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SPACE IN ACTION:

MOTOR ASPECTS OF PERIPERSONAL SPACE REPRESENTATION

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ABOUT THIS WORK

The present work aims to explore the characteristics of the portion of space immediately surrounding the body: the peripersonal space (PPS). Previous studies showed that this representation is highly plastic and that has a strict connection with the motor components of external world exploration. After an introduction regard the general characteristics of PPS (chapter 1), four studies exploring motor characteristics of peripersonal space will be presented in chapter 2 and 3. In particular, these works were conducted on young healthy subjects and athletes, with the aim to explore: the relation between PPS and the elaboration of motor programs (chapter 2 and 3); how experience could modify the evaluation of threats (chapter 2); the familiarity with a tool (chapter 3).

- Chapter 1 defines how the brain maps the space around the body, with focus on those works demonstrating that far and near spaces are coded in a modular way. Several studies have shown that multimodal neurons play a large role in the perception of stimuli external to the body. Many results on animals suggest that the encoding of space and motor acts in the space is not performed by a single cortical area, but by circuits that join parietal and premotor areas. The process of multisensory integration of information within the peripersonal space is also explored in humans studies. Functional and dynamic characteristics of PPS have been described in the second part of the first chapter, providing the necessary information on which studies of chapter 2 and 3 are based. The principal PPS functions are those dedicated to body defense and interaction with the external environment, to plan a more suitable action or reaction in a certain context. From these works emerges that the representation of spaces is a plastic construct. In fact, studies on animals, on healthy subjects and with neurological patients confirmed that active and prolonged use of an instrument to interact with objects can change the extension of peripersonal space, allowing a remapping of its boundaries.

- Chapter 2 explores the characteristics of the PPS focusing on its role on the body defense (Defensive peripersonal space – DPPS). Two works are presented in this chapter. The first one explores the role of the predictive motor system in modulating the DPPS during movement. In ecological contexts defensive mechanisms frequently occur during movement and for this reason we tested them not only in static conditions (as already done by other researchers) but also in dynamic conditions. Indeed, movements in different directions allow us to investigate the role of motor programs in risk assessment to stimuli entering or leaving our DPPS. The second study examined whether it is possible to change threat perception trough training, inducing a cognitive and somatotopical remodulation. In particular, we investigated if previously acquired sensorimotor experiences could modulate HBR in a boxing athletes.

- Chapter 3 focuses on the working PPS and on the motor experience associated to tool-use. In particular, we explored the PPS enlargement related with a familiar tool, with respect to an analogous instrument. In the first study, we evaluated, by means of a multisensory integration paradigm, how tennis players and novices to the sport of tennis perceived the PPS while holding a tennis racket. In the case of athletes, we also compared the effect of their personal racket, i.e., the one they regularly use during their sport activities, with a common one, to explore whether the two rackets were embodied in the PPS of tennis players in different ways. In the second study we replicated the previous protocol on a group of expert fencers. In addition, we asked whether the motor skills associated with the use of a specific tool, could influence motor representation in athletes. To explore this aspect, we tested cortical excitability of our subjects during motor imagery of a gesture related to their sport experience.

Index

CHAPTER 1 – GENERAL INTRODUCTION

1.1. The space around us[1]

When we look at the external world we do not notice physical boundaries or distinction between what is near us and what is in the far space. We perceive in the same way the paper we are writing in, someone waving at us, or a plane flying in the sky. Our everyday experience suggests that the space surrounding our body is a unitary, continuous construct.

On the contrary, lots of studies so far demonstrate that the portions of space are represented in a modular way in the brain and that the unified perception is built through the integration of distinct maps that are coding different spaces[2–4]. We can detect, locate, orient to, and reach for an object in order to be able to deal with it[5]. Every action we perform has a different value according to the sector of space in which it takes place[5–7].

Researchers have wondered how the external world map is formed in the brain, and how our body is represented in it. Different cortical circuits process various portion of space: in particular, the distinction seems to be linked with the distance from the body and the possibility of acting within it.

At least three kind of representations of space have been distinguished: the personal, that comprehend the body surface, the peripersonal, that is the space closely surrounding the body in which we can act and interact, and the extrapersonal, the space far from the body, outside arm’s length[3] (Figure 1).

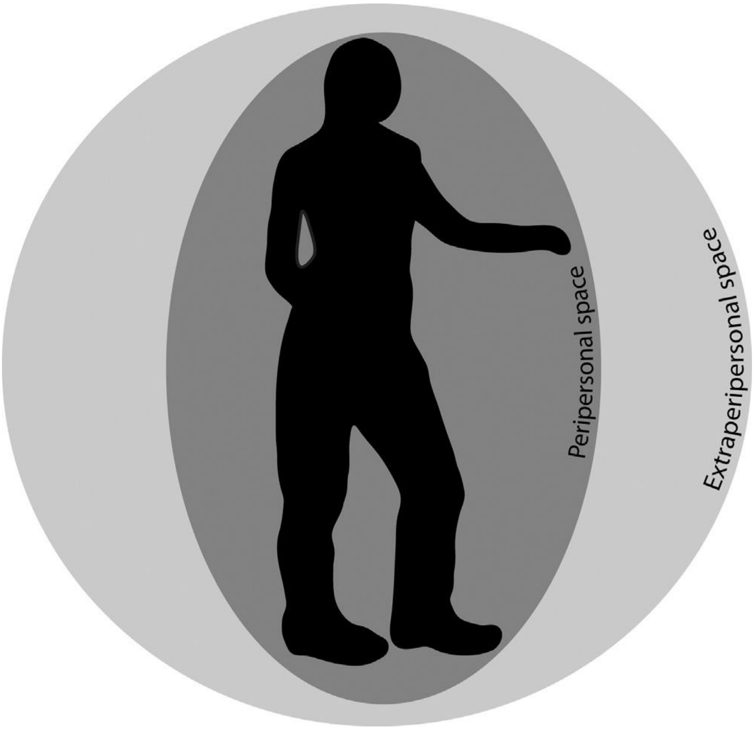

Figure 1: *Representation of the peripersonal space, directly surrounding the body, and the extrapersonal space, far from the subject.*
Source: Cléry et al. (2015) Neuronal bases of peripersonal and extrapersonal spaces, their plasticity and their dynamics: Knowns and unknowns. Neuropsychologia 70, 313–326.

The peripersonal space, hereafter PPS, is defined as a sensorimotor interface mediating the interaction between the actor and the external world[3]. It is the portion of the space where sensory signals originating from different sources can be integrated[8] and trigger motor responses[9].

In humans, most of the evidence supporting the different processing of the various sectors of space comes from studies with patients. In fact, neuropsychological conditions like neglect and cross-modal extinction provided important evidence for the dissociation between near and far space.

In 1997 and 1998 di Pellegrino and Ladavas first showed the impact of the stimulus' position in relation to the body in patients presenting tactile extinction[10,11]. People with unilateral brain lesion may fail to report a stimulus presented on the contralesional side when a competing stimulus is shown simultaneously on the ispilesional side, even though they can report either stimulus when it is presented alone[12,13]. This phenomenon has been called extinction[14]. Extinction may occur within different sensory modalities, presented singularly (unimodal extinction) or coupled (crossomodal extinction). Authors showed that presenting a visual stimulus *near* the ipsilesional hand can provoke the extinction of a tactile stimulus delivered on the controlesional hand. Notably, the extinction of the tactile stimulus did not occur when the concurrent visual one was presented *far* from the patient hand. Furthermore, authors showed that it was possible to improve patients' performance in detecting stimuli by presenting a second visual stimulus near the controlesional hand contemporaneous to the target one. This result showed dissociation between near and far representation, and a sensitivity of the PPS for stimuli originating from different sensorial sources. Further demonstrations of these different mapping modalities of near and the far space are provided by patients affected by neglect.

Neglect is a condition following brain damage to the right hemisphere, generally characterized by behavioral bias directed ipsilaterally to the damaged hemisphere and loss of spatial awareness for the contralesional side[15]. In humans, a double dissociation has been described between cases of neglect in near[16–21] and in far space[4,21–26].

In a study of 2000, Berti and Frassinetti[5] explored the possibility to change the spatial relations between the body and the external world in a patient who showed neglect in near space but not in far. The aim of the authors was to induce a remapping of far space as near, and vice versa, executing a task with different tools. Specifically, the patient had to execute a line-bisection task, indicating the midpoint of an horizontal line drawn on sheets placed in near or in far space. In both cases she had to perform the task with a stick, consenting a remapping of *far* in *near*, or with a projection lightpen, remapping *near* in *far*. Authors found that executing the task with the stick influenced the patient's computation of the space, changing far in near and worsening her performance when bisecting lines placed in far (intact) space.

Neglect and cross-modal extinction experiments showed with no doubt the dissociation in spaces representation, and that these are differently perceived by humans. Still, this studies, introduce further peripersonal characteristics. In fact, since the crossmodal extinction occurs between tactile and visual stimuli, Di Pellegrino[10] and Ladavas[11] works showed also the multisensoriality of the PPS. Berti[5] work, instead, showed that PPS representation is not fixed, suggesting that its representation is plastic and dynamic, and that tools could be integrated into it. These and other PPS properties will be described in following paragraphs.

1.2. Peripersonal space in the brain[27]

In everyday life we receive a lot of different, contemporary stimuli that originate from various sensorial sources and that must be integrated to create a coherent representation of the outside world. Single cells studies in monkeys proved the existence of multisensory neurons responding at the same time to stimuli in two or more sensory modalities. These bimodal, and also trimodal neurons have different receptive fields (RF) for each sensorial modality they are sensitive to.

Neurophysiological studies in non-human primates suggested that bimodal neurons are responsible for the dissociation in spaces representation, since their RFs tend to prefer stimuli presented in near or in far space. Two principal areas containing bimodal neurons and involved in space representation has been highlighted: the premotor and the parietal cortex.

1.2.1. Premotor neurons coding the near space

In a series of papers Rizzolatti and his group explored the area 4 and 6 in the periarcuate cortex of macaque monkeys, describing in this motor areas both somatosensory than visual properties[28,29]. They tested single neuron activity showing tridimensional objects of different shape and size, inside the visual responding region. The experimenters moved the objects from different angles and at different speeds towards the macaques, finding that a portion of periarcuate neurons require, to activate, that visual stimuli were presented in the space immediately around the animals, within their reaching distance. All of these neurons were also bimodal, responding to somatosensory stimuli too. Authors first coined the term “*peripersonal*” to refer both to the space surrounding the body of the monkey and to the neurons coding that portion of space.

Hereafter, we will refer to motor and premotor areas adopting the nomenclature suggested by Luppino and Rizzolatti[30], whom argued that the classical Broadmann’s distinction between area 4 and 6 did not consider their functional and anatomical properties. Inspiring from Matelli[31,32] area 4 (that corresponds to primary motor cortex - M1) was named F1. Area 6, instead, is divided in six parts, from F2 to F7 (Figure 2). In this organization, F5 and F4 areas present motor, somatosensory and visual responses: the first contains the “*mirror neurons*”; in the latter we found the higher concentration of bimodal neurons.

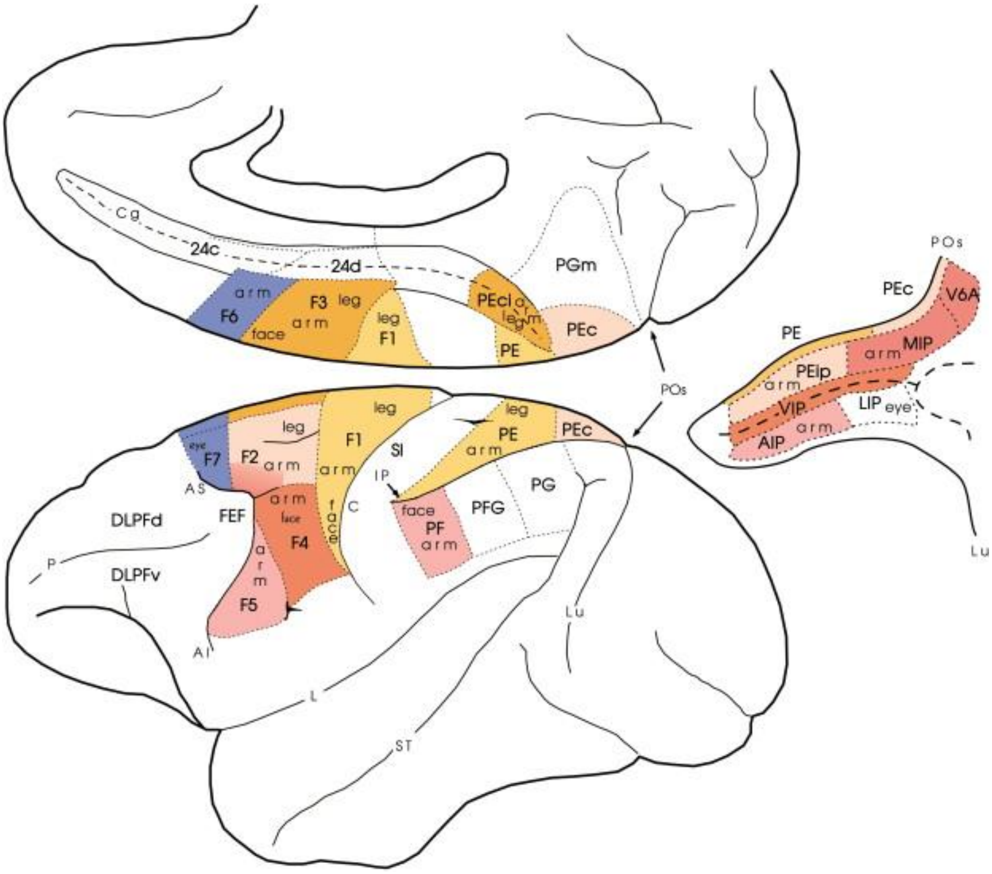

Figure 2: *Mesial and lateral views of the monkey brain showing the parcellation of the motor and posterior parietal cortices. The areas located within the intraparietal sulcus are shown in an unfolded view of the sulcus. AI, inferior arcuate sulcus; AS, superior arcuate sulcus; C, central sulcus; Cg, cingulate sulcus; DLPF, dorsolateral prefrontal cortex; L, lateral fissure; Lu, lunate sulcus; P, principal sulcus; POs, parieto-occipital sulcus; ST, superior temporal sulcus.*
Source: Rizzolatti & Luppino. (2001) The Cortical Motor System. Neuron, Volume 31, Issue 6, 889 - 901.

Fogassi and colleagues further investigates the properties of F4 neurons' receptive fields[33]. They explored tactile and visual RFs by touching the skin of the animals both with closed and open eyes and by varying gaze location. Authors found that tactile RFs were anchored to some body parts of the monkeys, especially the face, the arms or a region comprising face and trunk and/or arm. Further, they discovered that most of the bimodal neurons they studied presented non-retinocentric RFs. This means that the visual RFs are related to the body, and moving the eye does not change their localization in space. Namely, the neuron coding a location fires when a 3d object enter in that portion of space, both if the monkey is watching directly to the stimulus or elsewhere (Figure 3). Also, authors found that the extension of RFs neurons is not fixed, since increasing the speed of moving objects provokes the enlargement of the visual RFs. Furthermore, size of the tactile and visual RFs does not match, and their relation could vary.

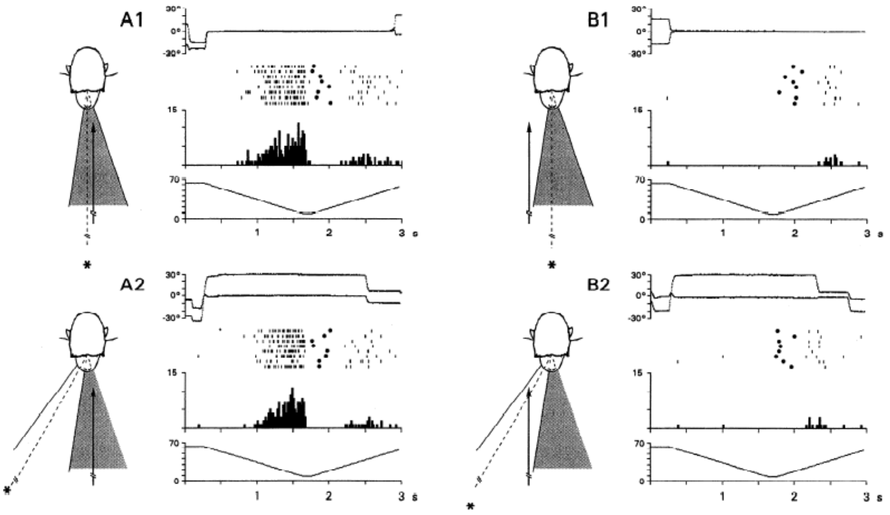

Figure 3: *Example of a bimodal neuron with RF indipendent of eye position. The neuron's tactile RF was located on the face. The visual RF is indicated by the grey triangle, whilst the asterisk represents the fixation point. In A1 and A2 the stimulus was moved inside the vRF along the sagittal plane. In B1 and B2 the stimulus was moved outside the vRF, along a trajectory parallel to that in A1, but in the opposite side of the face midline. Histograms represent neuron activity.*
Source: Fogassi, L. et al. (1996) Coding of peripersonal space in inferior premotor cortex (area F4). J. Neurophysiol. 76, 141–57.

1.2.2. Parietal neurons coding the near space

Another area found to be relevant for the coding of peripersonal space is the posterior parietal cortex (PPC). Despite being part of the visual pathway, the PPC is not purely visual and is involved in processing of multisensory spatial information, function useful to guide movements in space[34]. A specific part of PPC, located in the fundus of the intraparietal sulcus, is the VIP (ventral intraparietal area – See figure 2), which presents multimodal neurons responding also to at least four sensory modalities: visual, somatosensory, vestibular and auditory areas[34–43].

Colby[36] described some properties of VIP bimodal neurons, in addition to the selectiveness for stimuli presented near or very near the face of the monkey. Most VIP neurons were found to be strongly selective to direction and to speed of stimuli in movement. Each cell present, in fact, a greater sensitivity to stimuli moving in a preferred direction and a preferred speed. Also, neurons are not active in relation to saccadic eye movements and maintain their preference for near stimuli even when tested monocularly, suggesting that their response is not influenced by binocular disparity information. Later, Yang supported this finding, showing that VIP neurons dependent to disparity are less common than in other areas[44]. Some other VIP neurons do not show a preferred direction, but respond best to stimuli approaching the body of the animal, moving toward a particular point on the face, from any direction. VIP therefore seems to code the relative movement between the environment and the subject, more than the specific structure of the near objects[45]. Particularly, VIP neurons seem to be most sensitive to stimuli around the head, to near or approaching visual stimuli, and to vestibular head-motion signals[46].

