this way it is possible to evaluate if an output activated by condTMS in one brain region works, at a specific latency, as input producing significant effects on the motor cortex excitability indicating then a functional interplay between the cortical areas. As pointed out in (Cattaneo and Barchiesi 2011) experiments in which such paradigm is applied are based on *a priori* assumption on where to position the condTMS to test effects on a specific behavior. The main limitation of such approach is given by the anatomical variability of the functional active regions among individuals.

On-line repetitive TMS paradigm

Generally, lesion studies are a powerful tool to evaluate the causal relation between brain region and specific functions. In case of healthy human brains, it is possible to exploit the effects induced by TMS on the stimulated region to infer the relation of causality between such brain area and behavior. The basic concept behind such

application is that TMS can be used to perturb temporarily the function of a specific cortical target thus creating a transient virtual brain lesion. With the term repetitive TMS (rTMS) is intended a train of TMS pulses of the same intensity applied to a single brain area at a given frequency (from 1 Hz). The disruption of the cortical function during the stimulation is related to the intensity of the stimulation and the frequency (Kobayashi and Pascual-Leone 2003). In this work, we used an on-line paradigm, delivering the rTMS during the execution of the tasks. In this way we aimed to test whether disturbing the neural activity of the left OP the execution of a match to sample task or a haptic driven grasping task caused any sort of perturbation of the normal outcomes related (e.g. accuracy or hand preshaping).

Chapter 4

Cortico-cortical connectivity with primary motor cortex

Experiment I

Introduction

The human parietal operculum (OP) is a heterogeneous cortical region located above the caudal Sylvian fissure comprising Brodmann's areas 39, 40, 43, 3, 1, and 2 (Eickhoff, Grefkes et al. 2006; Eickhoff, Schleicher et al. 2006). It embeds 2 complete tactile sensory representations of the body and is considered an important station for processing somatosensory information ((Krubitzer and Kaas 1990; Krubitzer, Clarey et al. 1995). The portion of OP that contains such somatosensory representation is historically referred to as the secondary somatosensory cortex (SII). It has been showed that OP neurons respond to non-noxious and noxious cutaneous and muscular stimuli and to proprioceptive inputs generated by passive joint movements, in non-human primates (Krubitzer, Clarey et al. 1995) (Fitzgerald, Lane et al. 2006) and in human (Woolsey, Erickson et al. 1979) (Treede and Kunde 1995; Svensson, Minoshima et al. 1997) (Disbrow, Roberts et al. 2000; Del Gratta, Della Penna et al. 2002) (Mazzola, Faillenot et al. 2012). Parts of OP are also recruited during active movements. This activity has been considered the consequence of the proprioceptive reafference caused by movement (Faillenot, Toni et al. 1997) (Binkofski, Buccino et al. 1999; Binkofski, Buccino et al. 1999; Ehrsson, Fagergren et al. 2000; Ehrsson, Fagergren et al. 2003), but it also suggests a direct role of OP in the coordination of movement (Jäncke,

Kleinschmidt et al. 2001) (Reed, Shoham et al. 2004). The first aspect that we decided to study in order to investigate the role of such region in motor behavior was its connectivity with motor regions such as ipsilateral primary motor cortex (M1). Thus, probing the functional connectivity between OP and ipsilateral M1 is the aim of the first experiment which was addressed by using dual coil TMS. In a recent article, (Cattaneo and Barchiesi 2011) authors mapped the connectivity of the entire hemisphere with the ipsilateral M1 by means of such paradigm in subjects at rest. Among other results, we observed that TMS applied to the OP produced a short-latency effect on M1, supporting the presence of direct cortico-cortical connections between the 2 regions. These findings indicate that some OP neurons are just one synapse away from the motor cortex and are in agreement with anatomical studies in non human primates which show that in monkey OP, a substantial proportion of M1 neurons is stained following the injection of anterograde tracers (Mesulam and Mufson 1982; Mufson and Mesulam 1982; Pandya and Seltzer 1982; Cusick, Wall et al. 1989; Krubitzer and Kaas 1990; Stepniewska, Preuss et al. 1993; Cipolloni and Pandya 1999; Qi, Lyon et al. 2002; Disbrow, Litinas et al. 2003). Hence, in this first experiment we investigated by using dual coil TMS in baseline conditions the optimal stimulation point in the left OP cortex and we assessed the optimal intersitmulus interval between conditioning and test stimuli for eliciting short-latency modulation of M1.

Methods

Participants

Five right-handed volunteers took part in this experiment (3 females, age 25.6 ± 2.8 years). None of them had contraindications to TMS (Rossi, Hallett et al. 2009) and all

gave written informed consent in accordance with the Ethical Committee of the University of Trento (protocol n. 2009-033).

MRI and Neuronavigation

All MRI data were acquired with a 4T scanner (Bruker Medical, Ettlingen, Germany) using a birdcage transmit, 8-channel receiver head radiofrequency coil. Structural images were acquired using a 3D magnetization prepared rapid gradient echo sequence (3D MPRAGE) optimized for gray-white matter contrast [1x1x1 mm³ resolution, echo time (TE) =4.18 ms, repetition time (TR)=2700 ms, inversion time (TI)=1020ms, flip angle = 7°, Generalized Autocalibrating Partially Parallel Acquisition (GRAPPA) acceleration factor=2]. Anatomical images in Digital Imaging and Communications in Medicine (DICOM) format of each participant were then processed using the Brainvoyager software (Brain Innovation BV, The Netherlands) to produce a 3D surface reconstruction of the scalp and of the gray matter surface. The opercular sub-region that we aimed to cover with the grid was the OP4 part, defined according to (Eickhoff, Grefkes et al. 2006). We obtained the OP4 mask from the Statistical Parametric Mapping (SPM5) anatomy toolbox (Wellcome Department of Cognitive Neurology; <http://www.fil.ion.ucl.ac.uk/spm>) implemented in MATLAB 2007 (Mathworks Inc.) in the Neuroimaging Informatics Technology Initiative (NIfTI-1) format. Such mask was then converted in Brainvoyager-compatible format using the NIfTI converter plugin for Brainvoyager QX (available at <http://support.brainvoyager.com>). The participant's head and the condTMS coil were co-registered with the scalp 3D reconstruction by means of the Neuronavigation module of the Brainvoyager software, interfaced with the ultrasound tracker CMS20S (Zebris Medical, Isny, Germany). This allowed online updating of the position of the condTMS coil over the scalp.

Dual coil TMS

The stimulation paradigm used was the same as in (Cattaneo and Barchiesi 2011). Two different magnetic stimulators, each connected to a separate coil were used simultaneously. A Magstim 200 monophasic stimulator (The Magstim Company, Whitland, UK) was used to deliver the testTMS via a Magstim custom-made figure-of-eight coil with 50 mm diameter windings. A Magpro stimulator (Magventure, Skovlunde, Denmark) in biphasic stimulus modality produced the condTMS via an MC-B35 coil with winding diameter of 36 mm. The participants' heads were held still with a chin-rest incorporating an additional lateral head-constrain. The testTMS coil was permanently positioned on hand motor cortex of the left-hemisphere and held in place by means of a mechanical support. It was oriented with the handle pointing medially forming a 90° angle with the midline. The optimal coil orientation for trans-synaptic stimulation of corticospinal neurons in the hand representation in M1 is known to be of 45° (Mills, Boniface et al. 1992). In our protocol however, in order to allow the concurrent presence of the two coils on the scalp surface we adopted the configuration described above. Besides, other studies have shown that also a current orientation perpendicular to the midline elicits preferentially indirect waves in the cortico-spinal descending volley (Sakai, Ugawa et al. 1997). TestTMS was delivered at an intensity corresponding to 120% of individual resting motor threshold (Rossini, Barker et al. 1994) calculated for the right interosseus dorsalis primus (ID1) muscle.

Electromyographic (EMG) recordings

Motor evoked potentials (MEPs) were recorded from the right ID1 muscle. Recordings were realized by means of passive Ag/AgCl electrodes in a bipolar belly-tendon montage. The analog EMG signal was then amplified 1000x by means of a CED 1902

amplifier (Cambridge Electronic Design, Cambridge, UK) and digitized by means of a CED 1401 micro Mk-II unit (Cambridge Electronic Design, Cambridge, UK) at a sampling frequency of 4 KHz. The EMG recording was triggered by the testTMS pulse (time=0.0) by means of the Signal software (Cambridge Electronic Design, Cambridge, UK). In each trial EMG was recorded from -250 ms to +250 ms and stored on a PC for offline analysis.

General design

This experiment aimed at establishing the optimal position and ISI to produce short-latency effects by condTMS to M1. To do so we tested participants while sitting at rest and moved the condTMS coil over a grid of scalp points that covered the whole of the OP region. Moreover we tested different ISIs between condTMS and testTMS (see Figure 10). The analysis of the data was first carried out on a single-subject basis by computing statistical scalp maps of the comparison between condTMS+testTMS trials with testTMS trials. Finally, a group analysis was carried out to generalize the findings from single subjects.

Mapping procedure

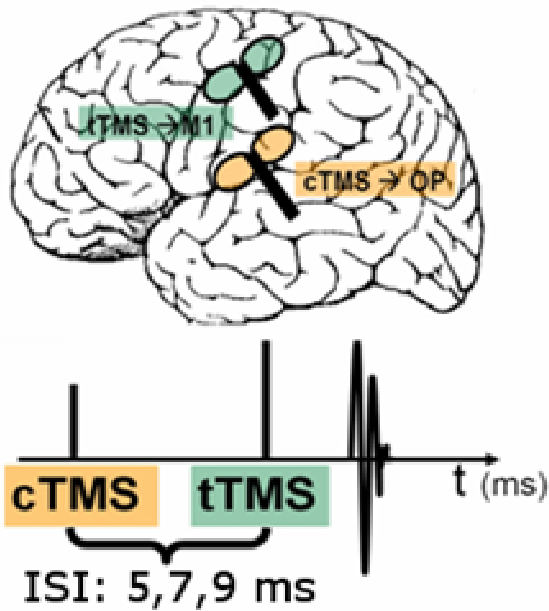
We built in each participant a 3x3 grid drawn over the left OP region (Figure 11 shows individual grids in the 5 participants). The condTMS coil was moved sequentially over the grid-points under online neuronavigation guidance and its intensity was set at 90% of resting motor threshold computed for ID1. Three different ISIs were used (5ms, 7 ms and 9 ms) in a separate blocks. The mapping procedure consisted in a sequence of condTMS+testTMS trials following a fixed spatial order over the grid. The moment of

TMS was manually determined in each trial by the operator whenever the condTMS coil had been moved over the following point of the grid. All 9 points of the grid were therefore tested at every cycle of 9 consecutive condTMS+testTMS . This cycle was repeated 16 times in order to have 16 repeated measures of condTMS+testTMS for each grid point. Trials with only testTMS were interleaved in the mapping sequence every 3 condTMS+testTMS trials. The session of stimulation described was repeated for three blocks, each with a different ISI. To summarize, each of the 3 blocks was composed by 144 (9 points x 16 repetitions) condTMS+testTMS trials interleaved with 48 tTMS-only trials for a total of 192 trials. Conditioned TMS paradigms conventionally use the grand average of test MEP amplitudes from the whole block as the denominator of the normalization ratio (for example see (Kujirai, Caramia et al. 1993) or (Ziemann, Tergau et al. 1998)). In the present work we performed lengthy experimental sessions in which the test MEP amplitude was likely to vary greatly in the course of the block for spontaneous fluctuations of vigilance or for technical aspects such as coil heating and switching. We therefore decided to adopt a quasi-instantaneous measure of test MEP amplitudes represented by the average of two adjacent testTMS trials moving along the timeline of the block in a sliding window, as described in (Cattaneo and Barchiesi 2011). Therefore each MEP from condTMS+testTMS trials was normalized to the mean of the 2 test MEPs preceding and following the conditioned trial.

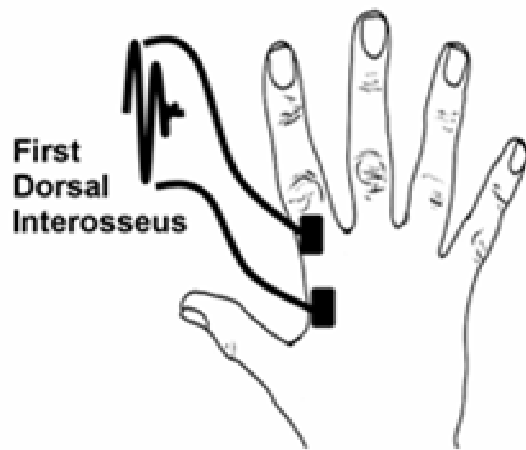
Computation of TMS statistical maps and group analysis

The data from each ISI was analyzed separately. As a result of MEP processing and normalization we obtained for each of the 3 ISIs a series of 16 normalized MEP amplitudes, each varying from 0 to $+\infty$, for each of the nine points composing the grid. Values >1 represented facilitatory effects of condTMS and values <1 represented

A Dual-coils TMS paradigm



C Motor Evoked Potential recording



B OP scalp grid points and cortical projection

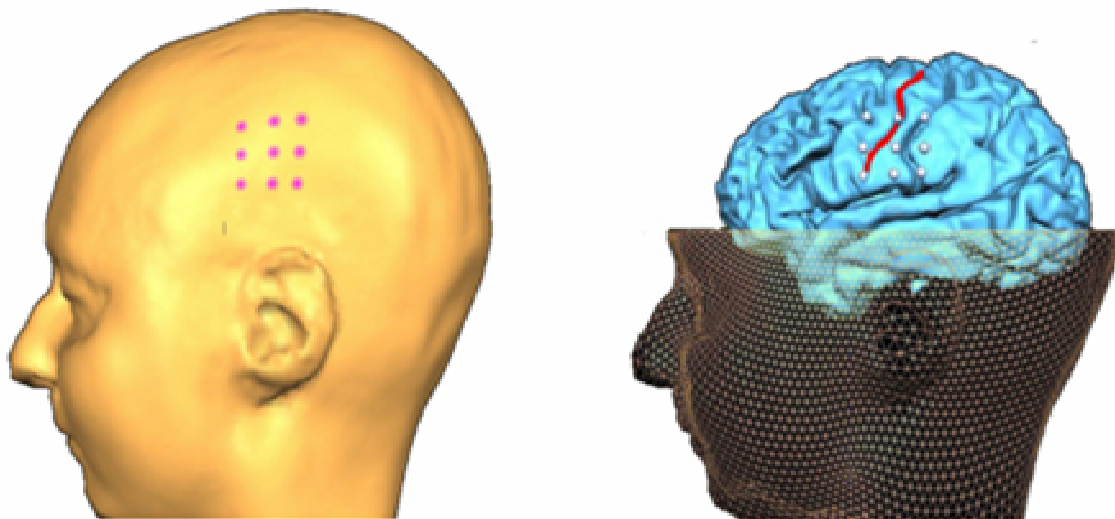


Figure 10. A: Dual coil TMS paradigm. CondTMS is delivered over the left OP; test TMS is delivered over ipsilateral M1. ISIs tested are 5,7,9 msec. B left: OP scalp grid points located over the left OP; B right: cortical projections of the scalp grid points. The central sulcus is drawn in red. C: Motor evoked potentials were recorded from the first dorsal interosseus by means of Ag/AgCl superficial electrodes in a belly tendon montage.

inhibitory effects of condTMS. Such values were transformed logarithmically in order to obtain a distribution of the data varying between $-\infty$ and $+\infty$. After this process, values >0 represented facilitatory effects of condTMS and values <0 represented inhibitory effects of condTMS. At last, each of the 9 distributions of 16 data was analyzed in a 2-tailed t-test for single samples, against the null hypothesis that the distribution had a mean value of zero. Significance level for the t-test was corrected for the 9 comparisons within the grid and therefore was set to $p = 0.05/9 = 0.0056$. In order to quantify the distribution of the effects of condTMS at the group level we performed an ANOVA on t-values with 2 factors: ISI (3 levels: 5, 7 and 9 ms) and POINT (9 levels corresponding to the 9 grid points).