For the importance of VIP area in multisensory spatial processing and for its high density of connection with different cortical regions, especially with F4 neurons, the VIP↔F4 circuit is often identified as the principal responsible for the peripersonal space coding. Despite functional homologies and strong communications, F4 seems to be dedicated principally to code the PPS around the limbs, whilst most VIP neurons represent the near space around the face and head. Further, electrical microstimulation of VIP and F4 neurons evokes defensive movement repertoire of the face and of the arms respectively[41,47].

1.2.3. Other regions involved in PPS representation

Several others motor and non-motor regions of the monkey brain contain bimodal visuo-tactile responses and contribute directly and indirectly to build the representation of the PPS.

The circuit formed by 7b - AIP (anterior intraparietal) - F5 (see Figure 2) codes grasping actions and seems to be involved in PPS representation. Area 7b’ cells have properties similar to VIP cells. In fact, this bimodal neurons code face and arm portion of space[4], responding preferentially to stimuli moving towards the animal. Conversely, area AIP is dedicated to code objects’ fine structure, rather than their actual position in space[48].

Circuit involving mirror neurons show sensitivity in particular to the physical properties of an object, to interact with it. This circuit is relevant to understand motor properties of PPS, because the possibility to grasp an object requires it to be at reaching distance, inside the peripersonal space. Specifically, three type of F5 cells exist: *Canonical* neurons, that respond during both the presentation of objects and the actions towards these objects[49,50]; *Mirror* neurons, which respond when generating an action and when seeing someone else performing the same action[51–54]; a subset of neurons presenting both *canonical and mirror* properties[4]. *Canonical* and *canonical-mirror* neurons respond to the presentation of a graspable object only when the grasping action evoked by the object is feasible, namely when the stimulus presented is in near peripersonal space, at reaching distance. In contrast, the response of *mirror* and *canonical-mirror* neurons during action observation is not affected by the location in space where the observed action is performed.

Another area that seems to be involved in the peripersonal space representation is the putamen, since bimodal neurons with tactile and visual RF were found there. The optimal kind of visual stimuli were moving objects entering near the tactile RF. In general, putamen neurons were often found to respond to voluntary movement[55].

1.2.4. The encoding of far space

Extrapersonal space coding seems to be related with the exploration of far space in a more sensorial way than PPS. The circuit responsible for the far space coding seems to involve a part of prefrontal lobe, the frontal eye-field (FEF), corresponding to area 8 (Figura 2). Some of FEF neurons are related to the coding of saccades, and are modulated by eye vergence[56–58] and binocular disparity[59], properties that consent the exploration of distant space and the perception of depth of far stimuli. FEF neurons presents also a retinocentric coordinates system. It was find that, in monkey, a specific damage of this area results in neglect in the controlateral space, more serious for the extrapersonal space and not associated with somatosensory deficits[60].

An area strictly connected with area 8 and physiologically similar to it is lateral intraparietal (LIP) area[61]. LIP neurons discharge even before saccades, even in task in which the saccades are expressively forbidden[6]. It has been suggested that the function of these neurons does not end with analyzing visual stimuli or planning saccades. Because FEF and LIP neurons share the function to locate an event in space, FEF↔LIP circuit have been considered the ideal candidate for *far* space representation. However, even if area LIP is considered by the most to be involved in extrapersonal space mapping, seems to contain cells preferring *near* stimuli. It has been proposed that each neuron had a preferred location in space, disposed along a fronto-parallel position, responding maximally to its preferred depth and way lesser in a spatial continuum. Main function of all LIP neurons seems to be detection trough eye-position error, namely the difference between current eye position and desired eye position[62]. Also Clery report area LIP as specific for PPS coding[4]. It is possible that its contribute to the *far* circuits is to orientate the eye to locate the external stimuli in retinotopic coordinates, preferred by FEF neurons.

1.2.5. The encoding of spaces in human

In humans, single cells investigation is not possible. Nevertheless, it is now known that two different circuits code peripersonal and extrapersonal spaces like in monkeys. Multimodal structures similar to those found in animals have been identified in possibly homologous cerebral areas in humans. Also in this case, parietal and premotor areas seem to be involved in PPS representation. It has been proposed that dorsal and ventral streams are responsible, respectively, for *near* and *far* coding. Dorsal visual stream, from occipital regions to PPC, is involved in visual guidance of actions[63] and could be relevant in PPS representation providing information to interact with external world. This hypothesis is, however, far from conclusive.

Hypothesis over different human brain circuits coding different portion of space has been supported by neurophysiological methods and imaging studies with healthy subjects and patients.

As showed in the previous paragraph, in humans most serious cases of neglect have been described either in peripersonal or in extrapersonal space. Similar dissociations have been observed in healthy subjects, through a phenomenon called ‘pseudoneglect’. It refers to the asymmetrical perception of objects in space, given by the right hemisphere dominance for visuospatial attention. The result is an ‘overattendance’ towards the left visual hemispace, opposite to the condition that usually characterise neglect syndrome[64]. Pseudoneglect is different through spaces. It is more sever in PPS than in extrapersonal[65], where the effect is reverted from a leftward bias (in PPS) to a rightward bias in far space[66]. Pseudoneglect has been used as an instrument to study space representation in healthy humans. In 2000 Weiss examined the line bisection performance of stimuli placed near or far from subjects in healthy humans[67]. Through Positron Emission Tomography (PET) recording they were able to distinguish different circuits related to act in *near* or in *far* space. These results support the idea that the ventral visual stream is primarily involved in attending to *far* space, whereas the dorsal visual stream is involved in attending to *near* space[63,64,68]. Further, in line with this hypothesis, repetitive transcranial magnetic stimulation (rTMS) has been shown to reduce the pseudoneglect when applied over posterior parietal cortex (PPC)[69,70], or to induce a temporary neglect-like condition of impaired perception[70]. In Bjoertomt study[70] experimenters applied on healthy subjects rTMS to disrupt different regions and tested the effect on a line-bisection task. They found that, apply rTMS over right inferior PPC provoked a severe peudoneglect in *near* space. On the contrary, disrupting right ventral occipital cortex caused pseudoneglect in extrapersonal space. This results support the dichotomy dorsal stream→*near* space, ventral stream→*far* space[71].

Because of rTMS applied over PPC seems to interfere especially with near space representation, and because functional magnetic resonance imaging (fMRI) studies found the right parietal lobe to be activated in healthy humans during line-bisection tasks in near space[72,73], this area seems to be a valuable candidate for PPS processing[70].

Some findings suggested that exists an area in human parietal cortex that shares some properties with macaque VIP. Bremmer and colleagues[74,75] demonstrate with fMRI the existence of a multimodal region in the depth of intraparietal sulcus that responds to polymodal stimuli conveying motion information. They also found an activation of the dorsal part of the ventral premotor cortex that remembers the direct projection between macaque VIP and PMv area. Despite pVIP multisensorial properties and its preference for moving objects, it is not always found a clear *near* space preference in this area.

Another area proposed for the peripersonal space representation is the dorsal parieto-occipital sulcus (dPOS), a dorsal stream region that is involved in reaching movements, that shows higher activation with the approaching of the stimulus. dPOS could be foundamental in utilization of gaze information to guide reaching and grasping actions in *near*. dPOS neurons would take advantage of vergence (and possibly accommodation) information to transform retinotopic to egocentric coordinates[76].

However, multisensorial properties of PPC - vPMC circuit have been proved to be fundamental for PPS representation. In a behavioral task of detection, subjects had to respond as soon as possible to a tactile stimulus presented on the hand, while ignoring contemporary audio stimuli[77] (see paragraph 1.3.2. and chapter 3 for a better description of this paradigm). Even if subjects were instructed to ignore audio stimuli, a sound presented near the stimulated hand reduced the response reaction time with respect to a sound presented in extrapersonal space. Authors applied rTMS over vPMC and PPc before the task, showing that the near sound did not facilitate anymore the RT, with respect to the disruption of V1.

A study on crossmodal extinction already presented show that human coding of peripersonal space shares some characteristics found in monkeys' single cells studies. In that study di Pellegrino and Ladavas[10,11] used contemporary tactile and visual, near and far stimuli to verify if extinction occurs on left controlesional hand. In the first place, the occurrence of extinction of stimuli belonging to different sensory category showed the multisensory properties of the PPS. This leads to think to the existence of bimodal neurons even in humans. Secondly, these studies confirm that PPS in human is represented in egocentric coordinates, anchored to specific body parts. In fact, when patients' hands were crossed, extinction of stimuli given to left hand occurs even if it is placed in the right hemispace.

Given that the dorsal visual stream plays a particularly important role in the visual guidance of actions[63,78], in humans as in monkeys perceiving the external world with motor aims seems to be fundamental in the creation of spaces maps.

1.3. Shaping multisensory action-space[79]

In previous paragraph we showed that spaces are represented in the brain by different circuits. But are peripersonal and extrapersonal space fixed? Is it possible to induce a remapping of spaces?

We have already seen that a recoding of spaces is possible. In fact it was found that visual RF of F4 neurons change on the basis of the speed of stimuli approaching the body, producing an expansion in depth of the RF[33]. So, PPS is not a fixed geometric entity, but being strictly related to the possibility to act in world, its extension could change in order to interact with the external object in a more efficient way.

We can figure at least two ways to interact with the external world: through defensive behaviours, that consist in the protection of the body, and goal-directed action. It is not clear yet if this distinction is conventional and refers to two sides of the same coin, or if it reflects a real distinction in brain coding. De Vignemont and Iannetti[80] raised this question, but their review shows that it’s hard to understand if different behaviours correspond to different categories. Two labels may be not exhaustive for the problem. Further investigation will be needed; meanwhile we will describe some PPS properties on the basis of these two categories.

1.3.1. Defensive peripersonal space

Dangerous stimuli could approach towards the body from the external world. This requires actions for avoiding potential threat or elicits protective behaviours.

In this optic PPS can be considered a “safety margin”[81] that represent the physical boundaries for a threaten stimulus to be dangerous for the body or not. The idea that there is a defensive zone surrounding the body precedes the concept of PPS itself. Heini Hediger, a biologist directing the Zurich zoo, noted already in 1955 that animals showed different behaviours depending on the proximity of other animals[80]. In an ecologic sense, a looming object represents a greater risk with respect to static stimuli. In particular, has been reported that people perceived threatening looming stimuli as having a shorter time-to-impact latency, comparing to neutral moving object[82]. A multimodal processing of visual looming stimuli has been suggested, supporting the existence of a PPS with defensive characteristics. It has been shown that when it can be predicted that a looming stimulus will impact with the body, the tactile sensitivity is enhanced at the predicted location. VIP and F4 bimodal neurons are the most likely responsible for the detection of dynamic looming visual stimuli[29,36,38,39,83], and this support their possible role in the definition of the defensive peripersonal space (DPPS)[4].

These areas (VIP and the polysensory zone (PZ) in the precentral gyrus) seems involved in maintaining the margin of safety around the body and to coordinate actions that defend the body surface, considering also that they show a preference for objects that are near or approaching the body. In addition, studies on monkeys showed that the electrical stimulation of both those areas induce set of defensive-like movements, like ducking, squinting, and blocking. These actions are characteristic for the defence of that part of the body where the sensory receptive fields of the neurons are located[84–86]. In particular, microstimulation of area VIP produces eye blinking and squinting[87], ear folding back against the head and shoulder shrugging[88] lifting the upper lip in a face grimace, the retraction of the face from the contralateral side of space and the lifting of the contralateral arm and movement of the hand into lateral or upper lateral space[89,90]. All this behaviours seem to be oriented to the face and head protection, in fact this movement repertoire is also observed following airpuffs delivered to the face[41,81]. Similarly, in F4 neurons with RF encoding the PPS around the head, microstimulation evoked a similar pattern of movements. In sites that codes PPS around the hands, instead, fast withdrawal of the hand to a protective posture behind the back is elicited[84,86]. Commonly to both areas, the electrical stimulation evokes goal-directed movements of the eyes[88,89,91]. These eye movements are reported to be slower than spontaneous saccades. At these regard, defensive behaviours have been descripted as composed by two phases[89]. In a first rapid phase it is possible to insert reflex reactions, mediated by subcortical structures like, for example, startle reflex[92,93]. A second, slower phase could require processing of stimulus location and movement, mediated by cortical areas like VIP and PZ.

A way to investigate the defensive peripersonal space is the Hand Blink Reflex (HBR). The HBR is a subcortical reflex inducing a blink of the eyes in response to a peripheral stimulation at the level of the wrist.

Contrary to other reflexes, the distinctive feature of HBR is that it is modulated by the hand position in space. In particular, the response dramatically increases when the stimulated hand is located close to the body, inside the DPPS of the face. This and others characteristics were described by Sambo and colleagues. These authors started from the knowledge that a blink reflex should be elicited both by upper and lower limb stimulation, and that the responses elicited were higher in the first than in the second case[94]. They argued that the proximity of the stimulus to the face might influence its dangerousness. This hypothesis was tested by comparing the responses of subjects holding their wrist in “far” and “near” positions: respectively, at a distance of about 60 cm from the ipsilateral side of their face or 4 cm from the face. Experimenters described that the magnitude of the HBR responses increased in near condition with respect to the far condition. They proved that this increase was definitely modulated by the presence of the hand in the DPPS and not only by the proximity by rotating the head of the subjects. Authors found that, if the hand was in “near” position, but the head of the subjects was rotated the HBR responses do not increase. On the contrary they tested the influence of the visual and sensorial information on HBR, by closing subjects’ eyes. Because HBR is modulated by the proximity of the hand even without visual inputs it can be assumed that somatosensory information were enough to modulate the reflex. Enough but not essential, as proved in a study from the same group. In this case, authors protect the face of the subjects with a wooden screen that covered their visual, then induced HBR stimulating in far and near condition[95]. They found that the HBR enhancement by hand-face proximity vanished even if the stimulated hand is near the face. This finding proves that the strength of the modulation is influenced by threat perception and risk assessment, because when the screen protects subjects' face the stimulus represents a minor danger. This suggests that the presence of the screen reduces the boundary of the DPPS of the face, and that the hand in the near position no longer enters in it. The relatedness with the defensive role of the peripersonal space is supported by the direct relation between the anxiety trait and the size of the DPPS[96]. More anxious individuals show increased defensive responses when threatening stimuli are presented at the same distance from the body, compared with less anxious individuals. This could be related with the fact that more anxious people perceive threatening stimuli as closer to their body than they actually are[97,98].

In the following works we investigated some properties of Hand Blink Reflex, related to its cognitive components and to the modulation of perceived threat. In Chapter 2 we will take advantage of the HBR to investigate the DPPS in a group of young healthy subjects and of a group of sportsmen.

1.3.2. Working peripersonal space

In the first paragraph we have seen that different tools could induce a remapping of space in patients with neglect syndrome[5]. Namely, a tool like a stick enables to act in a distant space like in near, whilst the opposite happens with instruments like light pens. Using tools modifies the possibility to interact with different portion of space, and immediately evoke the correspondent spatial representation. In case of neglect selective for near or far space, helding an object could immediately evoke one map or the other, and modulate symptoms. This proves that PPS has plastic properties, and that its boundaries are dynamic. Because the PPS shapes to integrate within it tools that change the way in which the body interact with the world, it seems to be strictly linked with the possibility to perform motor actions.

Tools, widen the reach of our limbs. To further explore this topic, Iriki and colleagues taught to a macaque to use a rake to catch food, otherwise outside their reaching space, and to pull it close to them[99]. They recorded the activity of monkeys' bimodal neurons in the intraparietal sulcus, isolating two kind of bimodal neurons, that they called distal-type and proximal-type neurons. The first type has somatosensory and visual RF normally anchored to the hand. The second type has sRFs on shoulder/neck reached by the arm. Authors showed that, only after monkey have used the tool for a while, distal-type vRF expanded to include the entire length of the tool. This enlargement, in fact, did not follow the passive holding of the tool, but requires active, intentional usage (Figure 4 a-d). Proximal-type vRFs, instead, expand to include all the surrounding space reachable by the monkey with the tool (Figure 4 e-g). Again, this shaping occurred only after active tool use. This finding proves the plasticity of PPS, in this case induced by the action dependent use of an object.

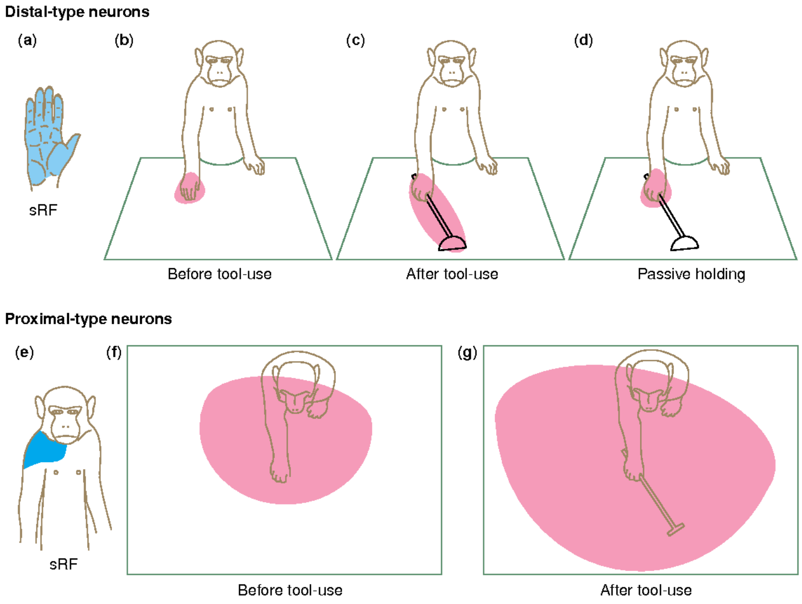

Figure 4. *Changes in bimodal receptive field properties following tool-use. (a) sRF (blue) of the distal-type bimodal neurons and their vRF (pink, b-d). (e) sRF of proximal-type bimodal neurons and their vRF (pink, f-g).*
Source: Iriki, A. et al. (1996) Coding of modified body schema during tool use by macaque postcentral neurones. NeuroReport, 7:2325-2330

In humans, similar properties for the tool use were found. Maravita and colleagues[100] tested multisensory integration and tool-embodiment with a visual–tactile interference paradigm. In this task subjects had to distinguish whether a tactile vibration was administered above or below one of their hand. Subjects held a tool in each hand and visual distractor LEDs were switched above or below the farthest tip of the tools, in congruent or incongruent positions with target stimuli. As expected, the interference on judgment was higher when the visual distractor was presented on the tool tip ipsilateral to the stimulated hand, compared to when it was lightened in the contralateral space. In half of the trials instruments were crossed so that the tip of the tools handled with the right hand was placed left, and vice versa. In this case, the interference was grater when the visual distractor was presented in contralateral space, since it corresponds with tool tip held by the stimulated hand. This results support findings of study with monkey presented before. As Fogassi showed[33], when the monkey moves the arm, the RF remained anchored to the specific body part. Also, after tool use neurons' RF expand to include the tool[99]. Here, active use of the tool seems to provoke a remapping of the *far* space into *near*, including the instrument as a functional extension of the body in space.

PPS remapping following tool use has been investigated also in patients with cross-modal extinction. Visual stimuli presented at the tip of a rake held with ipsilesional hand by patients induced more contralesional tactile extinction immediately after a brief tool use. The rake was likely embodied in personal space, and after tool use PPS widens, comprehending that portion of space that was unreachable before[101]. Again, passive holding or keeping the tool near the subject, but without any contact did not induce the remapping.

Tool use, shaping in PPS and actions are therefore linked to each other. It comes naturally to ask if the physical properties of a tool, and its effective functional properties are crucial in extending PPS. Namely, is the tool integration absolute or it depends by tool function? To answer this question Farnè et al.[2] designed a 60 cm long tool. They however placed the active part of the tool (the tines) at 30 cm from the handle. In this way they dissociate physical and functional properties of the same object. Author found a different amount of crossmodal extinction in patient after active use of a 60 cm long tool and the hybrid one 60 cm long with functional part at 30 cm. Because of the only difference between the two objects was the functional location, the decrease in crossomodal extinction they found in extrapersonal space proves the relation between the plasticity of PPS and the operational properties of tool used.

The active use of a tool is, indubitably, fundamental for its integration inside the PPS. However, the previously described examples showed an embodiment immediately after short use of the tool and lasting only for short time after that. In 2007 Serino and colleagues asked if the daily use of a tool could stably enlarge the peripersonal space, even without a training immediately preceding the test[77]. In particular, they investigated the PPS sizes in blind people, since they use a cane to navigate in the environment every day. To this aim they administered to a group of blind people and to a control group a multisensory integration paradigm, methods that took advantage of the PPS's multisensory properties. Subjects had to detect as fast as possible a tactile stimulus provided to the hand that held the cane. Concurrent task-irrelevant sounds were presented near the hand or in far space, at the tip of the cane. Authors showed that, in control group, reaction times to the tactile stimulus were speeded up when a concurrent sound was presented near the hand, rather then in far space. Nevertheless, after a 10 minutes training with a cane, the difference between the reaction time at a stimulus associated to a near and a far sound decreased. As regards blind people, they showed RT associated to far sounds even faster than those associated with near sounds. However, when they were tested handling a shorter tool the responses were similar to the control group. It seems that PPS representation seems to have a strongly adaptive value and that long term experience could induce stable plastic modifications enlargement of PPS. Notably, with this work authors proved also the existence of an auditory peripersonal space around the hand.

1.3.3. Functional meaning of spaces dissociation

Works of last decades widely demonstrated that representation of spaces in the brain is not unitary. The question is, what is the nature of space representation? Why was useful from an evolutionary point of view to maintain separate space representation, given that we have experience of one unitary phenomenon?

It has been told that space representation may not be a primary function of the brain, as action planning is. Localizing objects in space is fundamental to plan motor actions. It is possible to interpret the link between space representation and motor system in two ways: the space is perceived in a visuo-sensorial way, and only after interpreted by motor system[1]; Space is primarily sensorimotor. Various are the arguments supporting the second hypothesis. The properties of bimodal neurons, for example, seem to confirm it. In fact, bimodal firing rate seems to code mostly a potential motor action directed to a specific portion of space[33]. A bodily reference frame would make no sense in a sensory point of view, but a geometric reference system would be more efficient. Experiments that manipulated the possibility to interact with an object (motor hypothesis), instead of the physical/aesthetical properties of objects (visual hypothesis) presented a better correlation with the evoked responses. In this sense, it seems that the discharge pattern of neurons reflects the possibility to act with an object, a motor schema[102,103] oriented to a near portion of space. Study showing that speed properties of moving stimuli modulates neuron activity further support motor hypothesis. Another support to this hypothesis could be searched in discordant results present in literature with patients. It was shown that patients should suffer of a more severe neglect in one of each space. Aimola[104] proposed however that these deficits in near and far space processing depend on the ongoing task being performed by the subjects. In fact, he found that only some of the tasks proposed to patients induce a remapping of spaces and a worsening of patients' condition, and that this strictly depend by the motor goal of the task.

Studies about tool-use point out very clearly that active training is necessary to induce an integration of the instrument inside the PPS boundaries. Neither passive training, nor observing someone using the tool provoke a spatial remapping, and the visual hypothesis fails to explain the phenomenon.

The aim the work made along these years has been to further investigate the relation between the motor system and the peripersonal space. To do that we follow two lines of research that take advantage of the plastic properties of PPS and focused on defensive PPS and working PPS.

We explored the motor components of defensive PPS, to understand the role of the predictive motor system in modulating the DPPS. Moreover, we wanted to know if long term motor experience could devaluate the perceived danger of stimulus in a population of athletes that practice a combat sport.

Concerning the working PPS, we recruited athletes having a long and daily experience with tool-use to explore whether their motor experience with this implement induced and enlargement of PPS boundaries.

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CHAPTER 2 – DEFENSIVE PERIPERSONAL SPACE

2.1. Dynamic shaping of peripersonal space through predictive motor system: when the “near” becomes “far”.

2.1.1. Introduction

The studies presented in the following two sections (2.1 and 2.2) will focus on one of the two aspects of the PPS: its protective function. The defensive peripersonal space (DPPS)[1,2] has been recently investigated in humans by recording the Hand Blink Reflex (HBR), which is a subcortical response at the brainstem level elicited by the electrical stimulation of the median nerve at the wrist and recorded from the orbicularis oculi muscles[3–6]. In static condition, the HBR is modulated by the hand position in space: the response dramatically increases when the stimulated hand is located close to the face, inside the DPPS.

When we interact with the surrounding environment the modulation of the DPPS can become fundamental to prevent potentially dangerous situations. During voluntary movements, the central nervous system can estimate the final hand position using either motor outflow or sensory inflow (i.e., visual and proprioceptive inputs). These two sources of information can be combined in a predictive model, according to which, once the motor program is selected and sent to the periphery, an efference copy is formed to predict the future body state and the consequences of the movement, that, in turn, are compared with the actual state detected from the sensory feedback[7–9].

Understanding the role of the predictive motor system in modulating the DPPS during movement might be a first important step toward a full comprehension of the defensive mechanisms in ecological contexts when humans move in a possible dangerous environment. To this aim, we investigated the role of predictive motor mechanisms in dynamically shaping the DPPS during upper limb voluntary movements, by recording the HBR when participants were asked to move their right forearm up towards the face (Up-moving condition) or down far from the face (Down-moving condition). Indeed, movements in different directions could allow us to investigate the response to a dangerous stimulus entering or leaving our DPPS. In each condition, the HBR was elicited during the forearm's movement when the amplitude of the elbow angle reached three pre-defined values. In turn, these three positions determined three hand distances with respect to the face (far, intermediate and near).

We hypothesized that the predicted final consequence of the movement (either close to or far from the face) could affect the reflex response amplitude. Thus, on equal hand positions, comparing the Up-moving with the Down-moving condition, we expected to find a different modulation of the HBR depending on the direction of the hand movement.

During voluntary movements, intentional outflow and sensory inflow are both available to estimate the final position of the hand. Thus, to investigate the relative roles of these complementary sources of information in dynamically modulating HBR amplitude during movement, we designed two experiments, employing either passive movements (where only sensory inflow is present) or motor imagery (where, on the opposite, only intentional outflow is present). In the former experiment, the subjects were asked to stay relaxed while the examiner passively moved their right arm up towards or down far from their face; in the latter, the subjects stayed still, keeping their right hand either in far, intermediate, or near positions while imaging to move it up-to or down-from the face.

2.1.2. Materials and Methods

Participants

Twenty-eight participants, naive to the purpose of the experiment, were recruited for this study. They reported no previous history of neurological disorders or orthopedic problems for the right-dominant hand, as determined by the Edinburgh Handedness Inventory[10].

Firstly, participants were tested to assess whether they showed a reproducible HBR[3] (see Preliminary experiment). Thirteen participants were assigned to Experiment 1, Experiment 2 and Experiment 3A. Ten of them (about the 77% of the total number, 6 females and 4 males, mean age ± std = 22.4 ± 2.3) showed a reproducible HBR and were thus chosen to advance to the next stages of the experimental procedure. Fifteen participants were assigned to Experiment 3B; twelve of them showed a reproducible HBR and thus continued the experimental session (80% of the total number, 7 females and 5 males, mean age ± std = 23.1 ± 3.3). Participants gave written informed consent before taking part in the study. The study has been approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki.

Experimental set up

The HBR response was elicited by administering transcutaneous electrical stimuli to the median nerve at the right wrist, using a surface bipolar electrode attached with a velcro strap and connected to a Digitimer constant current stimulator (DS7AH HV, Digitimer Ltd, UK). As the stimulator provided constant current pulses, the trial-to-trial variability of the intensity of stimulation was negligible. Stimulus intensity was adjusted to elicit in each participant clear HBR responses (mean stimulus intensities were 27.7 ± 9.4 mA, range 15-42 mA). None of the participants reported painful sensations elicited by the stimulation. The stimulus duration was 200 µs and the inter-stimulus interval was ~30 s. A twin-axis electronic goniometer (TSD130B, BIOPAC System, Inc.) connected to a BIOPAC MP100 system was used to measure and record the elbow angle during movement execution. In Experiment 1 and in Experiment 2 this device allowed the automatic delivery of the electrical stimulation when the elbow angle corresponded to one of the three pre-determined stimulation positions.

EMG activity was recorded by means of two MP100 BIOPAC EMG channels from the orbicularis oculi muscles bilaterally, using two pairs of bipolar surface electrodes with the active electrode over the mid lower eyelid and the reference electrode laterally to the outer canthus. Signals were amplified and digitized at 1 kHz (BIOPAC MP100).

Experimental Procedure

The experiments took place in four different sessions. In the first session participants performed the Preliminary experiment during which the HBR responses were acquired in Static condition. Participants who showed a reproducible HBR in Static condition advanced to the next stages of the study. In the second session the selected participants executed Experiment 1 (Voluntary movement). Experiment 2 (Passive movement) and Experiment 3A (Motor imagery from intermediate position) were randomly executed in two other different sessions. At least one week passed between one experimental session and the following.

Participants were seated on a comfortable chair and kept the right elbow at the limit of a table, in a position allowing the right wrist to be in front of the ipsilateral eye while moving the forearm towards the face, but never touching it. The electrical stimulation was delivered, in static condition or during voluntary and passive movements, while participant's stimulated hand was located at three different positions relative to the face. In particular, when the elbow angle was 10° less than the maximal arm extension (far position, α1), the half of the difference between the angles of maximal arm extension and flexion (intermediate position, α2), and when the angle was 10° more than the maximal elbow flexion (near position, α3). Throughout the experiment participants were instructed to keep their gaze on a fixation point placed at 60 cm from the eyes.

<u>Preliminary experiment</u>: Static condition. This experiment aimed to make an initial selection of those participants who showed a reproducible HBR response. These subjects were admitted to the next sessions of the study. Further, we also tested the reliability of our set up by replicating the results known in literature. Participants were instructed, trial-by-trial, to put the arm in one of the three positions previously identified. After a randomly variable delay, the subject received the electrical stimulation, which was manually delivered by the experimenter. Twenty-four acquisitions were performed, 8 for each hand position. The order of the hand positions at which the participant received the electrical stimulus was pseudo-random.

Experiment 1: Voluntary movement (Figure 1A). The aim of the present experiment was to assess whether the HBR response was modulated during voluntary movement execution and was influenced by movement direction. Participants were asked to perform two sequences of movements with the right arm: elbow flexion-extension (block A) and elbow extension-flexion (block B). These two blocks were introduced to avoid that participants could predict the instant of the stimulation, and the order of blocks execution was balanced across participants. In both blocks, the electrical stimulation was delivered in each trial (flexion-extension or extension-flexion movements) during either elbow flexion (afterwards called Up-moving condition) or elbow extension (afterwards called Down-moving condition), when the angle measured by the goniometer reached one of the pre-set angle values (α1, α2, α3). At this time, an electrical signal was automatically generated by the goniometer and triggered the onset of the electrical stimulation. Ninety-six trials (2 blocks, 3 angles, 2 movement directions and 8 repetitions) were acquired. A minimum time of 30sec was kept as inter-trial interval. During this interval the subjects were asked to keep the arm relaxed. The Voluntary movement condition was preceded and followed by a HBR recording session in Static condition (4 repetitions x 3 stimulation positions before and after voluntary movement, for a total of 24 trials). This latter condition was introduced here and in each of the following experiments to test whether subjects' HBR responses in the three stimulation positions were comparable in the different days. Furthermore, this evaluation allowed testing possible effects on HBR amplitude due to habituation.

Experiment 2: Passive movement (Figure 1B). This experiment was performed to test the role that afferent (i.e., visual and proprioceptive signals) inputs could play in modulating the HBR response. Participants were asked to keep the right arm completely relaxed in a plastic splint while the experimenter moved it by means of a transparent wire connected to the splint and a pulley system. The passive movement was an elbow flexion-extension of the right arm. A second transparent wire was attached to the opposite site of the hand support (hand back) and adjusted in length to avoid that the distance between the hand and the face of the participant was less than 4 cm. At the beginning, the experimenter asked the participant to perform an elbow flexion and extension movement at natural velocity. When the experimenter thought to have understood participant’s natural movement velocity, she moved participants’ forearm and verbally questioned the subjects if he/she felt the movement velocity similar to his/her velocity. After participant’s agreement the experiment started. No differences were found between angular movement velocities evaluated in the Experiment 1 and the Experiment 2 (mean ± SD: Experiment 1, 100.13 ± 37 deg/s and Experiment 2, 99.58 ± 23 deg/s; $p = 0.95$).

The electrical stimulation was delivered during the passive movement when the angle measured by the goniometer reached the pre-set angle values ($\alpha 1$, $\alpha 2$, $\alpha 3$) during either elbow flexion (Up-moving condition) or extension (Down-moving condition) movements. As in the Experiment 1, an electrical signal, automatically generated by the goniometer, triggered the onset of the electrical stimulation. Differently from the Experiment 1, in order to reduce participants' expectancy, we introduced catch trials. We didn't introduce the blocks paradigm used in the Experiment 1 because in that condition no significant difference was found between the two blocks (see Results section). This allowed us to dramatically reduce the number of trials. Excluding the catch trials, a total of 48 trials (3 angles, 2 directions, 8 repetitions) were acquired. The Passive movement condition was preceded and followed by a HBR recording session in Static condition (4 repetitions x 3 stimulation positions before and after passive movement, for a total of 24 trials).

Experiment 3A: Motor imagery from intermediate position (Figure 1C). Before starting the experimental procedure, all the participants completed the Italian version of the Movement Imagery Questionnaire (MIQ-R[11]) to assess their ability to form kinesthetic and visual images. The MIQ-R is an 8-item self-report questionnaire, in which participants rated the vividness of their mental representations using two 7-point scales (associated to visual and kinesthetic imagery): 1 means ''really easy to feel/see'' whereas 7 corresponds to ''really difficult to feel/see''. All participants considered it fairly easy to form motor images and the scores indicated that they possessed good motor imagery abilities (mean ± SD = 18.8 ± 5.55). After that, they were instructed to put the arm in α1 and α3 positions in order to memorize them. Then, they were asked to keep the right arm in the position corresponding to α2 (intermediate position) and to kinesthetically imagine the right arm making a flexion (Up-moving condition, from α2 to α3) or an extension (Down-moving condition, from α2 to α1) movement at spontaneous velocity. At the beginning, for few trials, during motor imagery participants had to verbally report when the upper arm reached α1 or α3. When the experimenter learnt the time used by the participant to imagine to move towards one of the two positions, the experiment started. After each trial the subject was questioned whether the electrical stimulation was administered in correspondence to the position set for the current trial (α1 or α3), and imagined by the subject. In case of mismatch, the trial was repeated. As in the Experiment 2, catch trials were introduced to reduce participants' expectancy of the stimulus. Excluding the catch trials, a total of 16 trials were recorded for each subject (2 imagined directions, 8 repetitions). The Motor imagery condition was preceded and followed by a HBR recording session in Static condition (4 repetitions x 3 stimulation positions before and after motor imagery, for a total of 24 trials).

Experiment 3B: Motor imagery from near and far positions (Figure 1C). This experiment was performed to assess whether and how motor imagery influences the HBR response when the hand was close to or far from the face. A group of participants, different from those who performed previous experiments, were instructed to put the arm either in α1, α2 or α3, and to kinesthetically imagine one of the following movements at spontaneous velocity: a flexion movement from α2 to α3 (MI α2 to α3), an extension movement from α2 to α1 (MI α2 to α1) (the previous conditions replicated those proposed in Experiment 3A), a flexion movement from α1 to α3 (MI α1 to α3), and an extension movement from α3 to α1 (MI α3 to α1). As in Experiment 3A, at the beginning, participants had to verbally report when the upper arm reached the imagined final position to teach the experimenter about the time used to imagine the movement. After each trial, in case of mismatch reported by the participant between the arrival of the electrical stimulation and the stimulation position set for the current trial, the test was repeated. The four experimental conditions were executed in random order together and interleaved with catch trials. Excluding the catch trials, a total of 32 trials were recorded for each subject (4 imagined movements x 8 repetitions). The Motor imagery condition was preceded and followed by a HBR recording session in Static condition (4 repetitions x 3 stimulation positions before and after motor imagery, for a total of 24 trials).