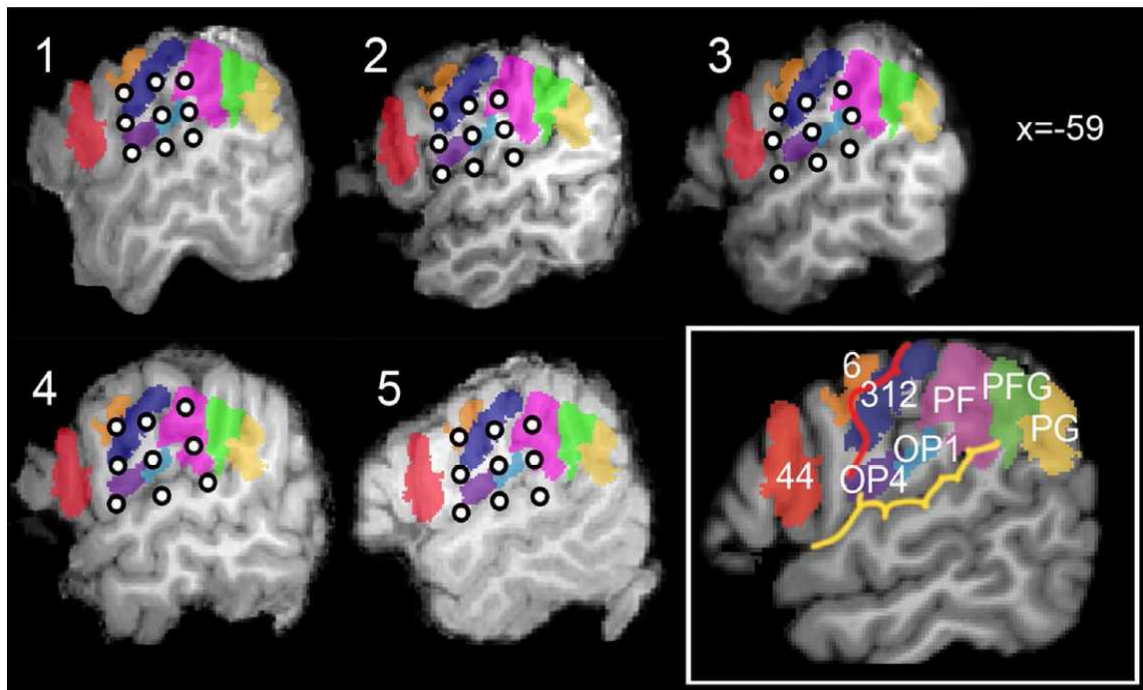


Figure 11. Individual MRI scans of the 5 participants to experiment I at the sagittal section corresponding to $x=-59$. Superimposed over the MRI scans is the probabilistic (50% probability) cytoarchitectonic maps and the projection from the scalp of the individual 9-point grids. The lower-right panel shows on a standard brain the labels of the cytoarchitectonic areas. The central sulcus is indicated in red and the Sylvian fissure in yellow.

Results

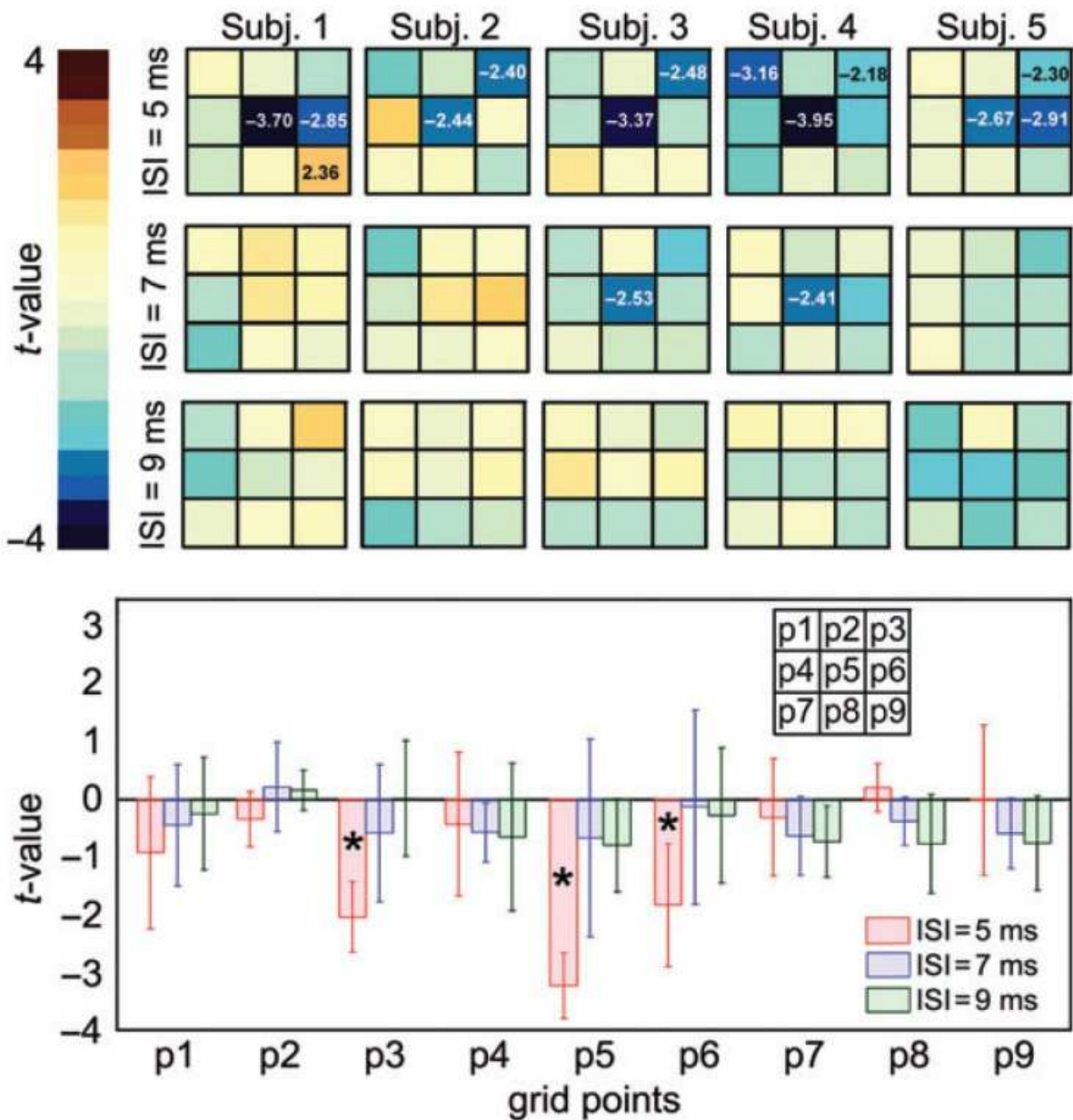


Figure 12. Upper panel: individual statistical maps of the t-values obtained contrasting the distribution of $\log(\text{condMEP} + \text{testMEP} / \text{testMEP})$ against the null hypothesis of the mean value = 0. Values of t are reported when they exceeded 2.13, i.e. the one corresponding to a p-value of $p=0.05$ at 15 degrees of freedom. The t-value Bonferroni corrected for 9 multiple comparisons ($p=0.0056$) corresponded to 3.25. Lower panel: mean t-values from all 5 participants. Asterisks indicate the grid points with significant differences of the mean values from chance level ($t=0$). The inserted grid indicates the nomenclature of the grid points. Note that orientation conventions of the grids are to be interpreted as overlying the scalp of the left hemisphere, therefore they are oriented with the cranial direction to the left and the caudal direction to the right.

The results of the mapping experiment show the existence of a short-latency modulation of condTMS delivered over OP on corticospinal excitability which is both temporally and spatially specific. The single-sample t-test performed on the points of individual grids produced significant results in all 5 subjects only at the ISI of 5 ms. The location of significant spots was distributed along the mid portion of the grid. All significant effects were inhibitory (Figure 12 upper panel). The ANOVA with GRID POINT and ISI as within-subjects factors showed that a significant distribution of t-values was present only for the 5 ms ISI. The ANOVA also shows that at the group level, a significant effect of condTMS was observed only for the grid point p5 (Bonferroni-corrected $p=0.02$) (Figure 12 lower panel). This corresponded to the ventral part of the postcentral sulcus, at the border between the OP4 and the OP1 region.

Discussion

The results of this experiment, carried out on participants in the resting state, show that condTMS delivered on the OP produces a short-latency inhibitory effect on the representation of hand movements in M1. In dual coil paradigms the presence of short latency condTMS-testTMS interactions is attributed to either the presence of direct cortico-cortical connections between the two stimulated spots or to the convergence of corticospinal efferent projections from the two areas. In the case of the OP, both hypotheses are possible, because the OP region is known to be directly connected to M1 (Tokuno and Tanji 1993) (Gharbawie, Stepniewska et al. 2011) (Gharbawie, Stepniewska et al. 2011) and to project fibers to the spinal cord (Galea and Dariansmith 1994). In the present work we did not control for changes at segmental level of motoneuron excitability produced by TMS of OP alone. We privilege the hypothesis that the short-latency modulation seen here is mainly due to cortico-cortical connections,

considering the very small contribution of the OP to the corticospinal tract, i.e. less than 3,5 % of contralateral corticospinal projections in the macaque (Galea and Dariansmith 1994). In this study, the effects of condTMS show striking temporal and spatial specificity. The modulation effects of OP condTMS are specific to the ISI of 5 ms (Figure 12), consistent with a direct link between OP and M1. This datum is analogous to the well-established short-latency effects of premotor cortex stimulation on M1 neurons known in non-human primates ((Tokuno and Nambu 2000);(Kraskov, Prabhu et al. 2011)) and humans (Davare, Montague et al. 2009) and it is likely that similar type of connections link OP to M1. We show that the conditioning effect is also restricted to a single small region along the post-central sulcus. This spot corresponds to the caudal border of the OP4 region where direct projections to the primary motor cortex in human subjects are thought to arise ((Eickhoff, Grefkes et al. 2006) (Eickhoff, Jbabdi et al. 2010)). These results are also in agreement with anatomical evidence in macaque monkeys showing that the OP sends dense projections towards the hand representation of the premotor cortex and M1 (Tokuno and Tanji 1993; Gharbawie, Stepniewska et al. 2011). However, in TMS studies the spatial relations between the stimulating coil and the underlying cortex is to be interpreted cautiously. The impact of TMS is likely to be maximum at sites different from that of the orthogonal projection of the centre of the coil onto the cortex and moreover it is not possible to assume that the effects of TMS are due to stimulation of a single cortical site but rather to the simultaneous activation of different though neighboring patches of cortex (Thielscher and Wichmann 2009; Thielscher, Reichenbach et al. 2010). In the present data, though we found that the most efficient coil position right over the post-central sulcus, the actual cortical origin of the short-latency effect of condTMS could be on a vaster region of the OP.

In the next chapter...

This fourth chapter focused on the characterization of the functional connectivity between the left parietal operculum and the ipsilateral primary motor cortex. Given such short-latency interaction, experiments described in the next chapter will focus on: a) assessing the role of parietal operculum as site of working memory for haptically acquired information on object geometry for guiding object directed actions (Experiment II), 2) providing evidences supporting the existence of different networks for processing haptic information whether such information is used for perception (Experiment IIIa) or action (Experiment IIIb) and characterizing the involvement of the parietal operculum in such circuits.

Chapter 5

Haptic driven grasping

Introduction

The results in support of direct cortico-cortical connectivity between OP and M1 obtained in Experiment I lead to a straightforward question: what could be the role of such connections? A first plausible hypothesis is that OP is involved in the direct transformation of somatosensory information in motor commands. Evidences supporting such hypothesis come from non invasive neuroimaging studies which show that, besides its well know involvement in passive tactile perception, its activation is present also during active tasks such as tactile discrimination (Burton, Abend et al. 1999), and motor related tasks such as complex object manipulation (Binkofski, Buccino et al. 1999) and motor learning (Eliassen, Souza et al. 2003). These outcomes lead to further investigations whose aim is to characterize the nature of the liaison between such cortical region and motor aspects. In support of the existence of anatomical connections there are anatomical studies on non human primates that show direct cortico-cortical projections between OP and primary motor cortex (M1) (Mesulam and Mufson 1982) (Mufson and Mesulam 1982) (Pandya and Seltzer 1982) (Cusick, Wall et al. 1989) (Krubitzer and Kaas 1990) (Stepniewska, Preuss et al. 1993; Cipolloni and Pandya 1999) (Qi, Lyon et al. 2002). In humans, the literature related to the study of such connections is quite poor. However, a recent reclassification of cytoarchitectonic parcellation of the OP region in humans has indicated that its dorso-rostral portion (labeled OP4) is characterized by its connections to M1 and is probably separated from

the classically defined the secondary somatosensory area (Eickhoff, Jbabdi et al. 2010). Moreover, in two our recent transcranial magnetic stimulation (TMS) studies (Cattaneo and Barchiesi 2011) and (Maule, Barchiesi et al. 2013) by applying a twin-coil paradigm of stimulation, beside mapping the optimal spot within the OP region for their interaction we found a very short latency (5ms) functional interplay occurring between OP and ipsilateral M1. Such results are in support of direct cortico-cortical connections and suggest a direct role of OP in the coordination of movement (Jäncke, Kleinschmidt et al. 2001) (Reed, Shoham et al. 2004) as a player involved in the direct transformation of somatosensory information into motor commands. The following experiments investigate with different experimental designs the nature of the involvement of OP in sensory motor integration.

In experiment II, we investigated the possibility that OP plays a direct role in haptically guided grasping. As well as in the Experiment I, we used the dual-coil TMS paradigm exploiting results previously obtained. Test stimuli (testTMS) were applied to M1 and conditioning TMS (condTMS) to the optimal spot of ipsilateral OP at 5 ms ISI (results obtained in Experiment I). OP-M1 connectivity was tested while participants were preparing the blindfolded grasp of an object, the variable size of which had been previously explored by the participant in the haptic or in the visual modality. In a complementary part of the experiment, we controlled for the validity of the effects of OP stimulation by implementing the same experimental paradigm with stimulation of the ventral premotor cortex (vPM), which is well known to play a role in visually rather than haptically guided grasping.

Experiment III is composed by two different experiments. In experiment IIIa and Experiment IIIb, on the basis of results obtained in Experiment II (Maule, Barchiesi et al. 2013) we decided to exploit the effect induced by on-line high frequency repetitive

transcranial magnetic stimulation (rTMS) delivered over the left OP in two different tasks involving the temporarily retention of the size related information acquired haptically. In experiment IIIa we tested the effects of online rTMS on the accuracy of participants performing a simple non-motor match to sample discriminative task of haptically explored diameter-variable cylinders. The aim was to test whether the stimulation affected the retention of the haptically acquired information on the size of the cylinder in a task where no motor plan was required. Differently, in Experiment IIIb we evaluated such effects on the hand preshaping in a delayed grasping of previously haptically explored diameter-variable cylinders. In this case, the effect of the noise introduced by rTMS was assessed on a task requiring a motor plan. In both experiments, we controlled for the validity of effect on OP, by implementing the same experimental paradigm on left primary visual cortex (V1) and with sessions without stimulation.

Experiment II

Methods

Participants

Twenty four healthy right handed volunteers (14 females, aged 32.2 ± 3.6 years) participated to this experiment. Twelve participants received condTMS over the left OP region (referred to as 'OP group') while the other twelve received condTMS over the left vPM region (referred to as 'vPM group'). None of them had contraindications to TMS (Rossi, Hallett et al. 2009) and all gave written informed consent in accordance with the Ethical Committee of the University of Trento (protocol n. 2009-033).

Electromyographic (EMG) recordings

Motor evoked potentials (MEPs) were recorded from the ED1 and the extensor indicis proprius (EIP) muscles. The ED1 muscle originates from the base of the first and second metacarpal bones and inserts on the proximal phalanx of the index finger. Its action is therefore that of abducting and flexing the index finger around the metacarpo-phalangeal joint. The EIP muscle arises from the distal third of the ulna and by intermediation of the dorsal aponeurosis, it acts upon the distal phalanx of the index. Its action is therefore that of extending the whole index finger. These two muscles therefore can act as antagonists in flexion-extension movements of the index finger, as in the experimental task adopted in this experiment. It should however be made clear that the two muscles are not obligatory antagonists because they can be partially synergistic in the abduction of the index finger (Brochier, Spinks et al. 2004). Recordings were realized by means of passive Ag/AgCl electrodes in a bipolar belly-tendon montage. The analog EMG signal was then amplified 1000x by means of a CED 1902 amplifier (Cambridge Electronic Design, Cambridge, UK) and digitized by means of a CED 1401 micro Mk-II unit (Cambridge Electronic Design, Cambridge, UK) at a sampling frequency of 4 KHz. A continuous recording of the EMG activity was carried out throughout the whole experiment by means of the Spike II software (Cambridge Electronic Design, Cambridge, UK) and stored on a PC for offline analysis. Peak-peak amplitudes of MEPs were extracted by an automatic algorithm in the Signal software (Cambridge Electronic Design, Cambridge, UK) seeking for the maximum and minimum EMG values in a time window from 20 to 40 ms after testTMS.

General design and experimental setup

Participants were tested in dynamic conditions during a delayed reach and grasp task. They were divided in two groups according to whether they received condTMS over the OP or over the vPM. In both groups, condTMS intensity was set at 90% of resting motor threshold computed for right ID1. The condTMS coil was positioned following the neuronavigation system on one single spot for every participant. In the OP group, condTMS was applied to a single point in the OP region that was established on the basis of the maps obtained in Experiment I, as a point along the postcentral sulcus, 2 cm above the Sylvian fissure. In the vPM group, the target of condTMS was established on the basis of previous dual-coil TMS experiments for the study of vPM-M1

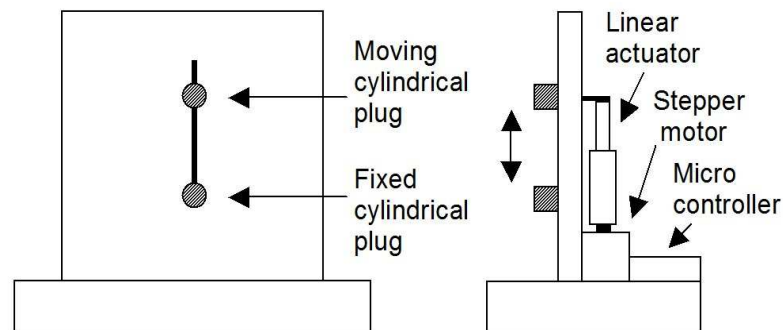


Figure 13. Schematics of the apparatus controlling inter-plug distance.

interactions (Baumer, Schippling et al. 2009; Davare, Montague et al. 2009; Davare, Rothwell et al. 2010); (Koch, Cercignani et al. 2010); (Lago, Koch et al. 2010) as a point along the precentral sulcus, 1.5 cm below the intersection with the inferior frontal sulcus. In this experiment also the ISI between condTMS and testTMS was fixed and corresponded to 5 ms for the OP group, which was defined as the optimal ISI in Experiment I and to 7ms in the vPM group (Davare, Montague et al. 2009). Subjects

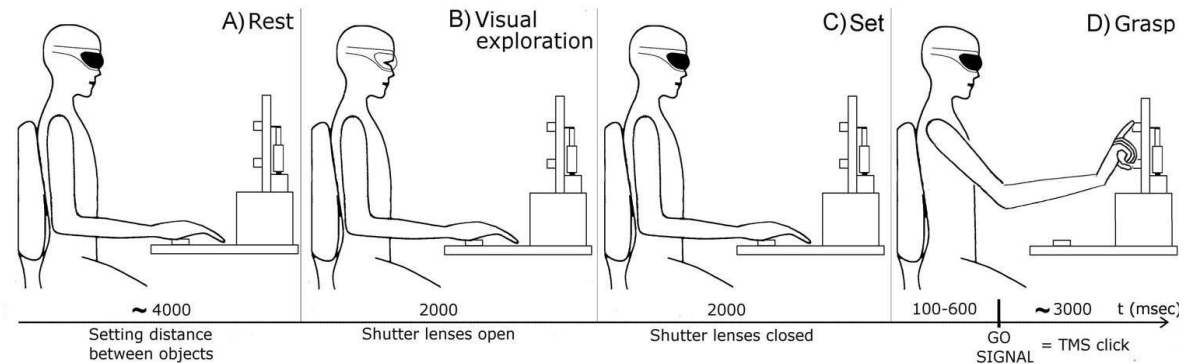
were sitting on a comfortable chair with their head placed in the chin rest. A custom-made (www.lmelettronica.it) apparatus holding two plastic plugs (Figure 13) was placed in front of them. The distance between the plugs was varied randomly at every trial. The lower plug was fixed to a vertical panel, the upper plug moved inside a vertical fissure in the panel, by virtue of a linear actuator connected to a stepper motor in the rear part of the panel. The whole automation was implemented by using an ArduinoUNO microcontroller (www.arduino.cc) which generated at each trial a random number that was converted in inter-plug distance. A linear potentiometer connected to the plugs assured feedback to the microcontroller on the inter-plug distance. The setup consisted additionally in A) a switch on the table on which the participant's right hand rested and which signaled the start of the Reach phase. B) a small touch-sensitive sensor placed over the upper plug in order to signal the time of contact of the hand with the object. C) a pair of computer-controlled liquid crystal shutter goggles (Plato translucent technologies inc.) worn by subjects in the whole experiment. Additionally participants were wearing earphones in order to hear acoustic cue-sounds and white noise played in the background. The participant's left hand was kept in a rest condition for the whole experimental session.

Trial structure and order

The participant's task is schematized in Figure 14 and consisted in a Rest phase, followed by exploration of the inter-plug distance, either visually or haptically, followed by a Delay phase in which participants waited for TMS. The TMS pulse served as the GO-signal and instructed the participants to release the home switch to reach and grasp the plugs with their index and thumb fingertips. TMS was therefore delivered when the

participant had already acquired the information on the inter-plug distance to be grasped, and was waiting to use that information to perform the grasp.

VISUAL EXPLORATION TRIAL



HAPTIC EXPLORATION TRIAL

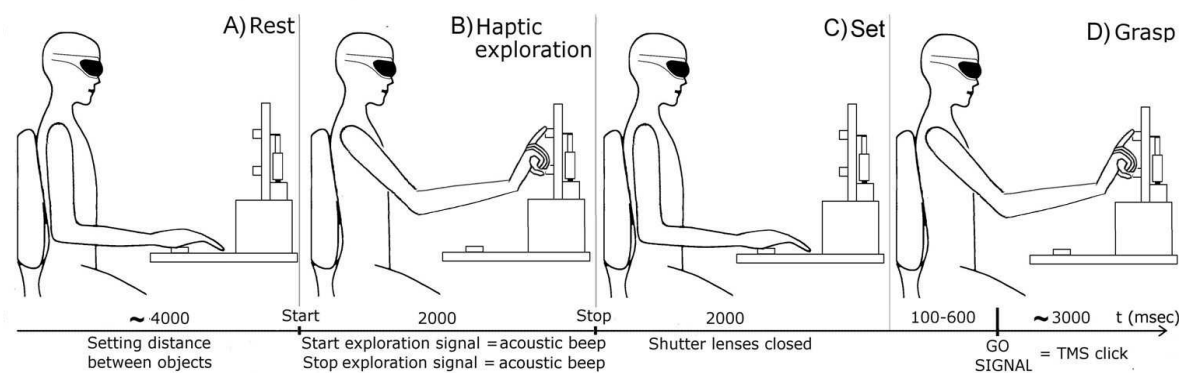


Figure 14. Top: Visual exploration trial. Bottom: Haptic exploration trial. Each trial is composed by four phases: A) Rest, change of inter-plug distance; B) Exploration: visual or haptic according with the type of trial (note that in haptic trials the vision of the object was occluded in this phase); C) Set: the subject waits for the GO-signal; D) Reach-grasp: the participant, with vision occluded in both trial types, performs a thumb-index grasping of the two plugs.

Visual and haptic trials were identical in the Rest, Delay and Grasp phases but differed in the Exploration phase. At the beginning of each trial, subjects were sitting with vision occluded by the shut goggles and with their right hand relaxed on the home switch while the distance between the two objects was being changed. In the subsequent exploration phase, the shutter lenses were opened for 2000 ms in order to allow vision of the objects

in Visual Exploration. Goggles instead remained closed in haptic trials and an acoustic cue-sound prompted the subjects to start tactile exploration of the plugs with their right hand. After 2000 ms another acoustic cue advised participant to finish the exploration and go back to the rest position. During this Delay phase, subjects were instructed to be ready to perform a thumb-index grasp of the plugs whenever they heard the GO signal. The GO signal occurred randomly between 2100 and 2600 msec after the end of the visual or haptic inspection. Once the plugs were grasped, subjects returned with their right hand relaxed on the home switch. The inter-plug distance was fully randomized between trials, the order of trials was fixed in the sequence: 1) Visual Exploration trial with cond+testTMS, 2) Visual Exploration trial with testTMS alone, 3) Haptic Exploration trial with cond+testTMS, 4) Haptic Exploration trial with testTMS alone. This basic sequence was repeated 50 times for a total duration of the experimental session of 35 minutes. For each trial, the set interplug distance and the timing of the go signal (TMS) were recorded in a log file by the Arduino system. The number of trials was 200 per each experimental session corresponding to 50 trials per each of the four conditions: 1) single pulse-visual trial, 2) single pulse-haptic trial, 3) dual pulse-visual trial and 4) dual-pulse-haptic trial.

MEP data analysis

The strategy of data analysis in this experiment was different from the conventional one used in experiment I. We did not compute the ratio between conditioned and test MEPs but focused on the modulation of raw MEP peak-to-peak amplitudes for each muscle. In each single subject, we calculated an index of correlation (Pearson's r coefficient) between the amplitude of MEPs obtained in single trials and the inter-plug distance of that same trial. The use of a statistical metric to describe the effect of an experimental

factor and a physiological process is widely accepted and validated in functional neuroimaging studies (for more details see (Friston, Frith et al. 1991; Friston 1995; Friston, Frith et al. 1995). In our work we decided to adopt this approach, and considered in our analysis the mutual combination of r-correlation coefficients between MEPs of the two antagonist muscles and the size of the grasped object. More precisely, we correlated MEP amplitudes with target size separately per each of the four conditions: 1) single pulse-visual trials, 2) single pulse-haptic trials, 3) dual pulse-visual trials and 4) dual pulse-haptic trials. Each r value was computed on 50 pairs of data. In our opinion the use of a statistical parameter to describe the distribution of single trial measures (MEP amplitudes) according to an experimental variable (the target size) is more reliable than the conventional approach based on the extraction of mean values of MEP amplitudes in factorial designs. The main reason for this is that mean values of MEP amplitudes are strongly influenced by outlier data and that MEP amplitudes are not distributed normally, being comprised between 0 and $+\infty$. The use of correlation coefficients linking the dependent variable and the dimension that is manipulated experimentally overcomes this potential source of noise and bias. Correlation coefficients were calculated in single participants and take account therefore of values from each single trial. They were computed separately for each of the two muscles, thus resulting in 8 r values for each subject, corresponding to a $2*2*2$ design with the factors: MODALITY (2 levels: haptic or visual), PULSE (2 levels: testTMS only or condTMS + testTMS) and MUSCLE (2 levels: ID1 and EIP). The r-values have to be interpreted in two ways. First, their absolute values indicate that MEPs from a given muscle are correlated with the size of the object that has been explored and that will be grasped. For each participant, positive and negative values of r indicate that the MEP size increases and decreases linearly with the size of the grasp target, respectively. Second, the two muscles are to be considered as mutual antagonists in the context of the present

movement. Therefore, besides their absolute values, also the mutual relation of r-values between the two muscles within each condition is informative because it indicates whether the MEP modulation reflects the actual reach-grasp EMG pattern, in which it is expected that r-values for the EIP muscle are more positive than those from the ID1 muscle. The r values were used as dependent variable in an ANOVA with the 3 within-subjects factors MODALITY*PULSE*MUSCLE and a between-subjects factor, i.e. the GROUP (2 levels: OP group and vPM group).