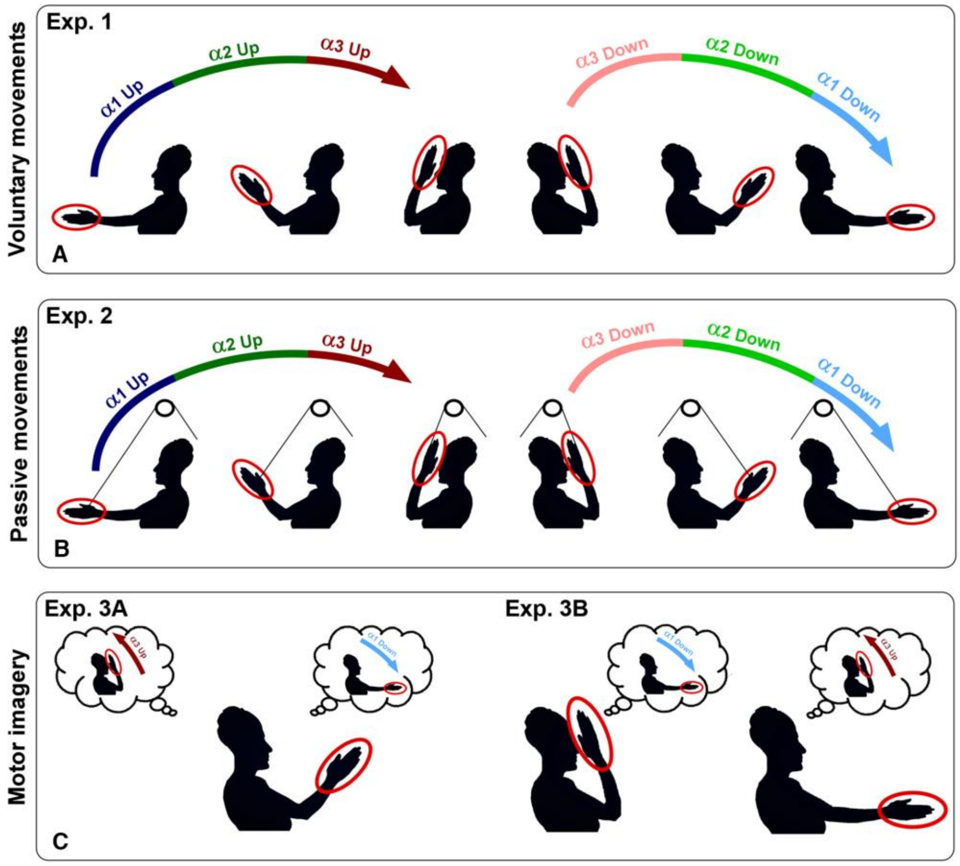

Figure 1. Experimental set up. *Panel A refers to Experiment 1, during which participants voluntarily performed either a flexion-extension or an extension-flexion movement of the elbow: while moving up towards (Up) or down far from (Down) the face they received an electrical stimulation in three pre-set positions (far, α1; intermediate, α2; near, α3). Panel B refers to Experiment 2 and shows the pulley system used by the experimenter to induce the passive flexion-extension (Up) and extension-flexion (Down) movements to participants' right forearm. The electrical stimulation was delivered in the same conditions as in Experiment 1. Panel C refers to Experiment 3A and Experiment 3B. In Experiment 3A the subject kept a static position corresponding to α2 and imagined either an elbow flexion movement towards the face (Up, from α2 to α3) or an elbow extension movement (Down, from α2 to α1), whilst in Experiment 3B the subject imagined either a flexion or an extension movement starting from a static hand position corresponding to α1 (Up, from α1 to α3) and α3 (Down, from α3 to α1), respectively.*

Data processing and statistical analysis

A custom made MatLab software was used to process the EMG signals. EMG signals from each participant were filtered and rectified. HBR responses were averaged separately in each condition and for each participant. Trials with an abnormal EMG activity preceding the HBR response were discarded by the analysis. The area under the curve (AUC, mV*ms) of each HBR average waveform was considered as outcome parameter. To compute AUC in each average EMG trace the software automatically analyzed a 130 ms-time interval from the stimulus onset that always contained the subject's blink. The resulting curve was then integrated to compute AUC. In all experiments, data were averaged across ipsilateral and contralateral recording sides (right and left eyes) according to the previous analyses proposed in literature[3].

In the Preliminary experiment, AUC values acquired in Static condition were compared by mean of a repeated-measure ANOVA with POSITION (3 levels: α1, α2 and α3) as within-subject factor. This analysis was used to confirm the literature and thus assess the reliability of our experimental set up. Further, it allowed us to identify the participants who showed a reproducible HBR response.

In order to compare the HBR responses in the Preliminary experiment with those associated to the Static condition in Experiment 1, Experiment 2 and Experiment 3A, the AUC values in the pre and post conditions of Experiment 1, 2 and 3A were averaged. Then, these data were statistically compared by means of a RM-ANOVA with POSITION (3 levels: α1, α2 and α3) and SESSION (4 levels: Preliminary experiment, Experiment 1, Experiment 2 and Experiment 3A) as within-subject factors.

In Experiment 1, Experiment 2 and Experiment 3A the AUC values measured in Static condition, before and after the different "dynamic" conditions, were subjected to three repeated-measure ANOVA (RM-

ANOVA) with POSITION (3 levels: α1, α2 and α3) and TIME (2 levels: pre, post), as within-subject factors.

In the Experiment 1, AUC data were analyzed by mean of RM-ANOVA, with POSITION (3 levels: α1, α2 and α3), BLOCK (2 levels: A and B), and MOVEMENT DIRECTION (2 levels: Up-moving, Down-moving), as within-subject factors. Furthermore, the HBR responses during voluntary movements (AUC values averaged over the blocks) were compared to those acquired in the corresponding Static condition (data were obtained by averaging AUC values evaluated in the pre and post conditions) by means of a RM-ANOVA with CONDITION (3 levels: Static, Up-moving, Down-moving) and POSITION (3 levels: α1, α2 and α3), as within-subject factors. In the Experiment 2, AUC values were statistically analyzed by mean of a RM-ANOVA with POSITION (3 levels: α1, α2 and α3) and MOVEMENT DIRECTION (2 levels: Up-moving, Down-moving). In order to compare AUC values evaluated during passive movement with those in Static condition (data were obtained by averaging AUC values evaluated in the pre and post conditions), a RM-ANOVA with CONDITION (3 levels: Static, Up-moving, Down-moving) and POSITION (3 levels: α1, α2 and α3), as within-subject factors, was performed. In the Experiment 3A, a paired t-test (2 levels: Up-moving, Down-moving), was adopted to evaluate HBR responses during the imagination of flexion and extension movements. Further, we performed an additional analysis where a baseline condition, during which the HBR response in Static condition corresponding to α2, was directly compared to HBR amplitudes during MI in both Up-moving and Down-moving conditions (RM-ANOVA, within factor CONDITION, 3 levels: Static α2, MI Up-moving, MI Down-moving). This allowed us to go deeper insight the mechanisms regulating the reflex response when the arm is actually in a Static condition but the motor system is involved in movement planning. In Experiment 3B, a 2x2 ANOVA compared the AUC values in Static condition when the arm was positioned in α1 and α3 with those obtained during MI when the subject imagined to reach α3 starting from α1 (α1 to α3), and imagined to reach α1 starting α3 (α3 to α1) (within subject factor CONDITION, 2 levels: Static and MI; within subjects factor POSITION, 2 levels: α1 and α3). Furthermore, we replicated the statistical analyses proposed in Experiment 3A concerning the comparison among between MI α2 to α3 and MI α2 to α1 by means of a paired t-test and the comparison among Static α2, MI α2 to α3, and MI α2 to α1 by means of a one-way ANOVA. Newmann-Keuls post hoc analysis was used to interpret significant interactions. Data in the text are reported as mean ± SE.

2.1.3. Results

<u>Preliminary experiment</u>: Static condition (Figure 2). The statistical analysis showed a significant effect of the factor POSITION ($F(2,18) = 7.49$, $p = 0.004$). Post hoc tests revealed a significant increase of AUC values in α3 (22.33 ± 2.55 mV*ms) with respect to α1 (17.86 ± 2.32 mV*ms, $p = 0.02$) and α2 (15.06 ± 1.26 mV*ms, $p = 0.003$). These results

confirmed the literature showing that when the stimulated arm is close to the face, inside the DPPS, the HBR magnitude is significantly higher than those evoked when the arm is in farther positions.

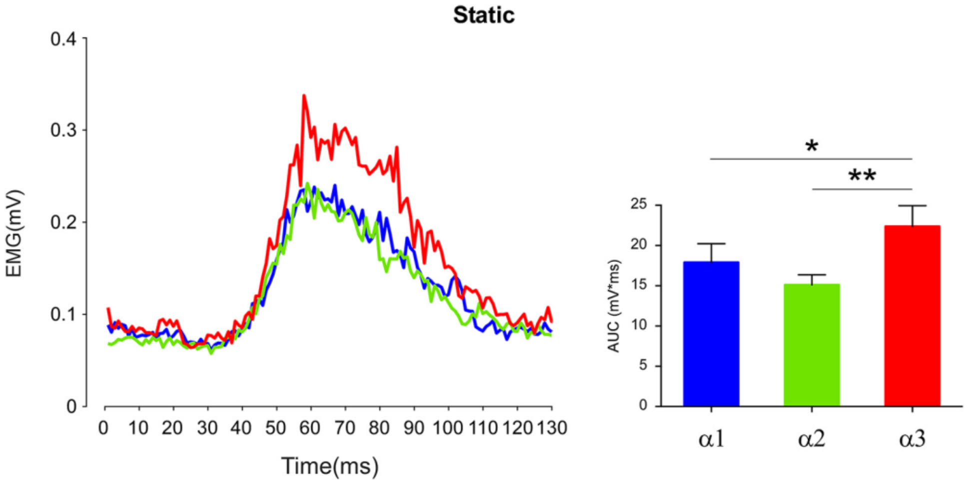

Figure 2. Preliminary experiment: Static condition. *Group-average, rectified HBR waveforms (left panel) and group-average HBR amplitudes (right panel, AUC, mV*ms) recorded when the arm was placed in the three stimulation positions: far (α1), intermediate (α2) and near (α3). Error bars refer to the standard error of the mean. ** refer to p<0.01.*

<u>Comparison among the Static conditions.</u> The results of the RM-ANOVA on AUC values related to the Static conditions acquired in each testing session showed a significant effect of POSITION (F(2,18) = 17.87, p = 0.00005), due to the significant increase of α3 with respect to the other stimulation positions (p always <0.004). No differences appeared among the SESSIONS (p = 0.33).

RM-ANOVA on AUC values evaluated in the Static condition during Experiment 1, 2 and 3A revealed an effect of the factor POSITION (Experiment 1: F(2,18) = 11.21, p = 0.0006; Experiment 2: F(2,18) = 8.19,

$p = 0.002$; Experiment 3A: $F(2,18) = 5.31$, $p = 0.01$) due to the significant increase of the AUC values in α3 with respect to α2 and α1 (p always < 0.05). No differences appeared in the HBR responses acquired in static condition before and after the “dynamic” experimental conditions (p always > 0.2).

Experiment 1: Voluntary movement. Single-subjects average data in each conditions are shown in Figure 3. RM-ANOVA analysis showed that MOVEMENT DIRECTION ($F(1,9) = 5.66$, $p = 0.04$) as well as POSITION ($F(2,18) = 6.94$, $p = 0.006$) significantly affected the amplitude of the HBR responses and a significant interaction between these two factors was found ($F(2,18) = 8.34$, $p = 0.003$). Post hoc analysis showed that during an elbow flexion movement (Up-moving condition) there was a significant increase of the HBR value in α3 (12.3 ± 1.7 mV*ms) with respect to α2 (8 ± 1.1 mV*ms, $p = 0.0003$) and α1 (8.7 ± 1 mV*ms, $p = 0.001$) (Figure 4A). Differently, during elbow extension movements (Down-moving condition) no difference in the AUC values was found in the three stimulation positions (α1 = 9.3 ± 1.3 mV*ms, α2 = 8.9 ± 1.1 mV*ms, α3 = 9.3 ± 1.1 mV*ms, p always > 0.7) (Figure 4B). Further, the HBR response in α3 when moving up to the face was significantly higher than that observed at the same position when the hand moved far from the face ($p = 0.001$). Finally, no difference between the two experimental blocks (i.e., elbow flexion-extension and elbow extension-flexion) was found ($p = 0.23$).

Further, when we compared HBR responses in Static condition and during voluntary movements, significant main effects of CONDITION ($F(2,18) = 32.94$, $p = 0.000001$) and POSITION ($F(2,18) = 13.64$, $p = 0.0002$) were found. Post hoc analysis revealed that AUC values in Static condition were significantly higher than those obtained during voluntary up and down movements (p always < 0.0005). Concerning the factor POSITION, the HBR response in $\alpha 3$ was significantly higher than in $\alpha 1$ and $\alpha 2$ (p always < 0.001). Furthermore, we found a significant interaction between CONDITION and POSITION ($F(4,36) = 4.52$, $p = 0.005$), suggesting that the position effect (i.e. a greater response in $\alpha 3$ position with respect to both $\alpha 1$ and $\alpha 2$) was present only in static condition (p always < 0.0002) and in up movements (respectively, $p=0.02$, $p=0.007$), whilst no position effect pertained to the down movements.

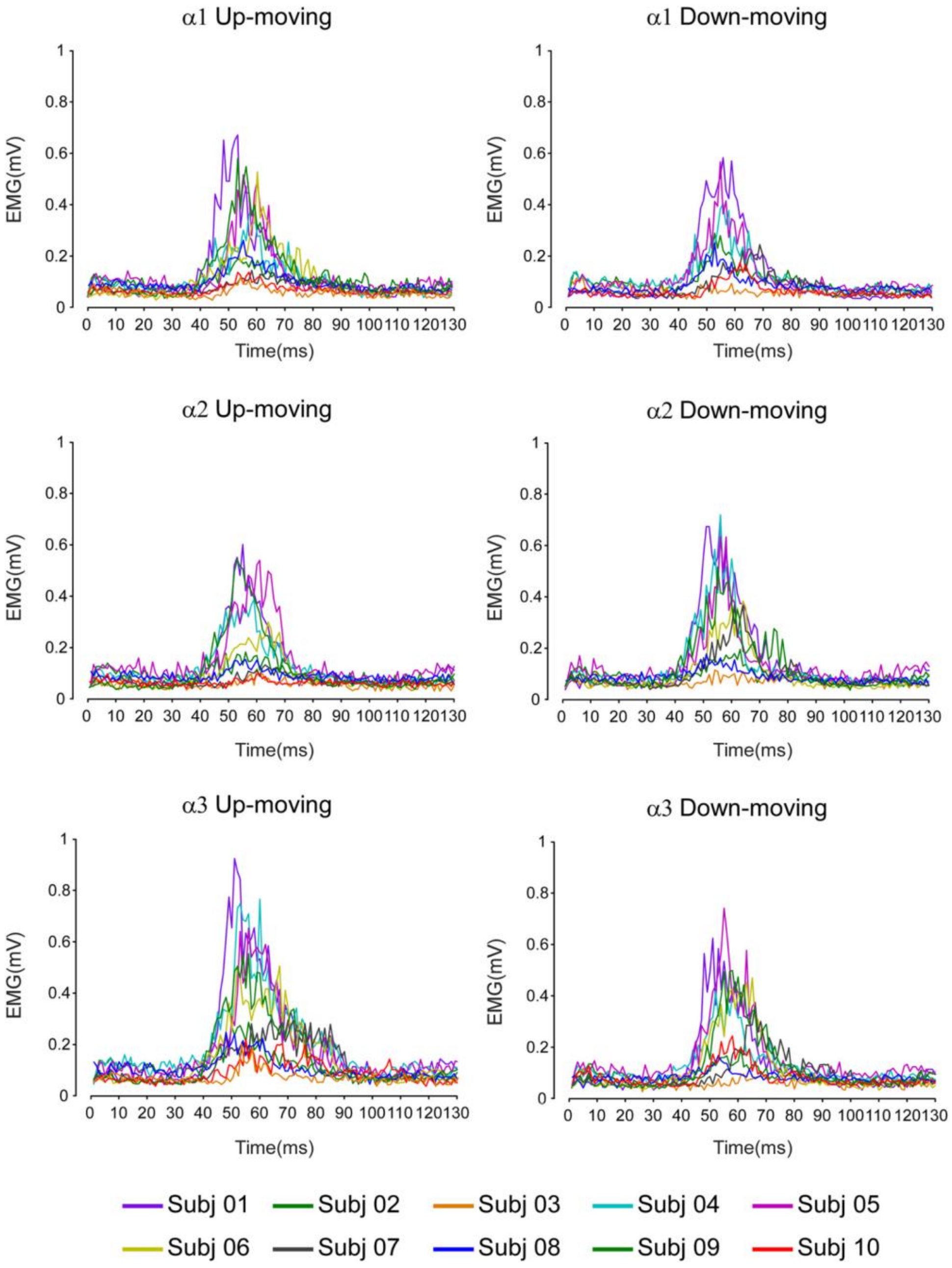

Figure 3. Experiment 1: Voluntary movements. *Rectified and superimposed average EMG traces (mean over the blocks and recording sites) of each participant for the near (α1), intermediate (α2) and far (α3) positions when participants performed up movement towards the face (Up-moving) or down movement far from the face (Down-moving).*