Analysis of EMG during the reach-grasp action

We also performed a direct analysis of the EMG activity of the actual reach-grasp movements performed in the last phase of the trial (Grasp phase), i.e. when subjects had already explored the target size. The epoch of the reaching movement was identified on the continuous EMG recordings with respect to home-switch release and plug-contact. The reaching epoch from each trial was then divided in ten decile time bins. Electrical muscular activity started slightly before the mechanical event of hand elevation and, obviously, continued after the contact with the target. Therefore in each trial we considered also two additional time bins immediately preceding and immediately following the reach, with duration identical to the deciles of that trial. This procedure resulted for each trial in a total of 12 consecutive time intervals of equal length. The average duration of deciles among trials and subjects was of 111 ms (SD: 12 ms). The rectified EMG activity was averaged in each 2 consecutive time bins, resulting in 6 values per trial corresponding to the time-course of EMG along the reaching movement. Pearson's r coefficient was computed between each of the 6 EMG values for the two muscles and the inter-plug distance of that trial. As with MEP analysis, the correlations were computed per each of the four conditions: 1) single pulse-visual trial, 2) single

pulse-haptic trial, 3) dual pulse-visual trial and 4) dual-pulse-haptic trial. Therefore each r value was computed on 50 pairs of data. The EMG-target size correlations were then analyzed with an ANOVA with 4 within-subjects factors: TIME (6 levels corresponding to the 6 consecutive time bins), MODALITY (2 levels, visual or haptic), PULSE (2 levels, single or dual pulse) and MUSCLE (2 levels, ID1 and EPI) and one between-subjects factor, GROUP (2 levels, OP and vPM) as between-subjects factor.

Results

Correlations between MEPs and target size

The results of the ANOVA performed on r coefficients obtained from the MEP amplitudes produced a significant main effect of MUSCLE ($F(1, 22)=7.99, p=0.01$) driven by the higher values of r -coefficients associated with the ID1 muscle compared to those associated to the EIP muscle. The most important finding however was a 4-way GROUP*MODALITY*PULSE*MUSCLE interaction ($F(1, 22)=19.78, p=0.0002$) which is illustrated in Figure 15. To explore the complex interaction we divided the analysis between the two groups in two symmetrical MODALITY*PULSE*MUSCLE ANOVAs, which both resulted in significant 3-way interactions ($F(1, 11)=15.34, p=0.002$ in the OP group and $F(1, 11)=8.18, p=0.016$ in the vPM group). We broke each interaction effect into two PULSE* MUSCLE sub-ANOVAs for each of the 2 sensory modalities. The results in the OP group showed a significant PULSE*MUSCLE interaction for the tactile modality ($F(1, 11)=23.03, p=0.0006$) but no interaction in the visual modality ($F(1, 11)=0.05, p=0.83$). Post-hoc t -tests indicated that the interaction was to be attributed to the fact that no difference was present between the r -coefficients from the two muscles in the single pulse modality (Bonferroni-corrected $p=1.0$), while a consistent difference

was present between the mean r-coefficients of the 2 muscles in the dual-pulse modality, i.e. when also OP had been stimulated with TMS (Bonferroni-corrected $p=0.00006$).

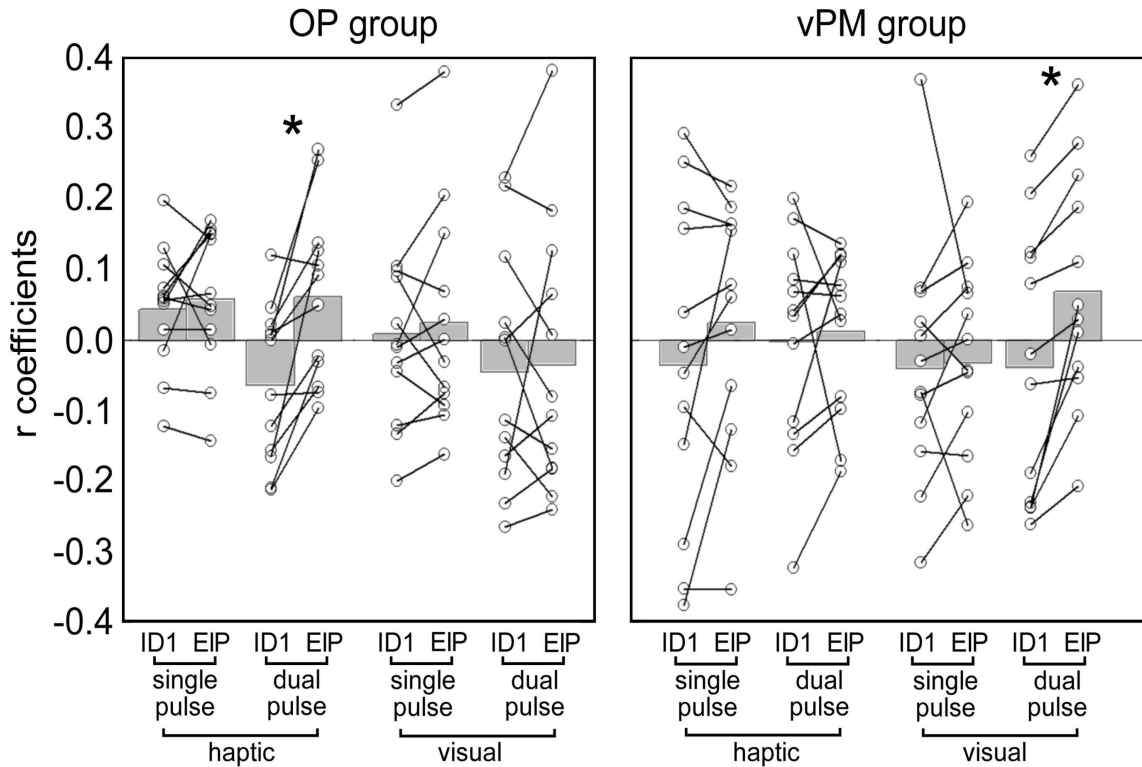


Figure 15. Grey columns indicate the mean r-coefficients calculated on the MEPs from the 2 muscles in the different experimental conditions. Line-circle elements indicate the individual data. The asterisks indicate the significant post-hoc comparisons.

On the contrary, the same analysis performed in the vPM group did not show any PULSE*MUSCLE interaction in the haptic modality ($F(1, 11)=0.96, p=0.35$) but did show a significant PULSE*MUSCLE interaction in the visual modality ($F(1, 11)=6.71, =0.025$). Post-hoc comparisons indicated that the interaction was ultimately due to a difference between r-coefficients from the two muscles in the dual-pulse trials (Bonferroni-corrected

$p=0.022$) but not in the single-pulse trials (Bonferroni-corrected $p=1.0$). Additionally, as a post-hoc analysis aimed at assessing whether any correlation was present between the MEPs in each category and the target size, we performed single-sample t-tests against the null hypothesis of mean $x = 0.0$. The results did not show any significant result (minimum $p=0.08$).

Effects of TMS on the subsequent reach-grasp movement

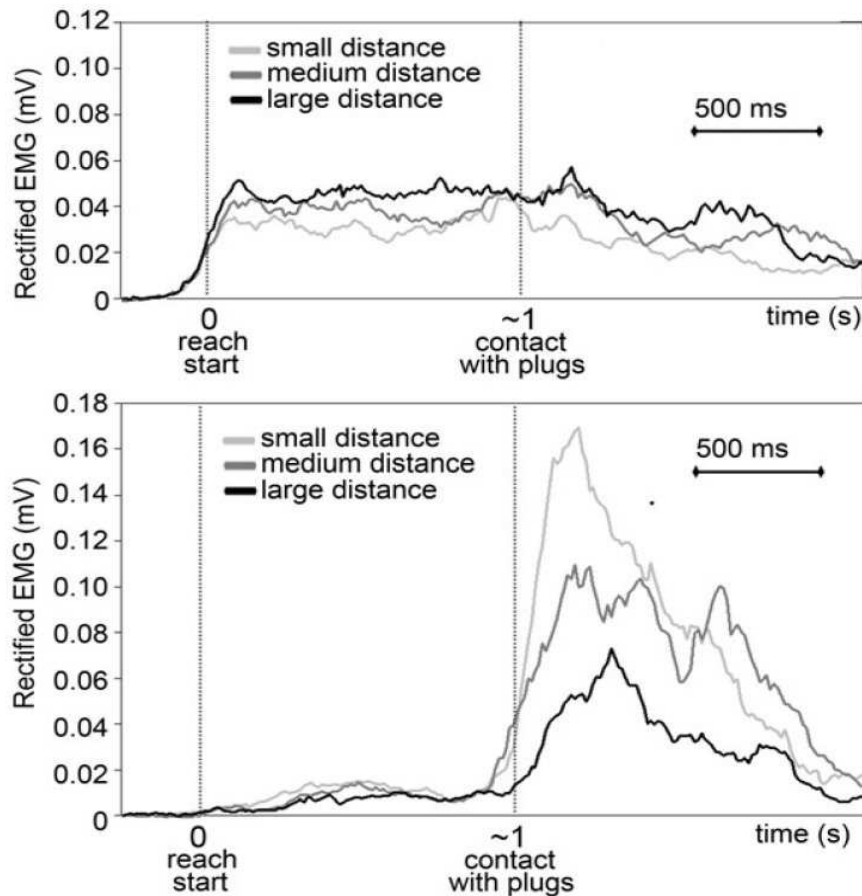


Figure 16. The rectified EMG recordings of the EIP and ID1 muscles from one representative subject are represented, to illustrate the relation of the EMG signal to the size of the object. The data from the single-pulse, haptic exploration conditions are shown. For purely illustrative purposes, the EMG traces have been averaged in 3 groups, corresponding to the lower (small), middle (medium), and upper (large) thirds of interplug distances. All traces are aligned to the start of the reaching, that is, when the participant's hand was lifted from the start-switch. In this subject contact with the plugs occurred around 1 s later. Notice that EIP muscle activity increases with increasing interplug distance and conversely ID1 activity decreases with the size of the target.

Figure 16 illustrates qualitatively in one representative subject the relation between the EMG recorded from the two muscles and the size of the object to be grasped. The results of the ANOVA showed a TIME*MUSCLE interaction ($F(5, 110)=62.57$, $p<0.000001$) which indicates the divergent time course of the r-coefficients from the two muscles during the Reach-Grasp phase. As shown in Figure 17 the IP muscle showed an activity that was correlated from very early in the reach with the geometry of the target object as indicated by the deviance from the zero value (dashed line in Figure 17).

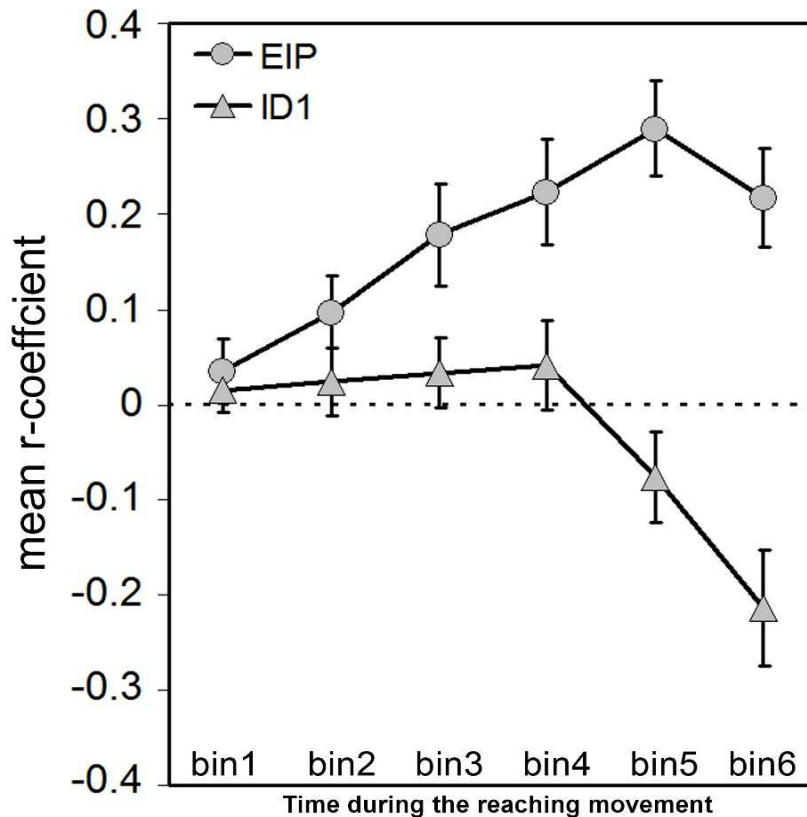


Figure 17. General time course of the r-coefficients linking each of the two muscles with the inter-plug distance to be grasped. Bin 1 corresponds to the period around the GO signal and bin 6 includes the contact with the plugs and the initial grasp phase. Bins 2-5 describe the reaching phase. The dashed line indicates $y=0$. Error bars indicate 95% CI.

The ID1 muscle on the contrary is specifically activated in relation to the object only very late in the reach and maximally during the grasp. The most complex result of the ANOVA was a GROUP*MODALITY*TIME*MUSCLE interaction ($F(5, 110)=3.43$, $p=0.006$) that is illustrated in Figure 18.

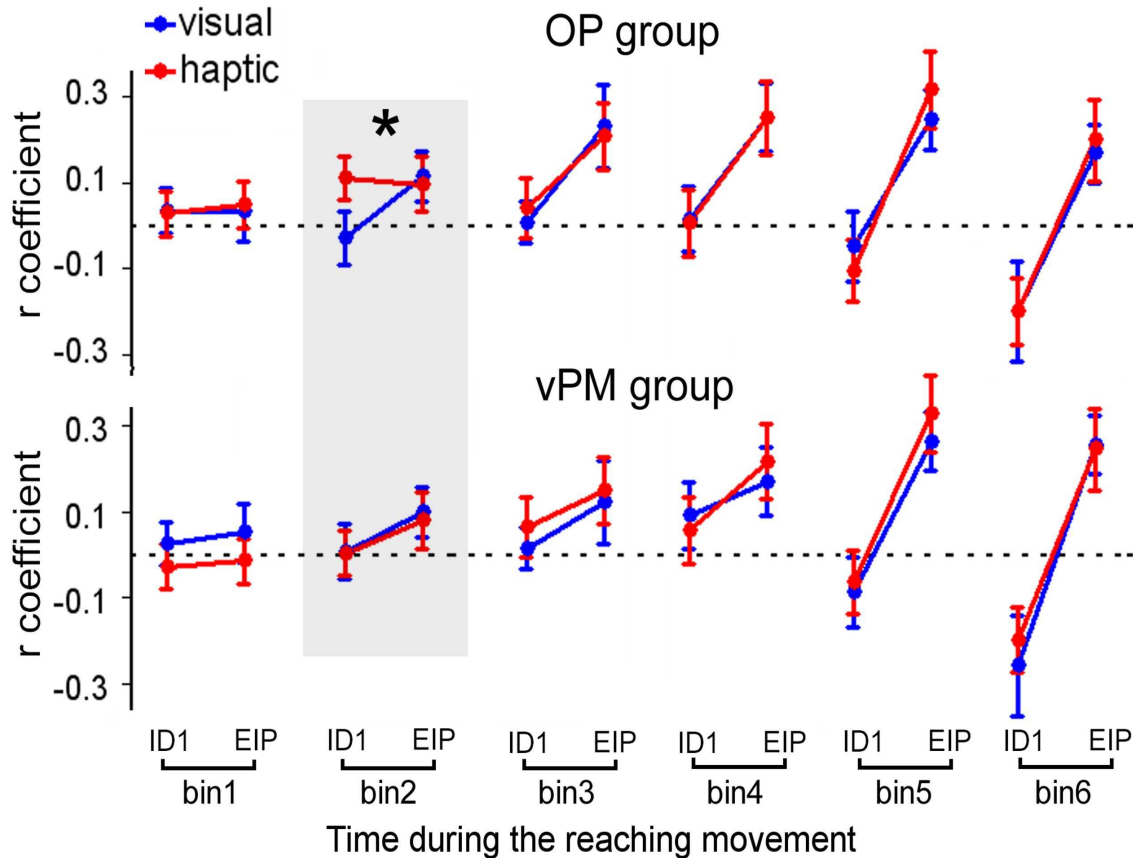


Figure 18. r-coefficients resulting from the correlation of EMG values in the Reach- Grasp phase with the inter-plug distance. Error bars indicate 95% CI. The 2nd bin, which resulted to be related to the 4-way interaction, is highlighted with a gray shade and an asterisk.

This interaction was further analyzed by means of 6 separate OUP*MODALITY*MUSCLE ANOVAs, which yielded a significant 3-way interaction only in the 2nd time bin ($F(1, 22)=7.10$, $p=0.014$). A further decomposition of the analysis indicated that only in the OP group a significant MODALITY*MUSCLE ($F(1, 11)=14.67$,

$p=0.003$) interaction was present. Post-hoc comparisons indicated that the interaction was due to the fact that in the visual modality the mean r -coefficients were significantly different between the two muscles (Bonferroni-corrected $p=0.027$) but not so in the haptic modality (Bonferroni-corrected $p=1.0$). Within the OP group, the results are not specific for the condition of dual pulse but are generally observed in the group of subjects who have been stimulated on the OP compared to those stimulated over the vPM.

Discussion

This experiment characterized the role of the OP-M1 interactions demonstrated in Experiment I. in processing haptic information for grasping. Previous data indirectly indicate that the motor functions of OP could be at least in part related to haptic knowledge of graspable objects' geometry. In humans, neuroimaging studies show that SII is active in tasks requiring the memorization of textures or 3D shapes acquired haptically (Bonda, Petrides et al. 1996) (Kaas, van Mier et al. 2013). In monkeys, single neurons in SII present a sustained activity when haptic information is memorized for the later performance of a discrimination task (Romo, Hernandez et al. 2002). Finally, one imaging study showed that SII is particularly active when monkeys must rely on haptic memory to perform a reach-to-grasp movement in the dark (Nelissen and Vanduffel 2011). Our experiment extends these observations by showing that OP stores haptic information on object shape that is then to be transferred to M1 once a reach-to-grasp plan is implemented. This is indicated by the critical finding that in trials with testTMS only, no information on a motor plan to grasp that particular object size could be extracted from the MEPs, ultimately suggesting that such information was not yet present in M1 at the time of TMS pulse. This conclusion arises mainly from the mutual

comparison of the r-coefficients between the two muscles, rather than from a significant deviation of r-coefficients in any of the conditions from the 0 value. In the first part of this experiment we applied condTMS to the OP region. Prior to the actual movement, the pattern of conditioned corticospinal output from M1 to the EIP muscle was differently correlated with the inter-plug distance compared to the ID1 muscle pattern. More specifically the EIP r-coefficients were higher than those from the ID1 muscle. This result is in keeping with the fact that we recorded two muscles that are partially antagonists when actually producing the hand shape required in the task. The EIP is more active when reaching for the manipulandum with a large inter-plug distance than with a small inter-plug distance. Vice-versa the ID1 muscle needs to be more relaxed when reaching for large inter-plug distances than when reaching for small inter-plug distances (Figure 16). The reciprocal activity in the two muscles is therefore a signature of the variations of inter-plug distance as confirmed by analyzing the ongoing EMG activity during the actual reach-grasp (Figure 17). Therefore, the significant PULSE*MUSCLE interaction following condTMS to the OP region can be explained exclusively by hypothesizing that muscle-specific information to be used for reaching-grasping is present in OP and is readily transferable to M1. These interactions are functionally relevant only when information relative to object geometry is acquired haptically but not visually (Figure 15). In the second part of the experiment we applied condTMS to vPM to a second group of participants. Taken together with the results of the OP group of participants the data show a clear double dissociation between sensory modality and stimulated cortical area. Indeed in the vPM group we found that the conditioned corticospinal output was predictive of the motor plan required to grasp an object of a given geometry only when that information was acquired visually and not haptically. The results in the vPM group are consistent with the effects characterizing PMv-MI interaction in the visual modality previously reported (Davare, Montague et al. 2009). Altogether, these results show that

SII and PMv are two essential components of the cortical grasping network in which they support highly complementary functions. It is likely that direct connections between these two areas are required to coordinate their activity and their interaction with M1 during grasp. As stated above while discussing the results of Experiment 1, the effects of the conditioning TMS on corticospinal excitability are likely to reflect cortico-cortical interactions. Differently from what observed in Experiment I, the net effects of condTMS are not merely inhibitory, but rather modulate the net output of M1 in the corticospinal system in a task-dependent pattern. As shown in previous twin-coil bifocal TMS studies and reviewed by (Koch and Rothwell 2009), the effects of conditioning TMS cannot be possibly classified as simply inhibitory or excitatory, but rather instantaneously indicate the transfer of information within a cortical network. The task used in the present experiment actually tests the capacity of the brain to hold in a working memory system the motor plan required to grasp an object of a given geometry. Some observations on this should be made. The issue of the neural site of tactile working memory for objects is not well defined because most studies focused on the retention of memory of textures rather than macro-geometrical object features. There is general agreement on the fact that haptic memory is supported by an expert and dedicated system which resides probably in a ventral network passing from the somatosensory cortex, the parietal operculum and insula and finally the frontal cortex as reviewed in (Burton and Sinclair 2000) (Pasternak and Greenlee 2005). Although our task was based on memory retention of object geometry, our data also suggest that OP plays a key function during grasping by transferring information to be used for movement to M1 (Milner, Franklin et al. 2007). From a further perspective, perception-action coupling theories generally are aimed at identifying several hierarchical levels of sensorimotor interactions, as reviewed by (Lebedev and Wise 2002), but do not take into account the possible differences between sensory modalities. In fact most models on perception-action coupling indicate

the premotor cortex as a pivotal node from which all information needs to pass to be transformed into movement. Our data suggest that the premotor cortex does not cover this role for all sensory modalities. Haptically-guided behavior relies on a distinct network in which the role of the parietal operculum is that of a “premotor” cortex where the term premotor does not refer to anatomy, but rather to its functional position in the circuit. In addition, we provide unprecedented indications that the OP region is causally involved in the use of haptic information for the reaching-grasping movements. We analyzed the actual pattern of ongoing EMG activity while subjects were reaching for the manipulandum and its correlation with object size (Figure 8). We found in the very early phases of hand pre-shaping in the haptic task a significant difference in EMG activity 16 between the OP and the vPM groups. More specifically, the physiological differentiation of EMG activity between the two recorded muscles (namely higher r-values for EIP than those for ID1 muscle) occurred later in the OP group compared to the vPM group. We did not find a significant effect of the PULSE factor. Our interpretation of this datum is that repeatedly targeting OP with spTMS produced a cumulative effect that was observed throughout the experimental session. The statistical results indicate that this sort of after-effect was present in both trials with only testTMS over M1 and in the ones with also condTMS over OP. Given the invariance of all other experimental conditions, including the sensory modality, the difference between the two groups can only be attributed to the site of application of condTMS. But how is it possible that it is evident also for testTMS-only trials? The observed changes in performance could be due to cumulative effects of TMS applied to OP along the whole experimental session. In other words this would be a repetitive TMS-like effect, resulting ultimately in a change in motor performance during the actual reaching for the object. In a post-hoc analysis we observed that the frequency of stimulation of OP was of 0.05 Hz. This is beyond 0.1 Hz that is considered the limit for carry-over effects of simple rTMS (Chen, Classen et al.

1997). However, in the absence of any other better explanation we hypothesize that the particular patterned repetitive stimulation resulting from the ordered sequence of trials applied here might escape the conventions established for simple frequency rTMS as has been shown, for example, for 0.1 Hz TMS coupled with peripheral deafferentation (Ziemann, Corwell et al. 1998). In the absence of a baseline condition (i.e. a condition without TMS) the interpretation of this finding is not univocal because the possibility exists that the difference between the two groups is due to the vPM stimulation. Given that vPM condTMS did not produce any modulation of MEPs in the haptic modality while OP condTMS did, this seems a less likely possibility.

Experiment III

Experiment IIIa

Methods

Participants

Participants: twelve healthy right handed volunteers (9 females, aged 26.6 ± 3.4 years) received on-line 4 Hz rTMS. None of them had contraindications to TMS (Rossi, Hallett et al. 2009) and all gave written informed consent in accordance with the Ethical Committee of the University of Trento (protocol n. 2009-033). All participants received TMS over two sites corresponding to left OP and ipsilateral primary visual cortex (V1) in two separated experimental sessions.

Stimulation sites

Given that in this experiment it was not possible to rely to the cortical anatomy of participants, the stimulation site corresponding to left OP was estimated on its average position computed on twenty-four anatomical scans provided in the experiment (Maule, Barchiesi et al. 2013). The coordinates of the OP spot, hereafter referred as X,Y are intended to be the scalp coordinates of the stimulation site. The coordinate system (X,Y) used as frame of reference was centered on the left external acoustic meatus; the X axis run parallel to the nasion-inion plane while the Y run along the dorso-ventral direction. The use of a Cartesian coordinate system is justified by the assumption that the site considered for the stimulation over OP is located in correspondence of a flat skull region

and thus the error on our estimation introduced by the head curvature is reasonably small. In each of the twenty-four anatomical scans the scalp spot corresponding to the left OP was previously marked on the reconstructed 3D mesh provided by BrainVoyager (<http://www.brainvoyager.com/>). The coordinates respect to the BrainVoyager reference frame were transformed into the new reference frame by applying the transformation matrix between the two systems previously obtained by computing the difference between the origins of the two reference frames. We then computed the average on the 24 coordinates obtaining the final averaged position for OP site. In order to identify this spot on participant's scalp, their head was adequately fixed with nasion-inion aligned and by means of a home-made dima representing the two cartesian axes placed in correspondence of the acoustic meatus it was possible to mark the two averaged coordinates (X,Y) of OP. In Figure 19 the X,Y coordinates of left OP respect to the new reference frame are plotted for the whole population. The V1 spot was identified as the O1 point according to the international standard 10-20 system.

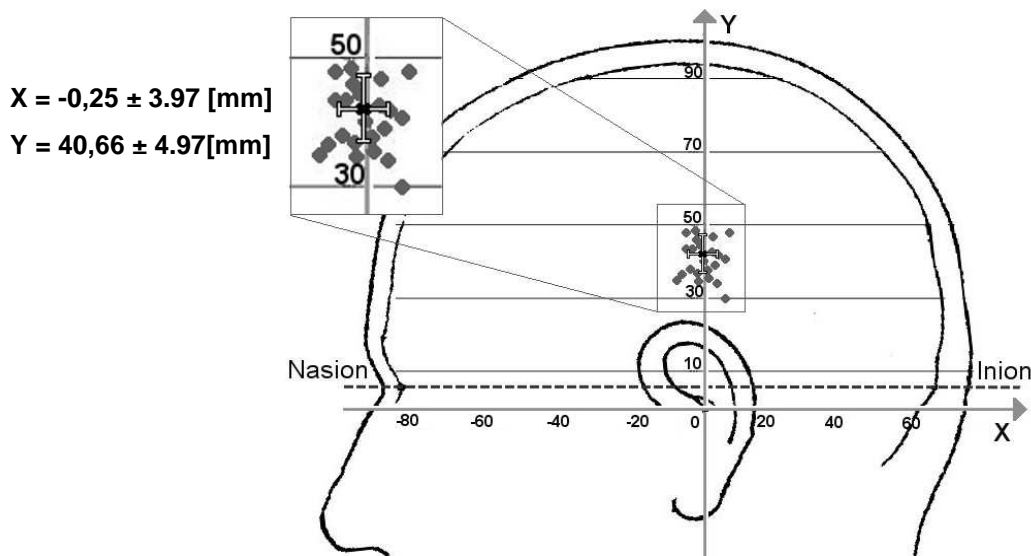


Figure 19. OP coordinates (X,Y) projected in out coordinate system. In the highlighted box, the spot representing the averaged coordinate of X and Y is represented in black. White bars represent the standard deviation along the two dimensions.

Stimulation procedure

The stimulation paradigm used is on-line high frequency repetitive TMS (rTMS) with $\nu = 4$ Hz. In this experiment, a single coil was used and moved from one TMS site to the other at the end of each experimental session. A further session without delivering TMS was run. The order of sessions was varied randomly across subjects. The stimulation was delivered with a Magpro stimulator (Magventure, Skovlunde, Denmark) via a custom-made figure-of-eight coil BCF-65 with 75 mm diameter windings. The intensity of the stimulation was set at 90% of the RMT ((Rossini, Barker et al. 1994)) computed for ID1. The subjects were sitting comfortably on a chair with both the forearms lying relaxed on the table. Their right hand was hanging out from the table, relaxed, ready to explore the object (see Figure 20). Their heads were held still with a chin-rest incorporating an additional lateral head-constrain. During the sessions in which the rTMS was delivered, the coil was permanently positioned over the stimulation site (left OP or left V1) and held in place by means of a mechanical support. Concerning stimulation over OP, the coil was oriented along the dorsal to ventral direction with the handle pointing the ceiling, while the stimulation of V1 was delivered with the coil's handle lying along the medial to lateral direction pointing towards left.

General design and experimental procedure

Participants were tested in dynamic conditions during a match to sample task performed with vision occluded. The study was composed by four experimental sessions: training session composed by 40 trials; OP stimulation session, V1 stimulation session, and no-TMS session each of them composed by 80 trials. Each trial was composed by three phases: A) haptic object exploration phase; B) delay phase; C) haptic object exploration phase. The objects explored consisted in two cylinders steel-made differing only in the

diameter dimension (cylinder 1: height = 30 mm, diameter = 15 mm; cylinder 2: height = 30 mm, diameter = 17 mm). The two cylinders were fixed on the two opposite points of a plastic-made wheel mounted on a vertical support (see Figure 20 top panel). The wheel was placed in front of the table at a suitable distance and height to allow comfortable exploration of the objects with the right hand. For each trial, the wheel was manually turned by the researcher. The subjects were therefore asked to explore haptically for 2 seconds the first cylinder presented (phase A). They were then required to stop the haptic exploration (phase B). After 2 seconds, they were asked to perform a second object exploration (phase C) of the second cylinder presented (see Figure 20 lower panel for the trial structure). Two possible objects were proposed during the phase C: the same cylinder previously explored or a cylinder differing from the previously explored only from the diameter. In the end, participants were required to answer the question: "Are the two explored cylinders different?". Their answer was stored for following analysis. It is important to highlight that the motor action were minimized as much as possible during the task and cylinders were presented immediately close to participant's fingers in a way that tactile exploration were performed without need of implement reaching. The rotation of the wheel occurred in each trial, even when the second cylinder presented was the same previously explored. For both the stimulation sessions, the 4 Hz rTMS occurred during the delay phase (phase B) for a total of 8 stimuli. During the training session, after the subject's decision, a feedback was provided by the researcher in order to allow the participant to become familiar with the objects. During the other three sessions no feedback was provided. An acoustic cue sound (1000 Hz, 50 ms duration) was announcing the beginning and the end of each phase. The beginning of each trial was triggered by the pushing of an electric button operated by the researcher. The control and the synchronization of the button, the cue sound, and the trigger for the 4 Hz rTMS was implemented by using the microcontroller ArduinoUNO

(www.arduino.cc). In each of the four sessions, the number of matching trials was balanced with the one of the non-matching as well as the direction of non-matching (small cylinder to large cylinder or large cylinder to small cylinder) and trials were presented randomly. The training session lasted about 7 minutes while three other three sessions lasted about 15 minutes for a total length of 52 minutes of experimental session.