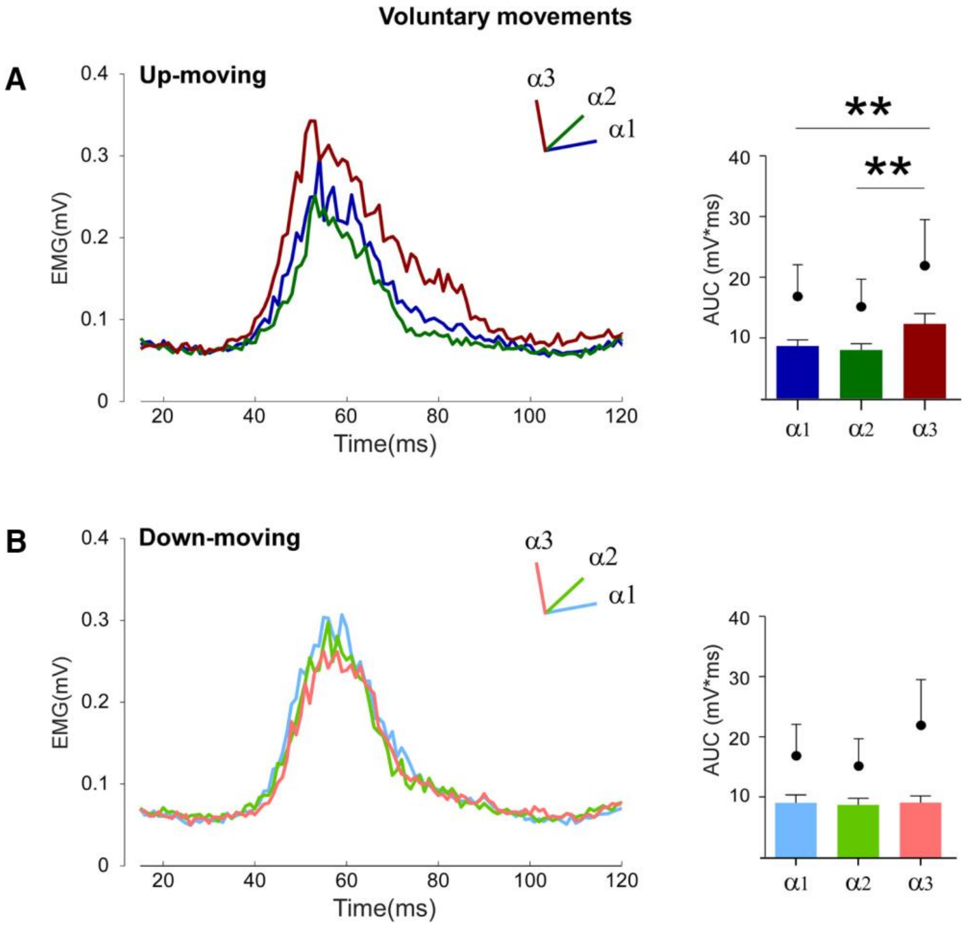

Figure 4. Experiment 1: Voluntary movements. *On the upper (A) and lower (B) panels are represented the group-average, rectified HBR waveforms (left panel) and the group-average HBR amplitudes (right panel, AUC, mV*ms) in the Up-moving and Down-moving conditions in the three stimulation positions: far (α1), intermediate (α2) and near (α3). Black dots refer to the group-average AUC values in the corresponding Static condition. Error bars refer to the standard error of the mean. ** refer to p<0.01.*

<u>Experiment 2</u>: Passive movement. The statistical analysis showed a significant interaction between POSITION and MOVEMENT DIRECTION (F(2,18) = 6.91, p = 0.006). As in the case of voluntary movement, during passive elbow flexion movements (Up-moving condition) the magnitude of the HBR responses significantly increase when the hand was near the face (α3 = 11.8 ± 1.8 mV*ms) with respect to far (α1 = 6.5 ± 0.9 mV*ms, p = 0.007) and intermediate (α2 = 7.3 ± 0.6

mV*ms, $p = 0.02$) positions (Figure 5A), whereas during extension movements (Down-moving condition) no difference was found among the three hand positions ($\alpha1 = 9.6 \pm 0.9$ mV*ms, $\alpha2 = 8.1 \pm 0.8$ mV*ms, $\alpha3 = 8.2 \pm 0.9$ mV*ms, p always > 0.3) (Figure 5B). Finally, the HBR response in α3 when the arm was passively moved up-to the face was significantly higher than that observed at the same position when the arm was moved far from the face ($p = 0.03$).

When we compared HBR responses in Static condition and during Passive movement, a significant main effects of CONDITION ($F(2,18) = 14.28$, $p = 0.0002$) and POSITION ($F(2,18) = 7.6$, $p = 0.004$) were found. Post hoc analysis revealed that AUC values in Static condition were significantly higher than those obtained during passive movements (p always < 0.0006), and that the HBR response in α3 was significantly higher than in α1 and α2 (p always < 0.0041). Furthermore, we found a significant interaction between CONDITION and POSITION ($F(4,36) = 4.14$, $p = 0.007$), suggesting that the position effect (i.e. a greater response in α3 position with respect to both α1 and α2) was present only in static condition (p always < 0.03) and in up movements (respectively, $p = 0.002$, $p = 0.006$), whilst no position effect pertained to the down movements.

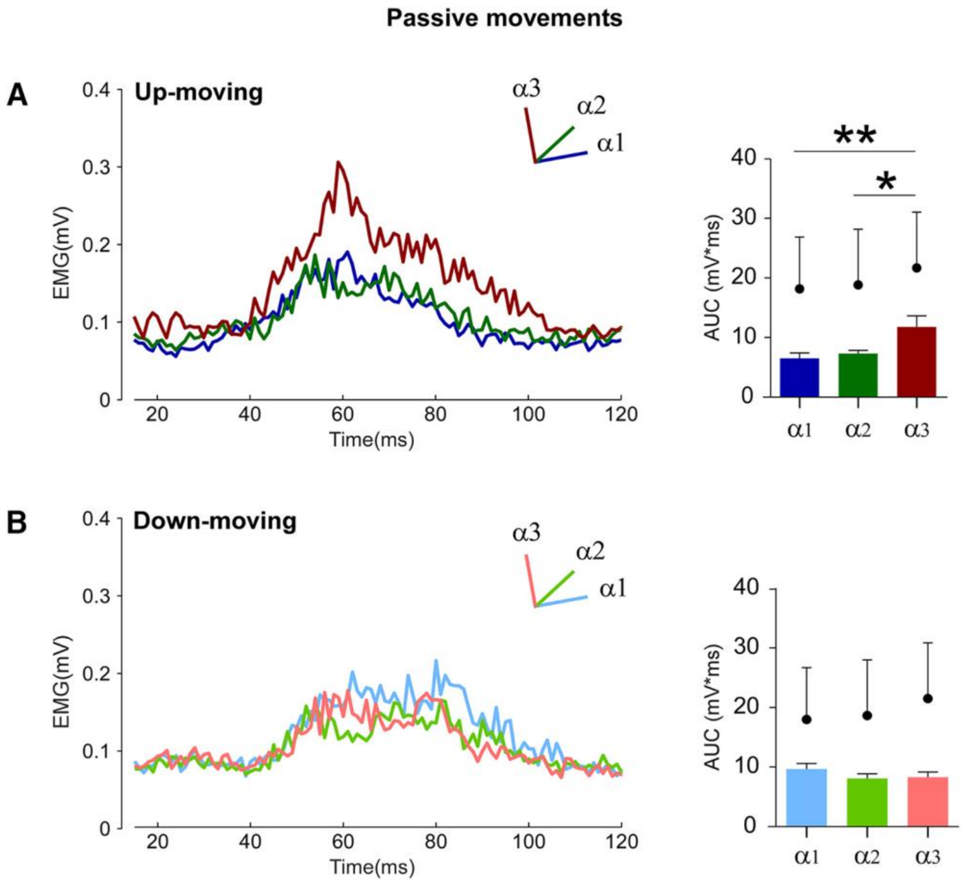

Figure 5. Experiment 2: Passive movements. *On the upper (A) and lower (B) panels are represented the group-average, rectified HBR waveforms (left panel) and the group-average HBR amplitudes (right panel, AUC, mV*ms) in the Up-moving and Down-moving conditions in the three stimulation positions: far (α1), intermediate (α2) and near (α3). Black dots refer to the group-average AUC values in the corresponding Static condition. Error bars refer to the standard error of the mean. * and ** refer to $p<0.05$ and $p<0.01$, respectively.*

<u>Experiment 3A</u>: Motor imagery from intermediate position (Figure 6). The results of the paired t-test showed a significant effect of the direction of the imagined movement: when participants imagined a flexion movement (Up-moving condition), from the intermediate to the near position, the HBR responses were significantly higher (10.9 ± 0.9 mV*ms) than when they imagined to extend their arm towards the far position (9.9 ± 0.7 mV*ms) ($t = 3.04$, $p = 0.01$). Further, when these

conditions were directly compared to a situation in which the subject kept the arm fixed in α2, the ANOVA showed a significant effect of the factor CONDITION (F(2,18) = 15.09, p = 0.0001). The post hoc analysis revealed that MI conditions, irrespective to the imagined movement direction, induced a reduction of the HBR response (p always < 0.0007).

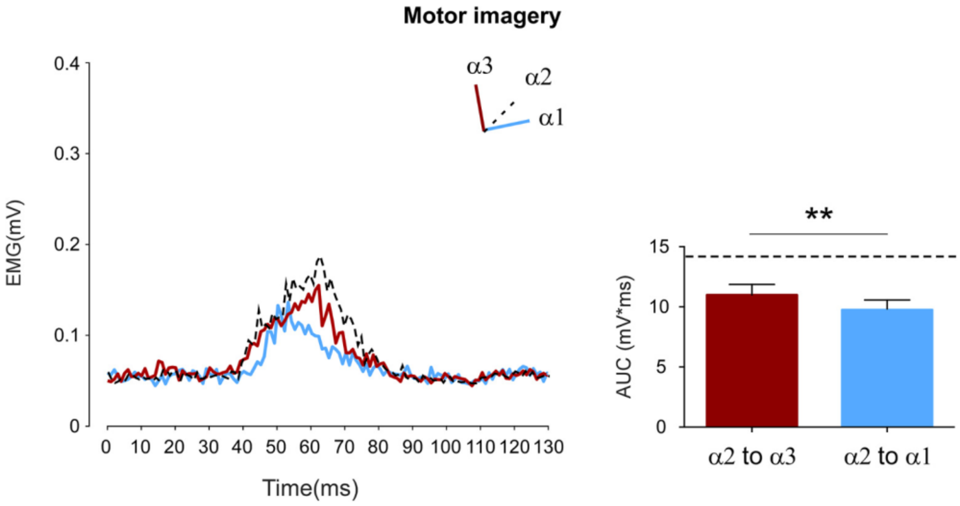

Figure 6. Experiment 3A: Motor imagery. *Group-average, rectified HBR waveforms (left panel) and group-average HBR amplitudes (right panel, AUC, mV*ms) when participants were asked to imagine to move the forearm from the intermediate to the near position (α2 to α3) and from the intermediate to the far position (α2 to α1). Dashed lines indicate the HBR waveform (left panel) and response amplitude (right panel) obtained in static condition corresponding to α2. Error bars refer to the standard error of the mean. ** refer to p<0.01.*

<u>Experiment 3B</u>: Motor imagery from near and far positions. The results of the paired t-test between the motor imagery α2 to α3 and α2 to α1 showed that AUC values associated to the imagined flexion movement (α2 to α3, 11.3 ± 1.0 mV*ms) were significantly higher (t = 3.07, p = 0.01) than those associated to the extension movement (α2 to α1, 10.2 ± 0.7 mV*ms), confirming the results described in Experiment 3A. Further, we

confirmed also that, when these conditions were directly compared to AUC values in Static condition corresponding to α2 (14.1 ± 0.9 mV*ms), a significant effect of CONDITION appeared (F(2,22) = 11.38, p = 0.0004). The post hoc analysis revealed that MI conditions, irrespective to the imagined movement direction, induced a reduction of the HBR response (p always < 0.01).

When the AUC values in MI conditions from α1 to α3 and from α3 to α1 were compared with those corresponding to the Static condition in α1 and α3 (Figure 7), ANOVA showed a significant main effect of CONDITION (F(1,11) = 19.86, p = 0.001), where AUC mean values in Static condition were higher than that recorded during MI. Further, a significant interaction CONDITION*POSITION (F(1,11) = 9.99, p = 0.001) was found. The post-hoc examinations showed that in Static condition the HBR response was significantly higher in α3 (17.7 ± 1.5 mV*ms) than in α1 (13.7 ± 1.1 mV*ms) (p = 0.0003), whilst no differences appeared between MI α1 to α3 (10.9 ± 0.8 mV*ms) and α3 to α1 (11.7 ± 1.1 mV*ms) (p = 0.31). Furthermore, AUC values in Static conditions were significantly higher than those obtained during MI starting from the same hand positions (static α1 vs. MI α1 to α3, p = 0.008; static α3 vs. MI α3 to α1, p = 0.0002).

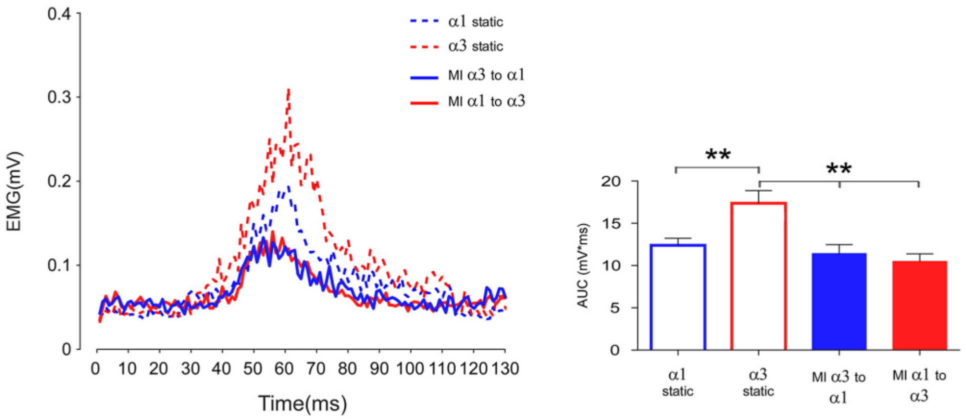

Figure 7. Experiment 3B: Motor imagery. *Group-average, rectified HBR waveforms (left panel) and group-average HBR amplitudes (right panel, AUC, mV*ms) in Static condition corresponding to α1 (blu dotted line and blue empty column) and α3 (red dotted line and red empty column), and when participants were asked to imagine to move the forearm from the far to the near position (α1 to α3, red straight line and red column) and from the near to the far position (α3 to α1, blues straight line and blue column). Error bars refer to the standard error of the mean. ** refer to p<0.01.*

2.1.4. Discussion

In this study, we sought for evidence that the predictive motor system can modulate a defensive response, the Hand Blink Reflex (HBR). The Preliminary experiment performed in Static condition showed that the HBR is significantly enhanced when one's own stimulated hand is located inside the DPPS of the face and this result is in agreement with the previously described "hand position" effect[3–5].

The Experiment 1 extended beyond the previous research on DPPS, passing from static to dynamic conditions. Firstly, we found a significant HBR enhancement in the near position also in dynamic conditions, i.e., when the moving stimulated hand entered the DPPS of the face. It is worth noting that, as previously suggested for Static condition[5], the HBR response was not linearly enhanced through the three stimulation positions, but a safety boundary exists: only inside this boundary the stimulus is potentially dangerous. Indeed, in both static and dynamic conditions, comparable HBR responses were found when stimuli were received in far (α1) and intermediate (α2) positions. But, mostly relevant is the HBR enhancement in the near (α3) position with respect to α1 and α2 only when the hand was moving towards and not down from the face, demonstrating the existence of a "hand movement direction" effect in HBR modulation, and suggesting that the space representation is dynamically shaped by the movement. Thus, what is crucial in HBR modulation in dynamic conditions it is not the actual position of the stimulated hand, but the final position where the hand is expected to be at the end of the movement. The lack of an increasing of HBR response when the hand from the far position moves towards the face might suggest that the dynamic shaping of the DPPS interacts with other aspects defining the safety boundary around the body. As mentioned above, the HBR enhancement has a not linear trend: i.e., a significant difference was present only between near position and the other positions. Thus, coherently, the directional modulation could occur only in the near position. We might also speculate that the "hand movement direction" effect can operate as an "energy saving" mechanism, that may reduce the defensive response when the stimulus is implicitly perceived as not risky, namely when the predictive motor system informs that the stimulus has been administered to the hand moving away from the face and, consequently, when "the near is becoming far".

During voluntary movements, two sources of information are available to the motor system in order to estimate the arm's final state: the sensory inflow, like the information coming from vision and proprioception, and the motor outflow (i.e., the copy of the motor commands). The results of Experiment 1 could not disambiguate between the role of these kinds of information in modulating the defensive response. One possibility is that this directional effect only pertains to the voluntary movements, when both the afferent and the efferent information are present. Alternatively, the directional effect could be present also when the afferent and the efferent sources of information are dissociated, as in the Passive movement (Experiment 2) and in the Motor imagery tasks (Experiment 3A and 3B). Our data verified this second hypothesis, showing a directional effect on the HBR modulation during both Passive movements and Motor imagery tasks.

Similarly to the voluntary movement, in the Experiment 2, dealing with passive movements, the HBR response increased when the hand received the stimulus near to the face only in the Up-moving condition, whilst no difference among the three hand positions was found in the Down-moving condition. This means that the modulation of the HBR response occurred also when only the afferent information (coming from vision and proprioception) was available to the system for predicting the consequence of the movement.

The results of the Motor imagery tasks (Experiment 3A and Experiment 3B) showed that the HBR response was significantly greater when the subject imagined to move up to than down from the face, although the arm was kept in the intermediate position (α2) and the position remained unchanged during the experiment. When the hand was placed close to (near, α3) or far from (far, α1) the face and subjects were asked to imagine to go down from (α3 to α1) or up to (α1 to α3) the face respectively, no difference was observed between HBR amplitudes (Experiment 3B). Although these two hand positions are shown to evoke divergent responses in Static condition, the motor imagery was able to abolish this difference, a result that underlines the role played by MI in modulating the reflex response. Moreover, when the hand positions corresponded to α3 and the subjects imagined to move far from the face (α3 to α1), the HBR response significantly decreased with respect to the corresponding static position. In agreement with the results of Voluntary and Passive movement conditions, these findings might be explained as a down-regulation of the HBR response when planning to move far from the face, albeit the hand was inside the defensive peripersonal space. Therefore, results from the Motor imagery tasks strongly support that the modulation of the defensive response occurred also when no actual movements were executed, but when only the efferent information (i.e., the efference copy of the motor program) was available to predict the final consequences of the movement. Converging evidences suggest that imagined and actual movements trigger similar motor representations[12–14] and share overlapping neural substrates[15–21]. In particular, motor imagery would engage the same internal forward models, i.e., the neural mechanisms that mimic the causal flow of the physical process by predicting the future sensorimotor state that are involved in action execution[22].

From an anatomical point of view, it has been proposed that the key mechanism generating the somatosensory evoked blink reflex may exist before somatosensory signals enter the common blink interneuronal networks. One possibility is that the appearance of the HBR may depend on the level of activity of a gating mechanism that exerts inhibition on the inflow of the somatic input. Therefore, HBR modulation may be the result of the integration of facilitatory and inhibitory mechanisms within the brainstem exerted by higher centres, such as the basal ganglia, cerebellum or cortex [23]. It has been proposed that the brainstem circuits mediating the HBR in humans undergo top-down modulation from higher order cortical areas (the polysensory zone in the precentral gyrus and the ventral intraparietal area) responsible for encoding the location of somatosensory stimuli in external space coordinates[4]. Particularly relevant for the dynamic context of our study is that the application of an inhibitory rTMS on the hand motor area determined a long-lasting reduction of excitability of the R2 component of the blink reflex[24], probably mediated by direct projections of motor cortical areas to the lateral medullary reticular formation[25]. Furthermore, in the MI context, the influence of cortical areas on brainstem neural circuits has been recently demonstrated during MI of a dynamic balance task[26]. Thus, we can speculate that when subjects move or imagine moving the hand throughout different positions inside the DPPS, the movement might differently activate the descending pathways originating from motor and associative cortical areas. These areas can influence the blink premotor activity, before entering the common blink-reflex pathway, shaping the amplitude of the HBR response.

Finally, the higher values of HBR response observed in the static condition with respect to the dynamic conditions of the three experimental sessions might suggest the use of two distinct neural mechanisms in modulating HBR in static and in dynamic conditions. Indeed, for each stimulation position, a significantly reduced HBR was found in dynamic with respect to static conditions. The decrease of HBR response during movement can be explained as consequence of the sensory attenuation, according to which the sensory effects generated by one's own actions are attenuated compared to the same effects generated externally[27–30]. It is important to note that, in our experimental context, during voluntary and passive conditions, the participant's movement triggered the electrical stimulator. Thus, the resulting stimulus can be interpreted as a self-generated sensory effect. We can speculate that, in the context of DPPS, a stimulus on which I can exert a direct control is interpreted as less dangerous and produces a lower defensive physiological response with respect to an externally generated stimuli, that is, by definition, outside from the subject's control. A similar explanation could account for the results of the motor imagery condition. Indeed, recent studies showed that the sensory attenuation occurs not only during action execution, but also during action preparation and planning[31]. Alternatively, the movement-related HBR decrease can be explained by the different processing of the proprioceptive inputs in static and dynamic conditions. We can suggest that in the static condition the proprioceptive inputs can give an exact information about the position of the hand with respect to the face. Conversely, when a subject is moving, the rapidly changing inputs generated during hand movements can make more difficult to estimate the hand position and therefore might reduce the amplitude of the reflex response. It is worth noting that the movement-related HBR decrease with respect to the static conditions is particularly relevant for the motor imagery condition, where, although the subjects did not actually move, the mere movement planning was sufficient to modify the reflex response.

Taken together, these findings provide physiological evidence for the role of the predictive motor system in dynamically shaping the defensive peripersonal space during movement.

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2.2. My arms, my shield: the defensive peripersonal space in boxers.

2.2.1. Introduction

In section 2.1 we used Hand Blink Reflex (HBR) to show that the safety boundary around the body is continuously shaped by the predictive motor system, through the integration of efferent and afferent signals during various dynamic conditions. In particular, the intensity of responses is higher when the hand moves towards the face, but decreases when the hand moves away from the face[1]. This shows that in dynamic conditions HBR modulation depends not only by the actual position of the stimulated hand, but also by the final position where the hand is expected to be at the end of the movement. In line with this assumptions a fine somatotopical and cognitive tuning of HBR has been reported by Sambo et al[2]. In particular, they showed that when a thin wooden screen is placed between the participants' face and their hand the HBR enhancement by hand-face proximity is suppressed. Thus, the screen reduces the extension of the defensive peripersonal space (DPPS), so that the hand is never inside the peripersonal space of the face, even in the "near" condition. All these findings indicate that the static or dynamic position of the hand inside the DPPS of the face induces an HBR enhancement, but its amplitude is significantly influenced by the activity of motor, cognitive and associative cortical areas[2,3]. On this basis, we can assume that HBR modulation strongly depends by previously acquired sensorimotor experiences.

In this context, the sense of agency, which refers to the feeling that we have control on our actions and, through them, on their effects in the outside world[4], might play a crucial role. Indeed, the sense of agency associated to flexion/extension upper limb movements can significantly differ as a function of the final outcome. Furthermore, it has been recently shown that reinforcement and learning might influence the subjective experience of agency over actions and outcomes[5]. Therefore, one could hypothesize that gaining a motor experience after an intensive and prolonged motor training, as occur in expert athletes, might affect the sense of agency. In fighting sport, the athlete learns to assume a specific position to protect a part of body. In boxing the danger for the athlete is represented by the punches of the opponent, especially those reaching the most delicate parts of the body such as the face. Therefore, to protect the face, boxers are trained from the beginning of their practice to have a good boxing guard position. Although at a first glance the hands are inside the DPPS of the face, boxers are confident to use their hands as a shield to protect the face from an external threatening stimulus. Also, it is anecdotally reported that expert boxers never close their eyes when the opponent punch reaches their hands. Since this sport situation can be considered the ecological counterpart of the HBR static experiment, we investigated whether this “shield effect” in boxers can shape the peripersonal space surrounding the face.

A group of boxers with different years of practice were enrolled and compared with a group of age-matched control subjects during a static HBR experiment hypothesizing that the expected HBR enhancement in the position close to the face was not present in boxers and that years of practice could have a role in this modulation.

Further to understand whether a possible shaping of DPPS was due to a broad experience of boxers in coping with dangerous stimuli or to an implicit sense of agency in maintaining a static position similar to the guard position, boxers were involved also in a dynamic experiment. We recorded HBR when participants were asked to move their right forearm up towards the face (up-moving condition) or down far from the face (down-moving condition). Indeed, movements in different directions could allow us to investigate the response to a dangerous stimulus entering or leaving the DPPS.

2.2.2. Materials and Methods

Participants

Twenty-six participants, naive to the purpose of the experiment, were recruited for this study. The Boxer group (n=13, 13 males, mean age ± SE = 28.27 ± 7.00 years) practiced for different amount of years: some of them participated to local and regional tournaments, but none of them competed at national level. They practiced boxing from 5 to 20 years.

The Control group was formed by volunteers who never practiced fighting sports (n=13 males, mean age ± SE = 26.86 ± 4.29 years).

They reported no previous history of neurological disorders or orthopedic problems for the right-dominant hand, as determined by the Edinburgh Handedness Inventory[6].

Participants gave written informed consent before taking part in the study. The study has been approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki.

Experimental set up

The HBR response was elicited by administering transcutaneous electrical stimuli to the median nerve at the right wrist, using a surface bipolar electrode attached with a velcro strap and connected to a Digitimer constant current stimulator (DS7AH HV, Digitimer Ltd, UK). Stimulus intensity was adjusted to elicit in each participant clear HBR responses (mean stimulus intensities were 5.41 ± 1.68 mA, range 1.6-7 mA, for the Control group and 8.43 ± 1.64 mA, range 5-9.9 mA, for the Boxer group). None of the participants reported painful sensations elicited by the stimulation. The stimulus duration was 200 μs and the inter-stimulus interval was ~30 s. A twin-axis electronic goniometer (TSD130B, BIOPAC System, Inc.) connected to a BIOPAC MP100 system was used to measure and record the elbow angle during movement execution, allowing the automatic delivery of the electrical stimulation when the elbow angle corresponded to one of the three pre-determined stimulation positions.

EMG activity was recorded by means of two MP100 BIOPAC EMG channels from the orbicularis oculi muscles bilaterally, using two pairs of bipolar surface electrodes with the active electrode over the mid lower eyelid and the reference electrode laterally to the outer canthus. Signals were amplified and digitized at 1 kHz (BIOPAC MP100).

Experimental Procedure

Participants were seated on a comfortable chair with their right elbow placed on a table, in a position allowing the right wrist to be in front of the ipsilateral eye while moving the forearm towards the face, but never touching it. The electrical stimulation was delivered while participant's stimulated hand was located at three different positions relative to the face. In particular, when the elbow angle was 10° less than the maximal arm extension (far position, α1), the half of the difference between the angles of maximal arm extension and flexion (intermediate position, α2), and when the angle was 10° more than the maximal elbow flexion (near position, α3). Throughout the experiment participants were instructed to keep their gaze on a fixation point placed at 60 cm from the eyes.

The experiment was composed of two phases that were performed in the same day: Static condition and Voluntary movement condition.

Static condition. At the beginning of each trial, participants had to assume with the right arm one of the three positions previously described, under experimenter's instruction. After a randomly variable delay, they received the electrical stimulation, which was manually delivered by the experimenter. Static condition was performed before and after Voluntary Movement condition (3 stimulation positions, 4 repetitions and 2 times), for a total of 24 pseudo-random acquisitions, 8 for each hand position.

Voluntary Movement condition. Participants were asked to perform an elbow flexion-extension with the right arm, with the goniometer attached on it. The electrical stimulator was automatically triggered by the goniometer when the moving arm of the subject reached the target position previously set by the experimenter. Target positions where the angle values previously identified (α1, α2, α3), and the stimulation was delivered both during the elbow flexion (afterwards called Up-moving) or elbow extension (afterwards called Down-moving) movement, for a total of 48 trials (3 angles, 2 movement directions and 8 repetitions). To reduce participants' expectancy, we introduced catch trials. A minimum time of 30 sec was kept as inter-trial interval. During this interval the subjects were asked to keep the arm relaxed.

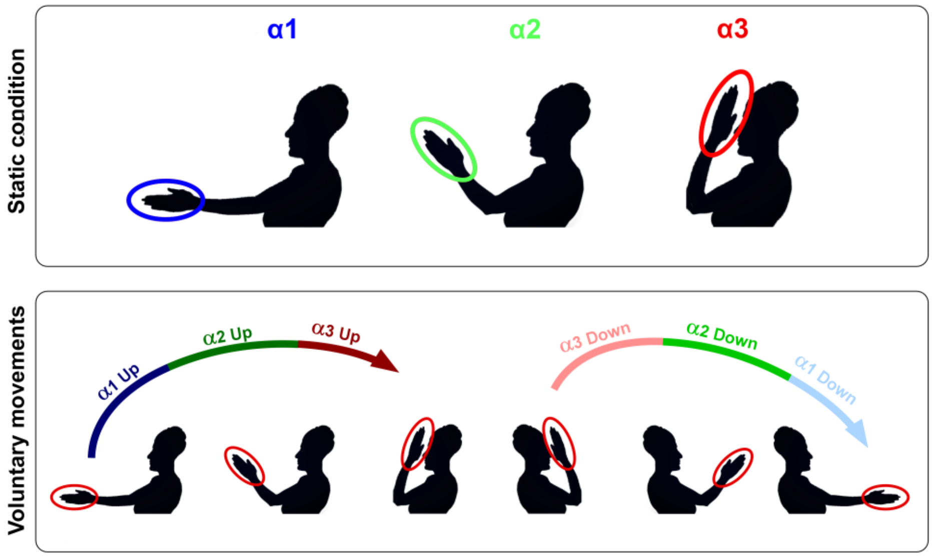

Figure 1. Experimental set up. *Upper panel refers to static condition, during which subjects kept the arm and receive the stimulation in three target positions: positions (far, α1; intermediate, α2; near, α3). Lower panel refers to Voluntary movements condition, during which participants voluntarily performed either a flexion-extension or an extension-flexion movement of the elbow: while moving up towards (Up) or down far from (Down) the face they received an electrical stimulation in three pre-set positions (far, α1; intermediate, α2; near, α3).*

Data processing and statistical analysis

A custom made MatLab software was used to process the EMG signals. EMG signals from each participant were filtered and rectified. HBR responses were averaged separately in each condition and for each participant. Trials with an abnormal EMG activity preceding the HBR responses were discarded by the analysis. The area under the curve (AUC, mV*ms) of each HBR average waveform was considered as outcome parameter. To compute AUC in each averaged EMG trace the software automatically analyzed a 130 ms-time interval from the stimulus onset that always contained the subject’s blink. The resulting curve was then integrated to compute AUC. In all experiments, data were averaged across ipsilateral and contralateral recording sides (right and left eyes).

In the Static condition, acquired AUC values were compared by mean of a repeated-measure ANOVA with POSITION (3 levels: α1, α2 and α3) as within-subject factor and GROUP (2 levels: Control group and Boxer group) as between-subject factor.

In Voluntary Movement condition, AUC data were analyzed by mean of RM-ANOVA, with POSITION (3 levels: α1, α2 and α3), and MOVEMENT DIRECTION (2 levels: Up-moving, Down-moving), as within-subject factors, and GROUP (2 levels: Control group and Boxer group) as between-subject factor. Newmann-Keuls post hoc analysis was used to interpret significant interactions.

Furthermore, for both Static and Voluntary Movement condition, we calculated for each subject of the Boxer Group the difference between the averaged AUC in the farthest and nearest positions. The values resulting from α3-α1 will be hereafter called ΔHBR. Pearson's correlation was applied to assess any relationship between the ΔHBR and the years of practice of subjects.

2.2.3. Results

Statistical analysis of the average amplitudes showed that the factor POSITION significantly affected the amplitude of the HBR responses

($F_{(2,48)}$ = 19.09, $p < 0.01$). Post hoc test showed a significant increase of AUC values in α3 (17.64 ± 1.59 mV*ms) with respect to α1 (13.91 ± 1.11 mV*ms, $p < 0.01$) and α2 (13.11 ± 1.14 mV*ms, $p < 0.01$).

Furthemore, a significant interaction between POSITION and GROUP factor was found ($F_{(2,48)}$ = 4.84, $p = 0.012$) and post hoc analysis revealed that the HBR responses of the Control group in α3 (20.64 ± 2.03 mV*ms) were significantly higher with respect to those acquired in the other positions (α1 = 14.95 ± 1.62 mV*ms, $p < 0.01$, α2 = 13.86 ± 1.42 mV*ms, $p < 0.01$). Conversely, this effect was not present in the Boxer group, where AUC values in α3 did not significantly differ from those in α1 and α2 (Figure 2).

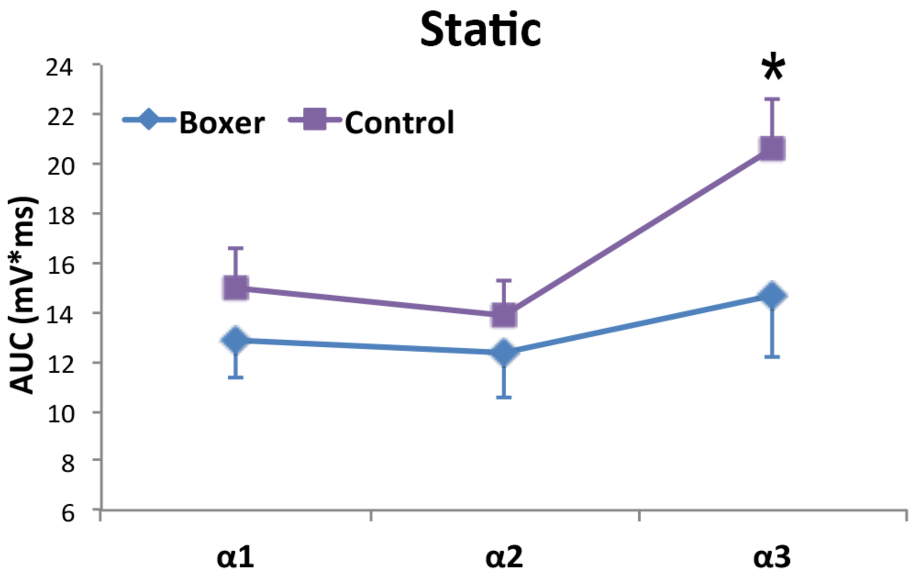

Figure 2. *Group-average HBR amplitudes (AUC,mV_ms) recorded when the arm was placed in the three stimulation positions: far (α1), intermediate (α2), and near (α3) in static condition. Purple lines indicate Control group, whilst blue lines indicate Boxer group. Error bar indicate standard error. *p < 0.05.*

In the Voluntary Movement condition, the results of the ANOVA on AUC averaged values showed significant main effects of MOVEMENT DIRECTION ($F_{(1,24)} = 6.16$, $p = 0.02$) and POSITION ($F_{(2,48)} = 18.70$, $p < 0.01$), and a significant interaction between these factors ($F_{(2,48)} = 18.84$, $p < 0.01$). Post hoc analysis showed that there was a significant increase of the HBR responses in α3 during the elbow flexion movement (Up-moving) with respect to all the other conditions (Up-moving α1: 9.87 ± 1.34 mV*ms; Up-moving α2: 10.31 ± 1.65 mV*ms; Down-moving α1: 10.39 ± 1.02 mV*ms; Down-moving α2: 9.97 ± 1.17 mV*ms; Down-moving α3: 10.66 ± 1.37 mV*ms. p always < 0.01) (Figure 3). No significant differences emerged between the two groups of subjects ($F_{(1,24)} = 0.09$, $p = 0.76$).

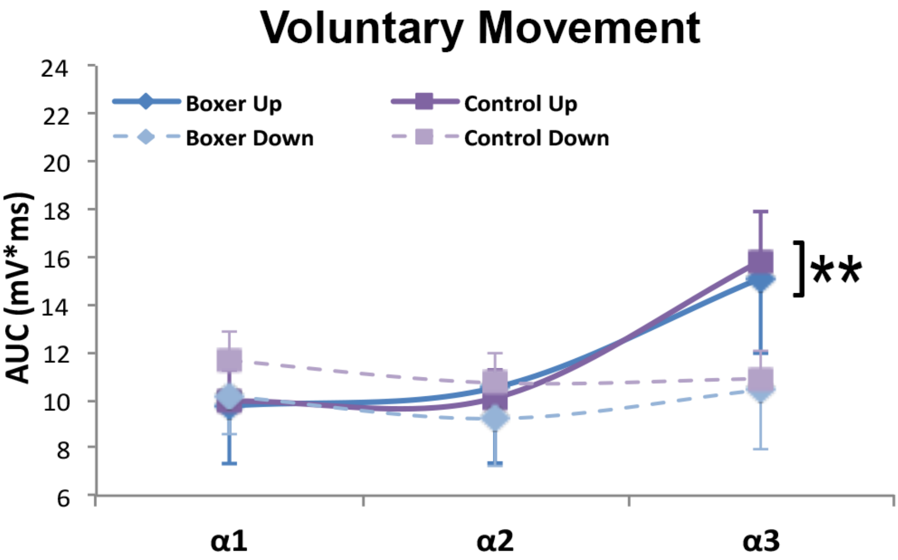

Figure 3. *Group-average HBR amplitudes (AUC,mV_ms) recorded in the up-moving (dark continuous lines) and down-moving (light dashed lines) in the three stimulation positions: far (α1), intermediate (α2), and near (α3) in static condition. Purple lines indicate Control group, whilst blue lines indicate Boxer group. Error bar indicate standard error. **p < 0.01.*

Figure 4 shows the relation between the years of practice of Boxer group and the ΔHBR of their responses in Static and Voluntary Movement conditions.