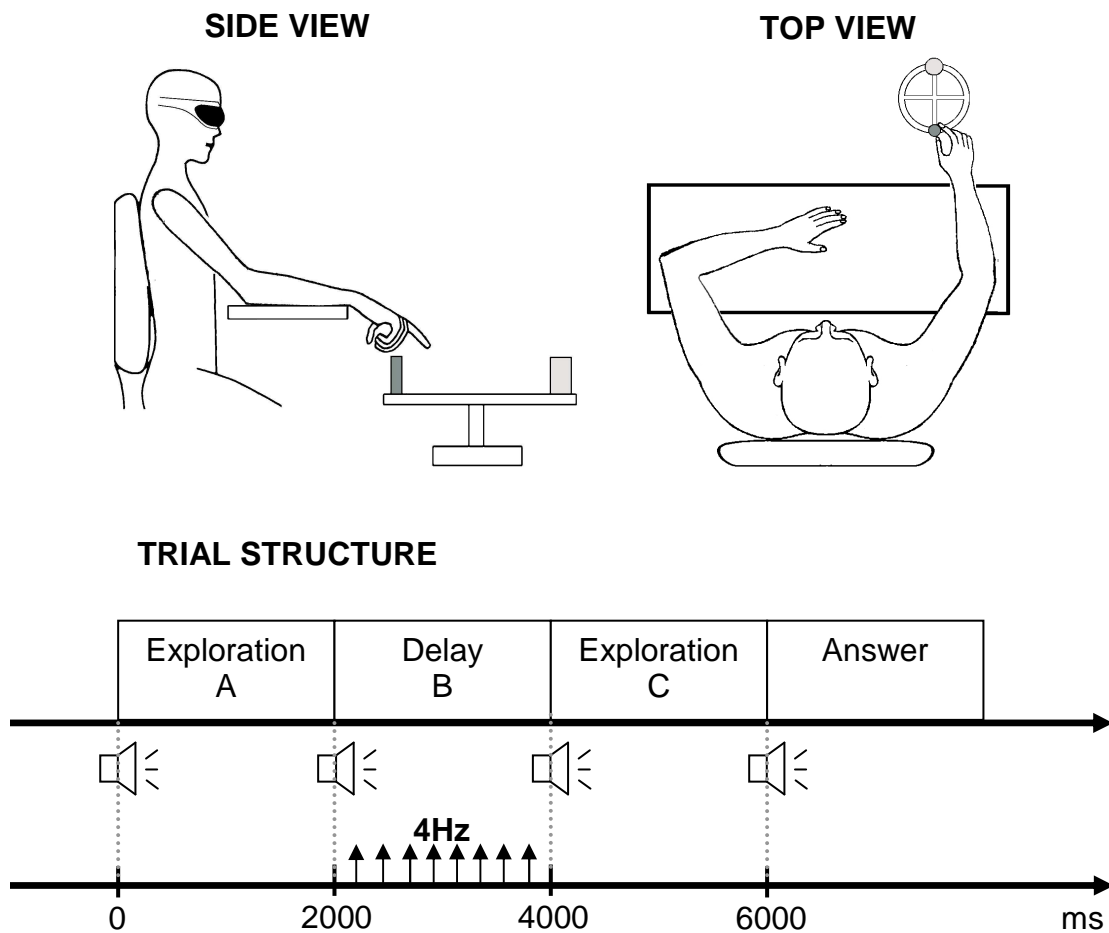


Figure 20. Top left: side view of the experimental setup. Top right: top view of the experimental setup. It is important to highlight that the motor action were minimized as much as possible during the task and cylinders were presented immediately close to participant's fingers in a way that tactile exploration were performed without need of implement reaching. Bottom: Trial structure.

Data Analysis

The aim of this experiment is to investigate the effects of rTMS on the subject's ability to perform a sample task. Since the task under investigation is a simple match to sample task ("Yes/No" task), we used the measure of sensitivity d' , as it is stated in the Signal Detection Theory (SDT) (Green and Swets 1966), to quantify the subject's ability to discriminate between the two cylinders. Henceforth, according to SDT, accuracy and sensitivity has been calculated as follows:

$$\text{Accuracy} \rightarrow P_c = (H+F)/2$$

$$\text{Sensitivity} \rightarrow d' = z(H) - z(F)$$

where H (hit rate) and F (false alarm rate) are the two SDT independent conditional probabilities of "Yes" answer, while z represents the inverse cumulative Gaussian distribution. We computed the sensitivity for each of the four experimental sessions; at the end, for each subject we obtained four values of d' that has been compared in a single factor (rTMS site) of three levels (left OP, left V1, no TMS) ANOVA analysis.

Results

The results of the on-way ANOVA performed on both the sensitivity and on the accuracy didn't produce any significant effect of the factor (SITE of STIMUATION). In particular, statistic obtained for the sensitivity (d') analysis was ($F(3,33) = 7.089$ $P = 0.6212$) and the one obtained of the accuracy (P_c) analysis was ($F(3,33) = 7.089$ $P = 0.8730$) (see Figure 21).

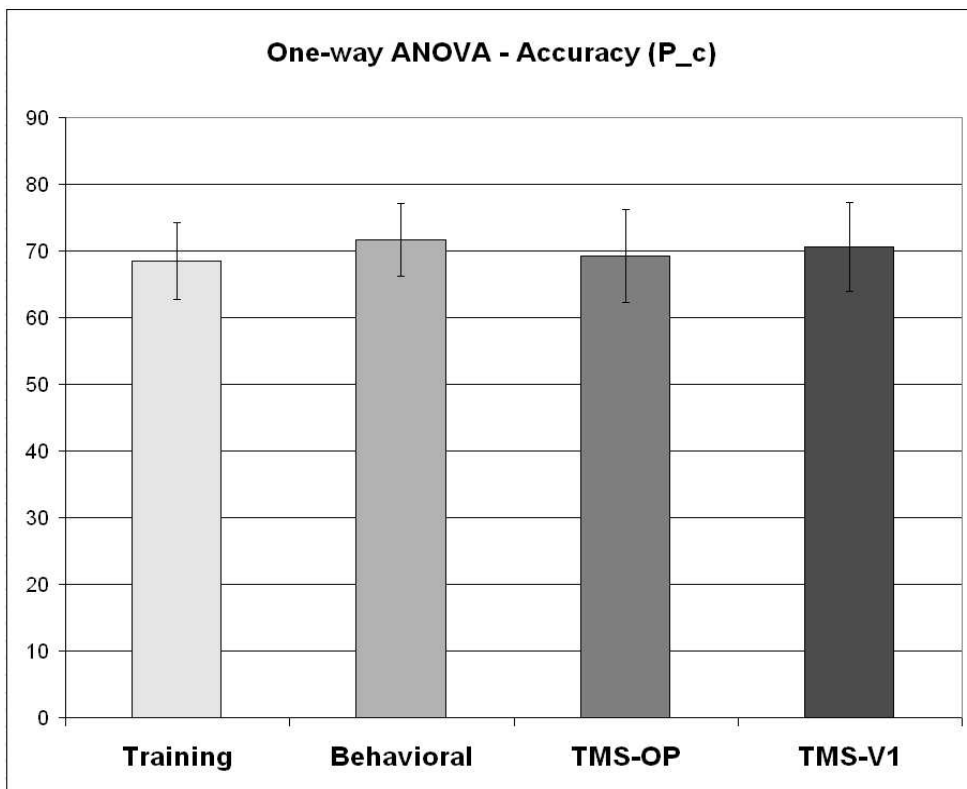
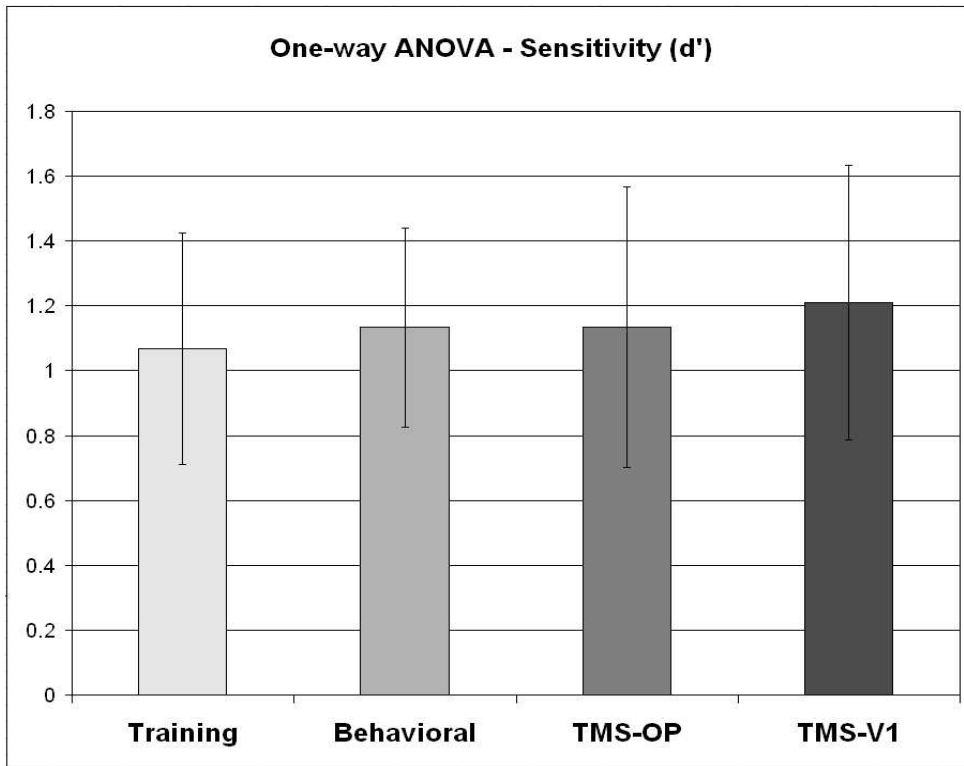


Figure 21. Results of on-way ANOVA statistic for the sensitivity (top) and for the accuracy (bottom). Different gray shades are used to indicate different experimental sessions. Black line lying on the bars represents the intervals of confidence ($\alpha=0.05$).

Experiment IIIb

Methods

Participants

Participants: Fourteen healthy right handed volunteers (6 females, aged 26.9 ± 5.7 years) received 4 Hz rTMS on-line. None of them had contraindications to TMS and all gave written informed consent in accordance with the Ethical Committee of the University of Trento (protocol n. 2009-033). All participants received TMS over two sites corresponding to left OP and ipsilateral primary visual cortex (V1) in a single experimental session.

Stimulation sites

The two sites of stimulation (namely left OP and ipsilateral V1) have been identified as in Experiment IIIa.

Stimulation procedure

The stimulation paradigm used is on-line high frequency repetitive TMS (rTMS) with $f = 4$ Hz. In this experiment two identical coils were used in a single experimental session. Trials without stimulation were interleaved randomly in the session. The TMS over OP was delivered with a Magpro stimulator while the stimulation over V1 was delivered with a Magpro compact (Magventure, Skovlunde, Denmark) both via a custom-made figure-of-eight coil BCF-65 with 75 mm diameter windings. The intensity of the stimulation was set at 90% of the RMT ((Rossini, Barker et al. 1994)) computed for ID1. The subjects were sitting comfortably on a chair with the left arm relaxed lying on the table. Their right

arm was lifted as showed in Figure 22 with thumb and index closed in such a way that they were touching each other's fingertips. Their heads were held still with a chin-rest incorporating an additional lateral head-constrain. The coils were permanently positioned over the stimulation sites (left OP or left V1) and held in place by means of a mechanical supports. The site corresponding to left OP and ipsilateral V1 was identified as described in Experiment IIIa. Concerning the stimulation over OP, the coil was oriented along the dorsal to ventral direction with the handle pointing the ceiling, while the stimulation of V1 was delivered with the coil's handle lying along the medio to lateral direction pointing towards the midline.

General design and experimental procedure

In this experiment participants were tested in dynamic conditions during a blindfolded reach-to grasp task. The study was composed by one single experimental session of 270 trials. Each trial was composed by four phases: A) rest phase; B) haptic object exploration phase; C) delay phase; D) blindfolded grasping of the previously explored object phase. The objects composing the stimuli consisted in four cylinders steel-made differing only in the diameter dimension (cylinder 1: diameter = 13 mm; cylinder 2: diameter 18 mm; cylinder 3: diameter = 23 mm cylinder 4: diameter = 28 mm). The height of all cylinders was 30 mm. The four cylinders were fixed horizontally on the external border of a plastic-made wheel mounted on a vertical support (see Figure 22) at a specific angle of 90 degrees. The wheel was placed in front of the table at a suitable distance and height to allow comfortable exploration of the objects with the right hand. For each trial, the wheel was manually turned by the researcher. The subjects were therefore asked to explore haptically for 2 seconds the first cylinder presented (phase B). They were then required to stop the haptic exploration (phase C). After 2 seconds, they

were asked to perform thumb and index grasping (phase D) of previously explored object. The rotation of the wheel occurred in each trial. The subjects were instructed to start every movement of different trial phases (namely exploration or grasping) with fingertips of thumb and finger touching each others and to go back to this reference position after performing either exploration and grasping actions. For each trial, the rTMS occurred randomly during one of three phases as well as the activation of the coil delivering the stimulation over OP or V1. Trials with no stimulation were furthermore randomly added during the session. Both the smaller and the larger cylinders were presented only as catch trials. This was done in order to avoid the production of long-term representation in memory of the two cylinders. The experimental design counted thus 3 factors (DIAMETER, SITE of STIMULATION and PHASE of STIMULATION); the DIAMETER had two levels (18 or 23mm) while the other two factors had three levels (stimulation over OP, V1 or NO STIMULATION - stimulation during phase A, B, or C). Ten trials were run for each condition ($2 \times 3 \times 3 \times 10 = 180$ trials). We interleaved randomly four catch trials for each conditions ($2 \times 3 \times 3 \times 4 = 72$) for a total number of trial equal to 252 trials and a total duration of the experimental session of about 55 minutes. An acoustic cue sound (1000 Hz, 50 ms duration) was announcing the beginning and the end of each phase. The beginning of each trial was triggered by the pushing of an electric button operated by the researcher. The control and the synchronized of button, the cue sound, and the trigger for the 4 Hz rTMS was implemented by using the microcontroller ArduinoUNO (www.arduino.cc).