A significant negative relationship was found in Static condition (*R = -0.61; p = 0.027*); namely, the more years of practice they have, the less were the differences between the HBR responses in near and far position (Figure 4A). On the contrary, no significant relationship was found between the years of practice in boxing and the ΔHBR of the Voluntary Movement condition (R = -0.28; p = 0.35; Figure 4 B*)*.

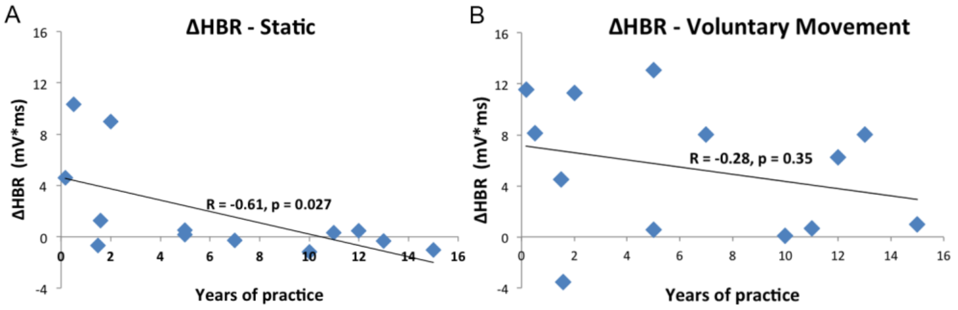

Figure 4. *Pearson's correlation between ΔHBR index and the boxers' years of practice. ΔHBR indicates the difference between subject-average response in $\alpha3$ and $\alpha1$ in static condition (A) and in voluntary movement condition (B).*

2.2.4. Discussion

The main finding of the present study is the lack of modulation of HBR responses in expert boxers when the stimulated hand was statically placed near the face, effect that was present in the control group. It is important to notice that the response trend was different in the two populations only when the stimulations were delivered in static condition. During the Voluntary Movement condition, instead, the behaviour of boxer and novice groups was basically the same, showing an increase of HBR responses when the stimulated hand in near position was moving towards the face. This dissociation between Static and Voluntary Movement conditions seems crucial to understand why this modulation occurs on boxer. We propose that the difference between the Static and Voluntary conditions was due to a different boxers' perception of the threat. We speculated that the near position mimics the guard posture, where boxers could perceive to be protected from the outside world. This result is in line with subjects' responses in Sambo study; indeed, when a wooden screen was placed in front of the participant's face the amplitude of the HBR responses in near position did not increase[2]. Authors explained their result assuming that the screen shortened the boundaries of the DPPS of the face leading to perceive the hand outside it, even if it was in the same near position. We hypothesized that an analogue "screen effect" automatically manifested in boxers as consequence of their sport experience, because in guard position they use hands as a shield to protect the face from an external threatening stimulus. In term of sense of agency, boxers learn that when they assume their guard position they are secure and protected from the opponent. Instead, when subjects are still moving, the stimulus caught them unprepared. We could imagine that this condition mimics a situation during which an opponent's hit reached the boxer when the guard is not completed. The result is that the perceived threat provided by the stimulus increase, and the HBR response shows a trend that is the same observed in control group.

Indeed, it is possible that in Voluntary Movement conditions boxers felt not to be in control of the parry action. When they are caught during a defense preparation they are not confident in parry efficacy. It has been shown that when subjects learn to associate an action with an effect, they lose their SoA when there is an action-effect mismatch. Not having the control over the effect lets boxers to assess a greater risk when moving

The main finding of this work is that motor learning might stably modify the perceived threat of outside events, and consequently the HBR, namely a subcortical reflex response. This is further supported by the correlation we found between HBR modulation in static condition and the years of boxer's experience; the higher the athletes' experience the lower the HBR modulation. This means that athletes' confidence on their "shield" increased the most experience they gain.

Taken together, these observations suggest that, as a result of sensorimotor experience, the brain can shape the DPPS by evaluating the harm probability through the assessment of the ability to cope with possible dangerous stimuli. Furthermore, this further corroborates that there is a modulation of the cortical circuits on the HBR.

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CHAPTER 3 – WORKING PERIPERSONAL SPACE

3.1. This racket is not mine: The influence of the tool-use on peripersonal space

3.1.1. Introduction

In this section and in the following one (3.1 and 3.2) we will focus on a second aspect of the peripersonal space: its goal-oriented function. As seen in chapter 1, this area is characterized by a high degree of multisensory integration of information originating from different sources, and this property differs from farther regions of space[1–4].

A great amount of studies, both on humans and monkeys, focused on the plasticity of the peripersonal space[2,5–7] and on the possibility to modulate its boundaries by integrating within it a tool. This phenomenon of embodiment causes the extension of the field of action[8,9] and the modification of interpersonal distances in social context[10].

Indeed, it is widely established that a brief period of tool use can temporarily modify the dimension of the PPS, whereas passively holding an implement for the same amount of time has no effect[11,12]. A step further was made by Serino and colleagues[13] who demonstrated for the first time that a prolonged use of an object can provoke a long-term expansion of the peripersonal space. This study showed a modification of the PPS in a population of blind subjects, who used a cane to navigate in their environment every day and to explore the world[13]. A plausible generalization of this result is that the PPS may undergo a long-term expansion in all those populations of subjects who daily experience the use of a specific tool, such as athletes.

The familiarity of a sportsman with his/her tool has already been studied in different areas. Neurophysiological studies have shown that a long-term use of an object during the athletic gesture can induce neuroplastic changes in the motor system[14]. For instance, Fourkas and colleagues found that, in tennis players, corticospinal excitability increased during motor imagery of tennis forehand, but not during other sports' specific gestures, underlying the key role of long-term experience in modulating sensorimotor representation. Coherently, Wang and colleagues showed an increased cortical excitability in badminton players when they imagined running a sportive gesture while holding the specific tool[15]. Further, a previous behavioral study from our group showed that motor imagery performance of athletes, who developed motor skills associated to the use of a specific tool, is reliant on the object used to practice movements and worsens with other non-specific tools[16]. Despite these studies demonstrated that the use of an object could affect motor representation, it has never been investigated how this would affect the PPS of athletes' who practiced a sport that requires the use of a tool. The purpose of this study was to investigate whether long-term motor experience with a specific kind of implement induces a stable modification of the PPS. To do that a multisensory integration paradigm[13] was administered on groups of tennis players and of novices to the sport of tennis, while holding a generic tennis racket.

We would take here a step further, glancing on what is directly experienced by the athletes when holding a racket different from their personal one. In his biography, Andre Agassi says about the use of a new racket: *"I feel as if I am playing with a broomstick. I feel as if I'm playing left-handed, as if I've suffered a brain injury. Everything is slightly off. The ball doesn't listen to me. The ball doesn't do what I say." (Agassi, 2009).*

Every athlete could claim that a notably difference exists between the own racket and any other, and that two rackets that are similar in the eyes of a novice could be perceived substantially different by a tennis player. Indeed, subtle differences, such as the distribution of weights along its length and /or the tactile sensation associated to the grip of one's own racket, might be appreciated by the athletes. For this reason, we investigated in the group of tennis players, whether the personal tennis racket, daily used during sport practice, evoked different responses with respect to the common tennis racket. Following the notion that tool remapping of PPS relies on motor knowledge[17] we hypothesized that athletes' ability gained during daily training with their personal tennis racket would reflect onto the PPS properties, which would expand to incorporate this tool.

3.1.2. Material and methods

Participants

A total of 34 participants, naive to the purpose of the study, took part in the experiment. They were classified in two groups on the basis of a questionnaire about their sport activities. The group of tennis players (n=14, 8 males and 6 females, mean age ± SE = 27 ± 2.41 years) was composed by agonist, but non-professional, athletes that had various levels of expertise: from 5 to 20 years' experience and from the IV to the II category of the Italian National Ranking, according to the Italian Tennis Federation (FIT). 20 healthy participants, novices to the sport of tennis, formed the control group (10 males and 10 females, mean age ± SE = 24.95 ± 1.28 years). Individuals who practiced sports that involved tool-use were not included in the study (see Table 1 for further details). All the participants were right-handed, as determined by the Edinburgh Handedness Inventory[18] and had normal hearing and touch. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee.

Table 1: *Characteristics of participants.*

	Tennis Players	**Novices**
Sex	8 Males 6 Females	10 Males 10 Females
Ages	27 (±2.41 SE) years	24.95 (±1.28 SE) years
Sports practiced	Tennis	Jim (3) Weightlifting (2) Running (2) Soccer (1) Volleyball (1) Water polo (1) Swim (1) Judo (1) No sport (11)
Years of practice	13.71 (± 1.31 SE) years	
Hours of playing/week	6.50 (± 0.83 SE) hours	6.91 (± 1.23 SE) hours

Experimental procedure

In order to investigate whether the tennis racket was integrated into the participants' PPS, a multisensory integration paradigm was employed[13] in three different sessions: no Tennis Racket (nTR), common Tennis Racket (cTR), personal Tennis Racket (pTR, only for tennis players).

Multisensory integration paradigm

Subjects sat on a chair with the back of the right hand always lying on a table. They performed a simple detection task during which they were required to verbally respond saying “tah!” as soon as they perceived an electrical tactile stimulus. The tactile stimulus was administered at the right wrist using a surface bipolar electrode attached with a velcro strap and connected to a Digitimer constant current stimulator (DS7AH HV, Digitimer Ltd, UK). Participants’ verbal responses were acquired through a microphone positioned around the neck.

A task-irrelevant sound (a 150-ms burst of pink noise), that subjects were instructed to ignore, was presented simultaneous to the electric stimulus. The sound was originated from either one of the two identical loudspeakers that were placed one in close proximity to the right hand, at about 30 cm from the body, and the other at a distance of about 68.5 cm from the other one. The volumes of the speakers were singularly regulated so that the intensity of the near and far sound was equal (70dB) as measured by a sound meter at subjects’ right ear. The tactile and the acoustic stimuli originating from the loudspeaker near the hand were delivered simultaneously. The far sound started slightly before the onset of the tactile stimulus, in order to compensate for the delayed arrival of the sound, due to the spatial distance. A custom-made MatLab® software managed the synchronization between the electrical and audio stimuli and the order of the trials.

Experimental design

The multisensory integration paradigm was repeated in several sessions, whose order was counterbalanced among the subjects. In each condition of the experiment, participants were blindfolded, standing with the back of the right hand lying on the table, in correspondence of the near loudspeaker. They had to answer verbally, as soon as possible, to the tactile stimulus, ignoring the non-target auditory stimulus. In the no Tennis Racket (nTR) subjects stand with their empty hand lying on the table (Figure 1A). In the common Tennis Racket (cTR) session both novices and tennis players repeated the multisensory integration paradigm holding with the right hand a 68.5cm long tennis racket that weighed 300g (the same object for everyone) at the level of the handle, which was settled in correspondence of the near loudspeaker. The remainder of the tool lied on the table so that the tip of the racket was placed in correspondence to the far loudspeaker. Again, participants were instructed to react to the tactile stimulus administered at their right wrist, ignoring the auditory stimulus. Finally, only the group of tennis players performed the personal Tennis Racket (pTR) session during which they executed the multisensory integration paradigm holding the tennis racket they regularly used to train themselves. The personal tennis rackets were long on average 68.5 ± 0.3 cm and all weighed 300g (Figure 1B). The grip dimension of all the personal tennis rackets was L3 and corresponded to that of the common Tennis Racket. Between the sessions subjects had the possibility to lift and settle the racket for the following session, but they remained blindfolded.

Every session of the experiment consisted of 90 trials randomly ordered: 30 trials where a tactile stimulus was coupled with the near sound (Near condition), 30 trials where the tactile stimulus was coupled with the far sound (Far condition), and 30 catch trials where subjects only heard either the Near (15) or the Far (15) sounds and they had to prevent themselves from answering. Catch trials were performed in order to avoid habituation. A familiarization phase, consisting of 3 repetitions of each experimental condition, including the catch trials, preceded the beginning of the experiment.

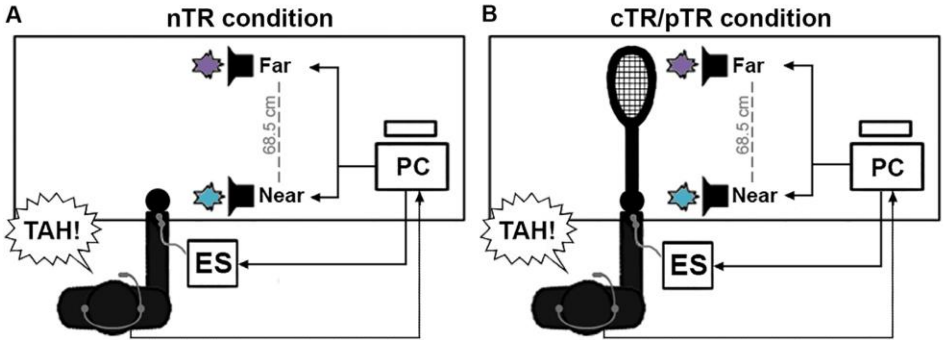

Figure 1. Experimental paradigm. *Participants sat on a chair with the back of the right hand lying on a table. They were requested to verbally respond (saying "TAH!") to an electrical tactile stimulus administered from the electrical stimulator (ES) in correspondence of the right wrist. Participants' verbal responses were acquired through a microphone positioned around the neck. Simultaneously to the electric stimulus, a task-irrelevant sound was presented either in close proximity to the right hand (Near) or at a distance of about 68.5 cm (Far). A personal computer (PC) controlled the order and the synchronization of the stimuli. Panel A refers to the set up in the no tool (nTR) session,*

when both novice and tennis players held no tool. In the Panel B the set up in the common Tennis Racket (cTR) session and the personal Tennis Racket (pTR) session are represented.

Data processing and statistical analysis

A custom-made MatLab software was used to analyze the audio traces of the subjects' verbal answers. From each trace, the reaction time (RT, ms) was calculated as the time elapsed between the onset of participant's verbal response and the delivery of the tactile stimulus in both Far (RT_{Far}) and Near (RT_{Near}) conditions. Responses higher or lower than 2 standard deviations from the individual mean RT value were treated as outliers and were removed from the analysis (always < 5% of the data set).

The mean RT values for both groups in nTR and cTR sessions were analyzed by means of an ANOVA with POSITION (Near vs. Far) and OBJECT (nTR vs. cTR), as within subjects factors, and GROUP (novices vs. tennis players), as the between subject factor, in order to evaluate whether the PPS was perceived differently by the two groups when they handled or not a tool.

ANOVA was replicated on tennis players' group with OBJECT (cTR vs. pTR) and POSITION (Near vs. Far) as within subjects factor. Then, following its results, an analysis of covariance (ANCOVA) was used to adjust the previous analysis to the years of experience of the athletes. In this case we calculated Bayes Factor (BF_{10}) for non-significant results.

Newmann-Keuls post hoc analysis was used to interpret significant interactions. Values are presented as mean ± standard errors.

3.1.3. Results

Statistical analysis showed that the origin of the sound significantly affect the reaction times (Figure 2). Indeed ANOVA showed an effect of the factor POSITION (*F(1,32) = 17.69, p = 0.0002*): the reaction times associated to Far audio stimuli (RT_{Far} = 368 ± 13 ms) were significantly higher than reaction times related to Near stimuli (RT_{Near} = 361 ± 12.24 ms). No significant difference appeared between nTR and cTR conditions (*p = 0.75*).

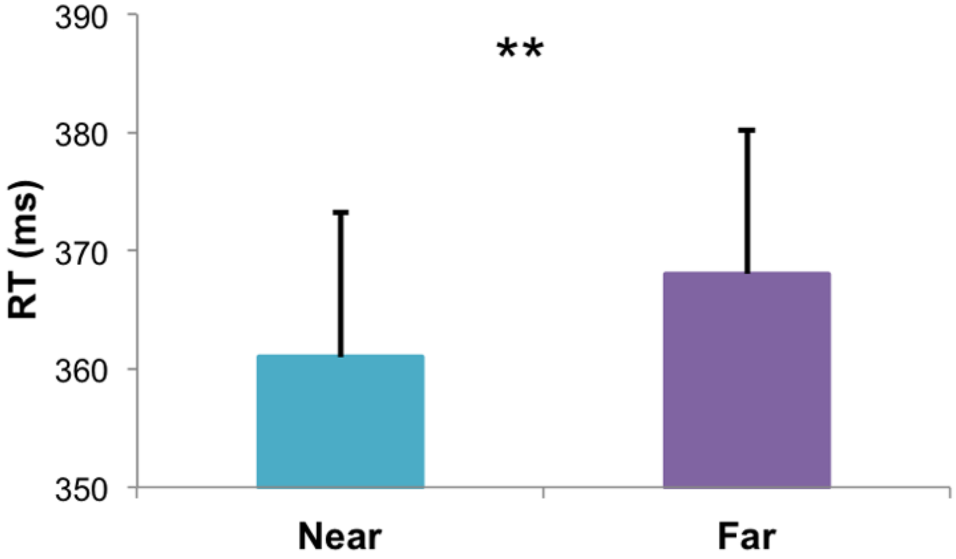

Figure 2. *Averaged reaction times (RT, ms) recorded in Near and Far conditions in Novices and Tennis Players groups in no Tennis Racket and common Tennis Racket sessions. The error bars refer to the standard error of the mean. **refers to p<0.01.*

Figure 3 shows the reaction time values in Near and Far conditions when tennis players handled the common Tennis Racket or the personal Tennis Racket. Statistical analysis showed a significant effect for the factor POSITION ($RT_{Far} > RT_{Near}$: *F(1,13) = 6.02, p = 0.029*), and a trend towards the significance (*p=0.087*) as regard the interaction OBJECT*POSITION. The ANCOVA showed a significant interaction between OBJECTS and POSITION (*F(1,12) = 6.31, p = 0.023*) (Figure 3). Post hoc analysis of this latter interaction showed that RT values associated to cTR were significantly higher than those associated to pTR. Furthermore, when the tennis players held the common Tennis Racket, reaction times to Far stimuli were significantly higher than those associated to Near stimuli (RT_{Far} = 371.47 ± 17.99 ms, RT_{Near} = 362.15 ± 16.75 ms, *p = 0.01*), whereas no significant difference was found between the RT_{Far} and RT_{Near} when subjects held the personal Tennis Racket (RT_{Near} = 347.35 ± 20.57 ms, RT_{Far} = 347.54 ± 19.13 ms, *p = 0.95*).