Hand aperture data recordings and analysis

The aperture of the thumb and finger was measured by means of a fleximeter. A fleximeter is a transducer consisting in a flexible stripe plastic-made embedding resistors

that change their resistance according with the bending/flexion of the strip. In this way it is possible to obtain an electrical signal varying according with the flexion of the transducer. The strip (70 x 5 mm, flat resistance = 25 K Ω ; bend resistance range = [45

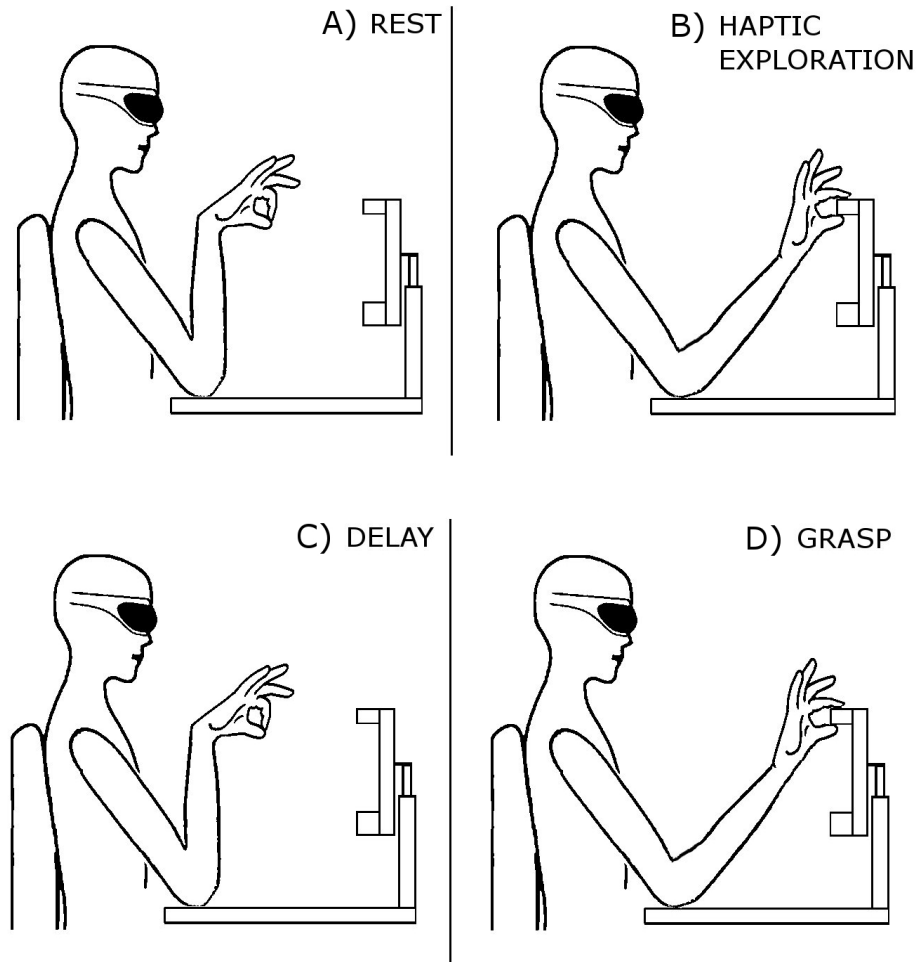


Figure 22. The task. Each trial is composed by four phases: A) Rest,; setting of the cylinder; B) Exploration of the cylinder with thumb and index grasping; C) Delay the subject waits for the acoustic go signal; D) Reach-grasp: the participant performs a thumb-index grasping of the cylinder

to 125 K Ω) was sticked tightly to the right palm of participants, running from the middle/proximal phalanx of the index finger, till the proximal phalanx of the thumb (see Figure 23). Since the grasping required in this study was a thumb-index grasping, the

signal produced by the fleximeter in such position was representative of the cinematic of the movement along the trial (see Figure 24). The signal was digitized by means of a CED 1401 micro Mk-II unit (Cambridge Electronic Design, Cambridge, UK) at a sampling frequency of 100Hz and recorded by means of the Signal software (Cambridge Electronic Design, Cambridge, UK). For each trial, the part of the signal related to the hand preshaping of the grasping phase was isolated from the rest of the signal in a time window ranging from about 5,5 s to 8 s (section 5 of the Figure 24) and the maximum value was extracted. Such value is intended to be representative for the maximum hand aperture during the preshaping phase before the effective grasping of the previously

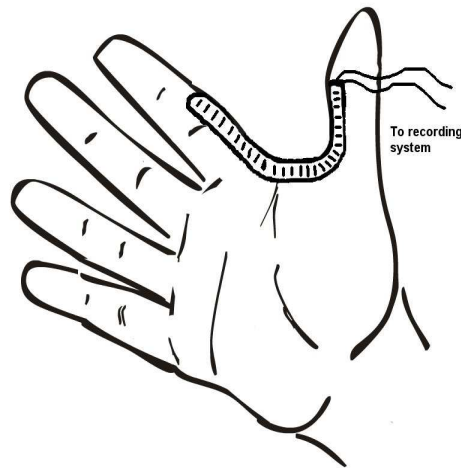


Figure 23. Fleximeter application. The fleximeter was stuck to the hand as showed in order to obtain an electrical signal variable according to the hand aperture indicating the hand preshaping during the thumb-index grasping (phase D).

explored cylinder. For all the trials belonging to a single condition the medians of the maximum values extracted were computed. The decision of adopt the median instead of using a classical value of average, is due to the fact that the shape of the distributions of

peak values belonging to different conditions were slightly skewed and in this case, it is known that the mean loses its ability to provide a good measure of central tendency of data. Moreover, in the electrical recordings, there was the unavoidable presence of artifacts introduced by the stimulation which might produce possible outliers into our data (Manikadan 2011). The final dataset for each of the fourteen subjects was composed by 18 values of the median of the peak distribution corresponding to a 2*3*3 design with the factors: DIAMETER (2 levels: 19 and 23mm), STIMULATION SITE (3 levels: left OP, left V1, NO TMS) and PHASE of STIMULATION (3 levels: during exploration, during delay and during grasping). Uche values were used as dependent variable in an ANOVA analysis with the three within-subject factors (DIAMETER, STIMULATION SITE and PHASE OF STIMULATION).

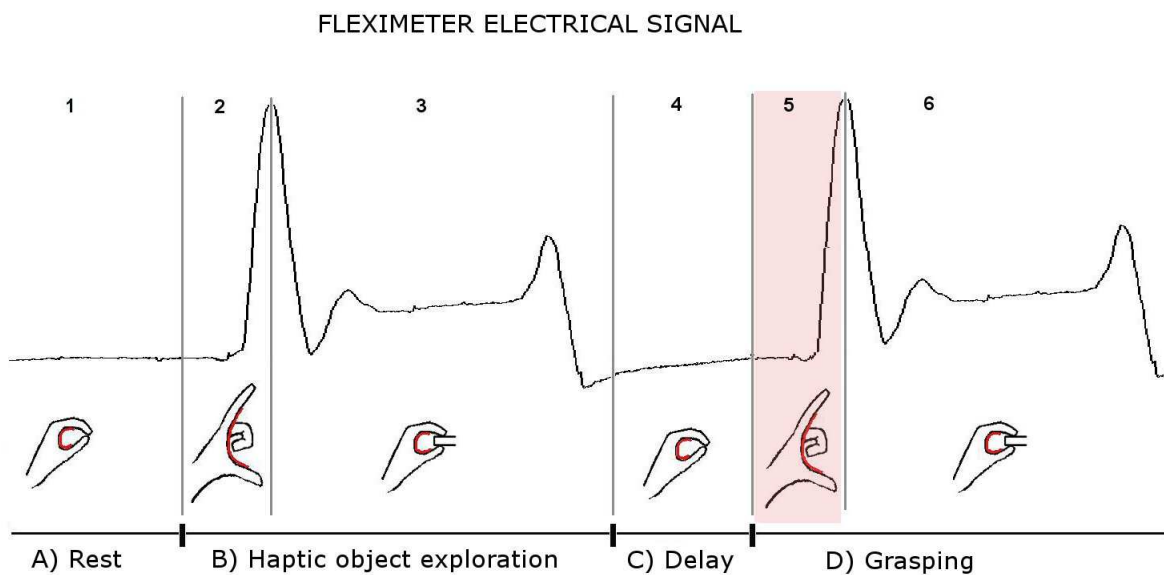


Figure 24. Fleximeter electrical signal. On the upper part of the figure the electrical signal obtained by the fleximeter during a whole trial is represented. On the lower part of the figure, the corresponding hand configuration producing the signal is reported.

Results

The results of the ANOVA performed on the median of peak values obtained from the fleximeter electrical signal during the hand preshaping before the grasping phase produced significant main effect of DIAMETER ($F(1,13) = 7.089$ $P = 0.0195$) due to the higher peak values obtained in correspondence of the grasping of the larger cylinder (see Figure 25). A further result is given by the 3-way DIAMETER * SITE of STIMULATION * PHASE of STIMULATION interaction ($F(4,52) = 2.605$ $P=0.0463$). To explore this interaction we computed different 2-ways ANOVA on data grouped according to the PHASE of STIMULATION. Such analysis didn't produce any significant effect of factors when stimulation was delivered during the exploration phase. Contrarily, we found a significant effect of the DIAMETER when stimulation occurred during the grasping phase ($F(1,13) = 9.01$, $P=0.01$). More interestingly a two-ways interaction (DIAMETER*SITE of STIMULATION) was obtained when stimulation was delivered during the delay phase ($F(2,26) = 5.44$, $P=0,01$). Results from a one-way ANOVA indicated that the interaction was to be attributed to the fact that no difference was present for smaller diameter between peak values obtained from different site of stimulation ($P=0,51$) while such consistent difference was present for the peak values obtained from the larger cylinder ($P= 0.0035$). Post-hoc t-test analysis indicated that the interaction was due to the facet that no difference was present between peak values when stimulation was delivered over the control site V1 and when no stimulation was delivered ($P=0.93$). Contrarily, a significant difference was obtained when stimulating left OP respect to V1 ($P=0.01$) and respect to not stimulating at all ($P=0.0038$). This difference is due to the fact that the stimulation over left OP induced a larger hand aperture. In Figure 26 the results obtained for the 2-ways interaction are showed.

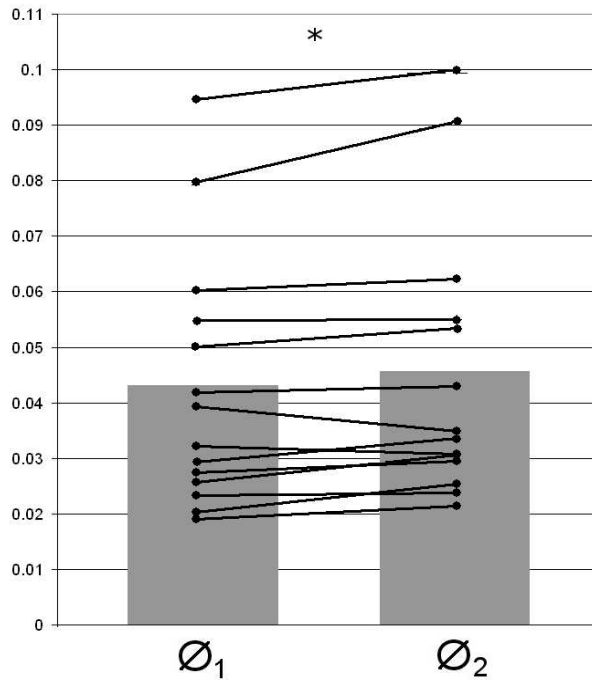


Figure 25. Main effect of the DIAMETER factor on the hand preshaping before the grasping phase.

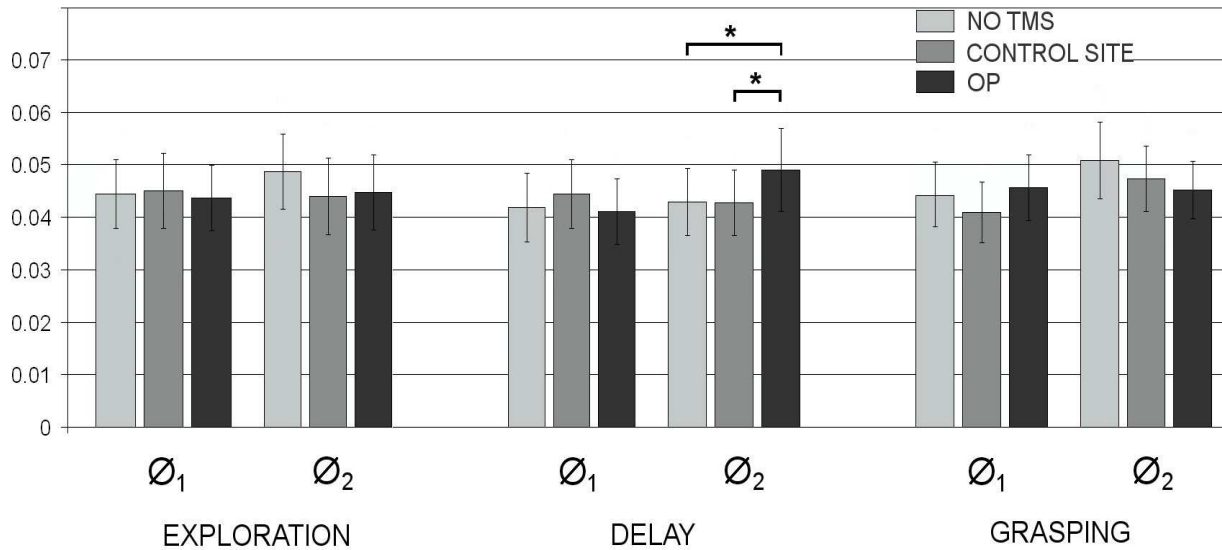


Figure 26. 3-way ANOVA interaction between factors DIAMETER, SITE of STIMULATION and PHASE of STIMULATION. The significant effect of rTMS delivered during the Delay phase is highlighted with the symbol * ($\alpha=0.05$)

Discussion of Experiment IIIa and Experiment IIIb

In this experiment we aimed to study whether rTMS delivered over left OP produced a significant perturbation of performance in two different tasks that both relied on the formation of an internal representation of the 3D properties of an object acquired in the haptic modality. Specifically, Experiment IIIa aimed to study the effects of the online rTMS on the participant's accuracy when performing an explicit discrimination task of an object's size. Experiment IIIb aimed to assess whether the same paradigm of online rTMS induced significant modification in the kinematics of hand pre-shaping when reaching for a previously haptically explored object. Both of the tasks required the short-term retention of the geometrical feature of the object (e.g. size), however the use of such information for the task accomplishment was different. The task in experiment IIIa presumably required to actively encode the size of the first cylinder, to maintain it in a memory buffer and to compare it to the size of a second cylinder explored with the aim to explicitly decide whether the two objects were the same or of different size. Experiment IIIb instead, required haptically exploring one cylinder, storing the size information in a memory buffer and, after a delay, using it implicitly to grasp the same object. The basic difference between the two tasks was that the haptic information was used for two different final aims: object perception (discrimination) and object-directed action (grasping).

A preliminary discussion is reserved for one result obtained in Experiment IIIb related to the main effect of the DIAMETER factor on the peak values (see Figure 25). The larger was the diameter of the cylinder previously explored, the higher was the peak value obtained indicating a larger hand aperture during the hand pre-shaping independently from the occurrence of the stimulation, site and timing. This result that reflects our expectation is in accordance with the evidences about the kinematics of visually guided

grasping in which increasing object size led to a proportional increase in the peak hand aperture (Bootsma, Marteniuk et al. 1994). This result also supports the use of the fleximeter and the as a system to measure the kinematics of such task-related grasping and the use of the median as a metric to represent our data.

Concerning the main discussion of our results, basically, we didn't observe any significant change introduced by rTMS in the participant's accuracy when haptic information was used for object discrimination. On the contrary, rTMS induced specific changes of the regular hand pre-shaping in the delayed grasping task. In the literature the absence of effect in a discrimination task find support in those obtained in (Stoeckel, Weder et al. 2003); in this fMRI study in which subjects perform tactile discrimination of size-variable objects, authors showed activation of S2 during exploration of objects, but not during the object discrimination phase. On the contrary, our results are partially in contrast with the literature that provides evidence for the activity of OP neurons during haptic discrimination task. In their recording study on non-human primates, Romo and colleagues (Romo, Hernandez et al. 2002) recorded neurons belonging to S2 in a vibrotactile discrimination task showing that such neurons were encoding the difference of frequency of the two haptic stimuli. A significant difference that could be taken into account in order to explain the discrepancy of our result is the nature of the stimuli. In our case there's involved a 3D geometrical feature of the object while in their case, monkeys had to discriminate between two different frequencies of vibration. Another difference could be attributed the region recorded from Romo and colleagues. They concentrated on neurons belonging specifically to S2 while in our experiment we assessed effects of rTMS one spot located over left OP (which is known to embeds more than only S2 field (Disbrow, Roberts et al. 2000) (Eickhoff, Grefkes et al. 2006)) whose coordinates was computed as the averaged coordinates on 24 subjects. We are

not able to tell if we were stimulation exactly over the S2 region. A limit of the experimental design of Experiment IIIa is that we investigate the effects of rTMS on the subject's performance during the delay phase, without accounting for the encoding phases of the cylinders. Such investigation could allow to assess the causal role of OP in encoding phase in a non-motor task such a discrimination task.

Beside such considerations, the difference related to the significance of effects between Experiment IIIa and Experiment IIIb might provide evidences in support of the hypothesis, already present in literature (Mishkin 1979; Murray and Mishkin 1984; Dijkerman and de Haan 2007) of separated cortical networks for processing haptically acquired information depending from the final purpose for which the input is acquired. Such model (see in Figure 27 a possible example adapted from (Dijkerman and de Haan 2007)), involving two different processing streams of acquired information has been previously proposed for the visual system (Goodale and Milner 1992) (Jeannerod and Rossetti 1993) under the concepts of visual ventral stream and dorsal stream. The former, is a network dedicated to process visual information for object perception in a scene-based frame of reference while the latter is engaged in the processing of visual inputs in a context of object-directed action which presumably occurs in an actor-based frame of reference. Results coming from our Experiment IIIb support the involvement of left OP in an "object-directed action" network for haptic modality compatible with the proposed dorsal stream for vision. The significant variation on hand preshaping introduced by rTMS when delivered during the delay phase, (a phase in which the haptically-acquired size information is likely to be present in an ongoing processing for the coming action) supports our previous hypothesis of OP as relevant player for the transformation of short-term memory of object geometry into a coherent pattern of muscular activation for grasping (Maule, Barchiesi et al. 2013). Such direct involvement

in an action-finalized network is furthermore supported from short-latencies (5ms) functional interaction with ipsilateral M1 demonstrated in (Cattaneo and Barchiesi 2011) and (Maule, Barchiesi et al. 2013) beside from various anatomical studies on non-human primates showing direct cortico-cortical connections between OP and M1 (e.g. (Tokuno and Tanji 1993) (Gharbawie, Stepniewska et al. 2011)).

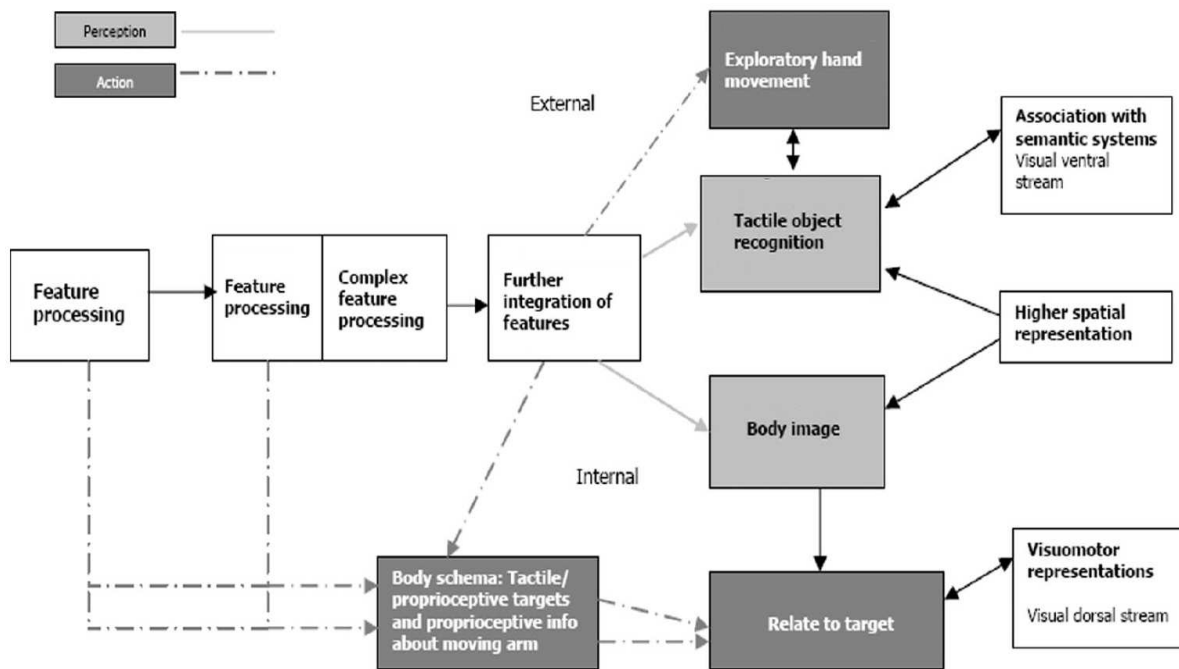


Figure 27. An outline of the two stream model proposed for haptic modality. Dark grey boxes and broken lines depict areas and projections involved in somatosensory processing for action. Light grey boxes and lines show the areas and pathways involved in somatosensory processing for perceptual recognition. Note also the distinction between somatosensory processing pertaining to the body (internal, shown in the bottom of the figure) and tactile processing of information concerning external stimuli such as objects (top). Adapted from (Dijkerman and de Haan 2007)

One striking and unexpected aspect of the the result of Experiment IIIb is that rTMS produced specific effects on hand kinematics only when it was applied during the delay phase and not during the exploration/encoding phase.

We hypothesize two possible explanations:

A) If the OP represented simply the storage site of a sensory memory, it would be expected that its role would be evident also when acquiring and storing the haptic information. The dissociation between the effects of rTMS during the encoding and the delay phases can be explained by a similarity with the model of the phonological loop in working memory (Baddeley 2003). In this model two systems interact in order to keep alive a verbal memory: a memory buffer and a rehearsal system. If we apply to haptic working memory a similar model, our data fit well with a role of the OP in the motor rehearsal module rather than in the pure memory buffer module. In other words, the present data seem to indicate that the part of the OP that we are functionally testing in our protocols has a role more similar to that of a premotor cortex than to that of a sensory cortex. By all means this partial conclusion does not exclude that the OP contains purely sensory representations, but in our opinion, at least some neuronal populations in the OP region are part of a premotor network rather than of a sensory one.

B) One alternative explanation that could actually encompass both a role of the OP as a sensory and as a premotor cortex is the following. It is well known that sensory representations in S2 are mainly bilateral. Therefore a compensation of contralateral non-stimulated OP could account for the lack of effects in the encoding phase of Experiment IIIb and even for the negative result of Experiment IIIa. On the contrary it is possible that the neurons in OP that directly access M1 contain a strictly unilateral representation of the body (as witnessed by anatomical connectivity data (Mesulam and Mufson 1982; Mufson and Mesulam 1982; Pandya and Seltzer 1982; Cusick, Wall et al. 1989; Krubitzer and Kaas 1990; Stepniewska, Preuss et al. 1993; Cipolloni and Pandya 1999; Qi, Lyon et al. 2002; Disbrow, Litinas et al. 2003)).

Our results support thus the hypothesis of two different streams for processing haptic acquired information depending on their eventual use (for perception or for action), a model which is dual to the one proposed for visual modality. As well as in the visual domain where investigations on certain types of visual illusion ((Aglioti, Desouza et al. 1995; Haffenden and Goodale 1998)) or studies on visual agnosia (Goodale, Milner et al. 1991) or optic ataxia (Jeannerod, Decety et al. 1994) are taken to show the dissociation between vision for action and for perception, also for haptic modality such dissociation has been showed with tactile illusions (Westwood and Goodale 2003) and studies on patients (Caselli 1991) (Reed and Caselli 1994). With our experiment we provided further insights in support of the hypothesis that the anatomical and the physiological organization of the somatosensory system should reflect the final use of haptically acquired information hypothesizing an active and direct interplay between OP and ipsilateral M1. We furthermore provided evidences that might suggest an analogy between the role of OP in haptic modality the one of PMv in visual modality supporting the involvement of OP in the production of the object-directed movement acting as premotor region, as a site for retention of haptically acquired information related to geometry of explored object.

Chapter 6

Conclusions

By summarizing the different results obtained with the described experiments it is possible to hypothesize the following conclusions:

- Data obtained in Experiment I provide further evidences related to cortico-cortical connectivity of OP with primary motor cortex, confirming results obtained in (Cattaneo and Barchiesi 2011). Our outcomes allowed us to identify the optimal stimulation site in the left OP that elicited short-latency modulation of M1 as a point along the postcentral sulcus, 2 cm above the Sylvian fissure. Moreover we were able to characterize the functional interaction by finding the optimal interstimulus interval between conditioning and test stimuli that elicited modulation of excitability of motor cortex equal to 5 ms, a data in support a monosynaptic connection between those two regions.
- Results of Experiment II indicate that OP and vPM play a fundamental role in the transformation of short-term memory of object geometry into a coherent pattern of muscle activation for grasping. Each of the two areas is specialized for one sensory modality, the OP contains information acquired haptically and the vPM contains visual information. Despite this segregation, the role of the two cortical stations appears very similar in storing macroscopic sensory information to be used in an open-loop manner for object-directed motor behavior.

- With Experiment III we provided evidences supporting the hypothesis of the existence of two separate neural pathways involved in the processing of haptic information depending on the final purpose: for conscious perception and recognition or for guidance of actions. Without excluding the purely sensory nature of the OP region, we hypothesized on the basis of our results that at least some neuronal populations belonging to such parietal region might play a significant role in a premotor network as an element working for the maintenance of the haptic acquired information which is following used to produce a suitable object-directed action.

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Figure 2. Cytoarchitectonic maps A: Roberts and Akert (1963). Abbreviations: Allo, allocortex; CS, central sulcus; Ins a, agranular insular cortex; Ins d, dysgranular insular cortex; Ins g, granular insular cortex; IP, intraparietal sulcus; IPD, anterior subcentral sulcus; OFO, orbitofrontal cortex; PrCo, precentral opercular cortex; SA, arcuate sulcus; SP, principal sulcus; SSII, second somatosensory cortex; tr, transitional zone.)). B: Lateral view of the cerebral hemisphere of macaca mulatta. Abbreviations: AN, annectent gyrus; AS, arcuate sulcus; CING S, cingulated sulcus; CC, corpus callosum; CF, calcarine fissure; CS, central sulcus; IOS, inferior occipital sulcus; IPS, intra parietal sulcus; LF, lateral (Silvian) fissure; LS, lunate sulcus; OTS, occipitotemporal sulcus; POMS, parietoccipital medial sulcus; PS, principal sulcus; RS, rhinal sulcus; STS, superior temporal sulcus. C: Intraparietal, lateral and cingulated sulci are opened in order to show the location of the zones lying within the sulci (Pandya and Seltzer 1982).

Figure 3. Architectonic map of the insula and surrounding regions. Abbreviations: AI, first auditory area; AII, second auditory area; CS, central sulcus; I, insula; Ia-p, insula agranular periallocortical; Idg insula dysgranular, Ig, insula granular; OF, orbitofrontal cortex; OF a-p, orbitofrontal cortex agranular periallocortical; OFdg orbitofrontal cortex dysgranular; OFg, orbitofrontal cortex granular; OFO, opercular cortex; PA, postauditory cortex; PF, anterior inferior parietal cortex; PI parainsular cortex; POC, prepiriform olfactory cortex; Prco, precentral operculum; RI, restroinsular cortex; SI, primary somatosensory cortex; SII second somatosensory cortex; STPg, supertemporal cortex granular; TPdg, temporopolar cortex dysgranular (Mesulam and Mufson 1982).

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Figure 9. Group activation maps after stimulation of the left hand. Left: contralateral activation of SII/insula. Right: Ipsilateral activation of SII/insula. (Mazzola, Faillenot et al. 2012). On the low-right: the new mask representing the regions of interests used in the analysis. Parietal operculum is composed by OP1, OP2, OP3 and OP4 (Eickhoff, Schleicher et al. 2006). Anterior insular cortex is composed by anterior short gyrus (ASG), middle short gyrus (MSG) and precentral insular gyrus (PreCG). Posterior insula includes Ig1, Ig2, Id1 and the post-central gyrus (PostCG) according to the probabilistic maps based on cytoarchitectonic maps included in (Eickhoff, Stephan et al. 2005).

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mean t-values from all 5 participants. Asterisks indicate the grid points with significant differences of the mean values from chance level ($t=0$). The inserted grid indicates the nomenclature of the grid points. Note that orientation conventions of the grids are to be interpreted as overlying the scalp of the left hemisphere, therefore they are oriented with the cranial direction to the left and the caudal direction to the right.

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Figure 17. General time course of the r-coefficients linking each of the two muscles with the inter-plug distance to be grasped. Bin 1 corresponds to the period around the GO signal and bin 6 includes the contact with the plugs and the initial grasp phase. Bins 2-5 describe the reaching phase. The dashed line indicates $y=0$. Error bars indicate 95% CI.

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