Bayes factor BF_{10} evaluated on non-significant factors OBJECTS and POSITION was 1.17 and 0.31, respectively. These findings suggested only anecdotal evidences according to the criteria by Jeffreys[19].

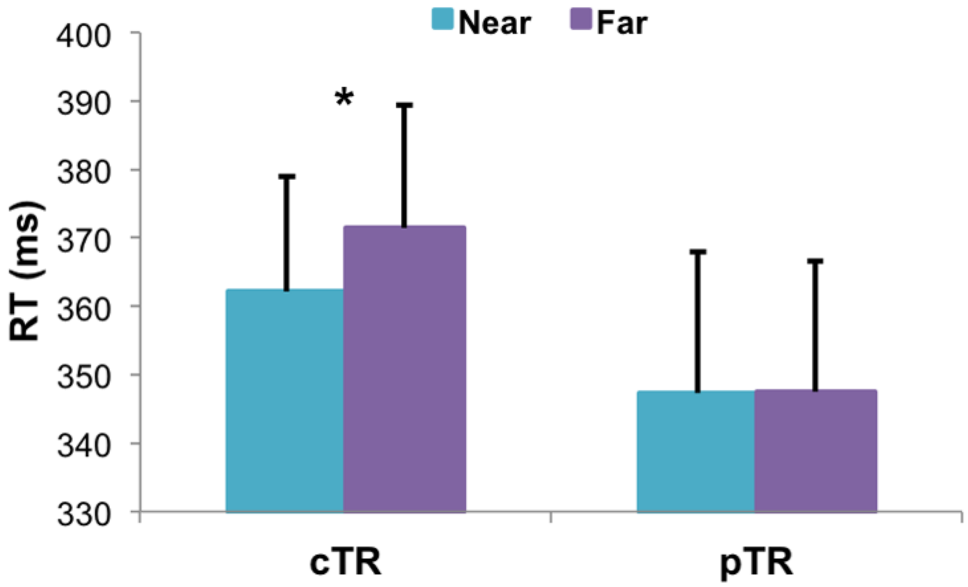

Figure 3. Averaged reaction times (RT, ms) in the Tennis Players group recorded in Near and Far conditions in the common Tennis Racket (cTR) and in the personal Tennis Racket (pTR) sessions. The error bars refer to the standard error of the mean. *refers to $p<0.05$.

3.1.4. Discussion

The main finding of the present study is that the long-term experience of the tennis players with their own tennis racket caused the embodiment of this tool in the PPS and this result become more evident with increasing years of experience. In fact, when years of experience were added as covariate, the difference between the reaction times in the Near and Far conditions measured by means of a multisensory integration paradigm decreased only when tennis player handled the tennis racket they used for daily practice. This modification was not generalized to the generic tennis racket.

Further, no differences appeared between tennis players and novices when they held nothing: namely, both groups showed higher reaction times when the tactile stimulus was coupled with the Far sound compared to the Near sound ($RT_{Far} > RT_{Near}$). Our findings are in line with those described by Bassolino and colleagues[20] in a study where they used a multisensory integration paradigm to describe the PPS properties in a group of subjects that were habitual PC-mouse users. In particular, they tested the extension of the PPS when no tool was involved in both the “expert” and the “naïve” non-dominant hand. They found that, even if subjects were long-term users of the tool, their perception of the peripersonal space did not change, until they held the object.

This is also in line with the former literature about the peripersonal space[13,21,22], and suggests that, despite the motor experience gained during years of practice with a tool that enlarges the space of action, the athletes’ perception of the PPS is similar to that of the novices when they moved freely, without handling an object.

No differences between the two groups appeared when the participants were required to perform the multisensory integration paradigm handling a generic tennis racket. Indeed, as in the no-tool condition, only the effect of the sound position was noticed, irrespective to the individuals' experience with the sport of tennis, causing RT_{Far} higher than RT_{Near}.

Until now the expansion of the peripersonal space after tool use has been described in a lot of different contexts and populations, but never in the sportive framework. The PPS has been studied in blind subjects, population that uses a cane as tool to explore the surrounding environment, and it was shown that the PPS enlarged to embody the tool[13]. The extension of the PPS was also investigated with different types of implements, like a wheelchair[23], a total body tool, or the computer mouse, which can be considered a "virtual tool" because the space where it is used and the space where it exerts an effect are not physically connected[20]. Further, it is known that the boundaries of PPS enlarge immediately after a few minutes of practice with an unfamiliar object, but this change does not occur when the object is only passively handled before the test[24]. As a consequence, the authors suggested that the remap of the PPS crucially depends on the motor experience acquired by the subject during the use of the tool. In another work, Canzoneri and colleagues showed that long term modifications of the PPS are possible even without exercising immediately preceding the test if the tested object is familiar to the subjects and is become of daily use[25].

In the present study the group of novices had no previous experience with the tennis racket and did not considered this tool as familiar. Hence, the lack of difference between the two groups in the common Tennis Racket condition can be ascribed neither to the familiarity the novices might have with the tennis racket, nor to a preceding training, since they did not perform a training session before the test. Therefore, the explanation might be sought in the response of the tennis players and in the lack of modulation of the PPS when they handled a generic racket. Indeed, at first, one might hypothesize that the tennis racket is an object familiar to these athletes, which is consequently embodied and could determine an expansion of the PPS. However, both the lack of difference between the two groups when they handled the common Tennis Racket, and the difference encountered when the tennis players handled cTR, but not pTR, suggest that the daily experience with a specific tool crucially influences the perception of the PPS, especially after many years of practice. Here, the two rackets used during the experiment had no macroscopical structural differences: lengths, weight and size were the same for all the participants and corresponded to those of the common Tennis Racket. Also, both of them laid on the table during the experiment, so that subjects could not be able to perceive differences in the dynamical properties.

Thus, we propose that the differences emerged in responses given with the two rackets could be ascribed to the level of experience the subject reached with her/his own sport tool. Subtle differences between the two tools might have been detected by athletes, as for instance, the distribution of weights along its length and /or the tactile sensation associated to the grip of one’s own racket. Going deeply, we could propose that a greater familiarity with an object allows to dedicate more attentional resources to a portion of wider space[26]. This could automatically trigger the affordance of actions beyond their reachable space, mechanism that is known to enlarge the PPS[27].

Therefore, we suggest the occurrence of a durable extension of the boundaries of the PPS from the space around the hand to the space around the implement used by the athletes, but only when the instrument is the specific one used during playing. This means that the plasticity of the PPS does not depend only on the function that can be accomplished with a tool, but it is also related to the familiarity of the tool itself, results of the experience gained over years of practice.

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3.2. Neurophysiological correlates during motor imagery and peripersonal space modulation in expert fencers: effect of tool use

3.2.1. Introduction

Practice and experience lead to structural and functional changes in the brain. Skilled professionals with multiple years of training and practice, such as athletes and musicians, showed significant changes in multiple brain regions[1–4]. Some sports require that athletes interact with an object. In some cases, like soccer and basketball, the object is one for all. In some other sports athletes have their own tool, which became an important part of the action, like in tennis and hockey. It has been proved that in expert athletes the long-term physical training with sport-related tools leads to functional and structural changes in multiple brain areas (in soft tennis[5]; badminton[4]; table tennis[6] and tennis[7]), and is also reflected in behavioral measures[8]. Since the development of internal models of action is a requisite for motor learning and for the production of skilled actions[9–11] one can suppose that even tools must be integrated in it. This has been investigated in athletes, through motor imagery. Motor imagery (MI) is the mental process that consists of a motor task in the absence of movement and muscular activity[12]. Fourkas showed that cortical excitability in athletes increased when they imagined to execute a gesture related to their sport rather than related to other sports, but without any tool[7]. Later studies confronted the role of the objects, rather than of the performed action, comparing the effect evoked by the tool specific for a sport and the implement unrelated with the sport in question by means of behavioral tasks and neurophysiological methods. Bisio found that, in tennis players, handling the racket rather than an umbrella during motor imagery induced a better isochrony between real and imagined sport-related movement[8]. In the same year, Wang showed also a facilitation in cortical excitability when badminton players held during motor imagery a badminton racket, with respect to a plastic bar[13]. These results suggested that after a long-term training with a sport implement, the object is integrated in athletes' motor plans. Indubitably, to learn actions associated with tool-use is a multisensory process.

A tool modifies our field of action, changing our efficacy in space. Clinical evidence indicates that both short- and long-term familiarity of specific body parts (e.g., hands) with specific tools or objects may induce their integration into the human body schema[14–17]. As reported in the previous chapter, daily-used rackets and common rackets are differently embodied in the peripersonal space of athletes[18]. Even though the two objects used in that study were two tennis rackets equal in dimension and weight, athletes recognized their personal tool, and this resulted in a reduction of the reaction time in the far condition. This suggested that what the athlete considered as far space while handling the common racket became the near space while handling the personal racket. Whether the origin of this modification in space perception is related to an inclusion of the personal sport-tool in the internal motor representation needs to be demonstrated.

The aim of this work is to verify whether is possible to find an integration specific for a familiar tool in internal motor representation. In particular, we asked if the tool used during training affects the primary motor cortex (M1) excitability in a different way during MI task in elite athletes.

To do so we enrolled 12 fencers with national and international career. We compared the embodiment of their personal èpèes with the embodiment of a common one. Then, we tested the cortical excitability with TMS during the preparation and MI of a gesture related to fencing while subject held their personal or the common èpèe.

3.2.2. Material and Methods

Participants

12 expert fencers (6 males and 6 females, mean ± SE = 26.00 ± 2.78 years) took part in the experiment. Only subjects specialized in èpèe, one of the three weapons used in fencing, were selected. The group was composed by agonist athletes of national and international level. Subjects underwent a questionnaire to explore their athletic career. The years of experience went from 10 to 33 (mean ± SE = 15.75 ± 2.14) and the hours of weekly practice went from 5 to 28 (mean ± SE = 12.00 ± 1.67). Two of them started their

careers with a different weapon (foil or sabre) but only used the sword for most of their sports career.

All the participants were right-handed, as determined by the Edinburgh Handedness Inventory[19], had normal hearing and touch and had no contraindication to TMS. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee.

Experimental procedure

Subjects underwent two experimental sessions, in two different days in random order. In order to investigate whether the èpèe was integrated into the participants' PPS, a multisensory integration paradigm was employed[20]. To verify if the tool was included in the internal motor representation a warned reaction time task[21] was applied while holding the swords.

Multisensory integration paradigm

Subjects sat on a chair with the back of the right hand always lying on a table. They performed a simple detection task during which they were required to verbally respond saying "tah!" as soon as they perceived an electrical tactile stimulus. The tactile stimulus was administered at the right wrist using a surface bipolar electrode attached with a velcro strap and connected to a Digitimer constant current stimulator (DS7AH HV, Digitimer Ltd, UK). Participants' verbal responses were acquired through a microphone positioned around the neck.

A task-irrelevant sound (a 150-ms burst of pink noise), that subjects were instructed to ignore, was presented simultaneously to the electric stimulus. The sound was originated from one of the two identical loudspeakers that were placed one in close proximity to the right hand, at about 30 cm from the body (near position), and the other at a distance of about 110 cm from the other one (far position). The volume of the speakers was singularly regulated so that the intensity of the near and far sound was equal (70dB) as measured by a sound meter at subjects' right ear. The tactile and the acoustic stimuli originating from the loudspeaker near the hand were delivered simultaneously. The far sound started slightly before the onset of the tactile stimulus, in order to compensate for the delayed arrival of the sound, due to the spatial distance. A custom-made MatLab® software managed the synchronization between the electrical and audio stimuli and the order of the trials.

Experimental design

The multisensory integration paradigm was repeated in several sessions, whose order was counterbalanced among the subjects. In each condition of the experiment, participants were blindfolded, standing with the swords in the right hand lying with the back on the table, close to the near loudspeaker. They had to answer verbally, as soon as possible, to the tactile stimulus, ignoring the non-target auditory stimulus. In the common Èpèe (cE) session subjects underwent the multisensory integration paradigm holding with the right hand a 110 cm long èpèe that weighed 750g (the same object for everyone) at the level of the handle, which was settled in correspondence of the near loudspeaker. The remainder of the tool lied on the table so that the tip of the sword was placed in correspondence to the far loudspeaker. In personal Èpèe (pE) session performed the multisensory integration paradigm holding the sword they regularly used to train themselves (Figure 1). The personal èpèes were all long 110 cm and weighed 750g. Between sessions subjects had the possibility to lift and settle the swords for the following session, but they remained blindfolded.

Every session of the experiment consisted of 90 trials randomly ordered: 30 trials during which a tactile stimulus was coupled with the near sound (Near condition), 30 trials where the tactile stimulus was coupled with the far sound (Far condition), and 30 catch trials where subjects only heard either the Near (15) or the Far (15) sounds and they had to prevent themselves from answering. Catch trials were performed in order to avoid habituation. A familiarization phase, consisting of 3 repetitions of each experimental condition, including the catch trials, preceded the beginning of the experiment.

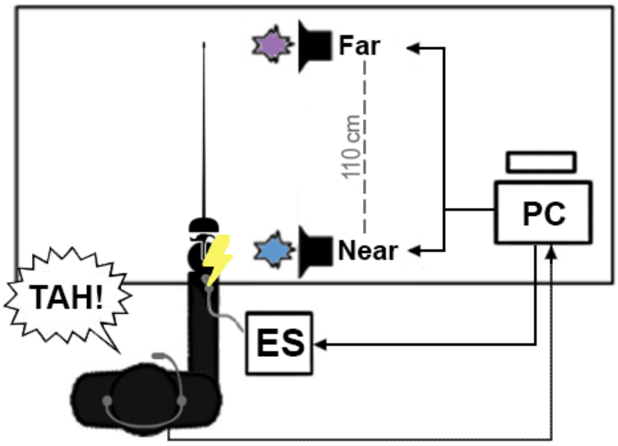

Figure 1. Multisensory integration paradigm. *Participants sat on a chair with the back of the right hand lying on a table. They were requested to verbally respond (saying "TAH!") to an electrical tactile stimulus administered from the electrical stimulator (ES) in correspondence of the right wrist. Participants' verbal responses were acquired through a microphone positioned around the neck. Simultaneously to the electric stimulus, a task-irrelevant sound was presented either in close proximity to the right hand (Near) or at a distance of about 110 cm (Far). A personal computer (PC) controlled the order and the synchronization of the stimuli. Figure refers both to the set up in the common èpèe (cE) session and in personal èpèe (pE).*

Data processing and statistical analysis

A custom-made MatLab software was used to analyze the audio traces of the subjects' verbal answers. From each trace, the reaction time (RT, ms) was calculated as the time elapsed between the onset of participant's verbal response and the delivery of the tactile stimulus in both Far (RT_{Far}) and Near (RT_{Near}) conditions. Responses higher or lower than 2 standard deviations from the individual mean RT value were treated as outliers and were removed from the analysis (always < 5% of the data set).

The mean RT values were analyzed by means of an ANOVA with OBJECT (cE vs. pE) and POSITION (Near vs. Far) as within subjects factor.

Newmann-Keuls post hoc analysis was used to interpret significant interactions. Values are presented as mean ± standard errors.

Warned reaction time task

TMS was used to evaluate changes in the left M1 excitability during different tasks. Intensities were expressed as a percentage of the maximum output of the stimulator. TMS was performed with a single Magstim 200 magnetic stimulator (Magstim Company) connected with a figure-of-eight coil with wing diameters of 70 mm. The coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45° angle to the sagittal plane inducing a postero-anterior current in the brain. This orientation was chosen based on the findings that the lowest motor threshold is achieved when the induced electrical current flows approximately perpendicular to the line of the central sulcus[22].

The coil was placed in order to evoke good responses both in the right abductor pollicis brevis (APB) and extensor carpi radialis ECR muscles. Prior to the experimental procedure, the intensity of stimulation was individually defined to reliably elicit peak-to-peak motor evoked potentials (MEPs) amplitude of a minimum of 1 mV in both muscles at rest (S1mV). Twenty trials were recorded for each condition, and the average MEP amplitude was taken as MEP size.

EMG recording

MEPs were recorded using silver disc surface electrodes taped to the belly and tendon of the muscles. The ground electrode was placed at the elbow. MEPs were recorded from right APB and ECR muscles using silver disc surface electrodes taped to the belly and tendon of the muscles. The ground electrode was placed at the elbow. Electromyographic signals (EMG) were digitalized, amplified and filtered (20 Hz to 1 kHz) with a 1902 isolated pre-amplifier controlled by the Power 1401 acquisition interface (Cambridge Electronic Design Limited, Cambridge, UK), and stored on a personal computer for display and later offline data analysis. Each recording epoch lasted 400 ms, of which 100 ms preceded the TMS.

Experimental design

Experiment consisted in 6 sessions performed during the same day, for a total duration of about two hours (Figure 2): BASELINE session, MI no-tool session, REST sessions while handling cE and pE, MI tools sessions while handling cE and pE.

During MI no-tool, REST and MI tools an auditory tone that served as ‘‘warning’’ cue was emitted and followed later by a second auditory tone that served as the ‘‘go’’ cue 2 to 3 seconds later (Figure 2). Subjects were instructed to stay alert after the *warning* sound, and to imagine a gesture related to fencing right after the *go* sound. Sounds cues were produced with a customizable microcontroller board (Arduino). In MI no-tool and MI tools sessions, subjects were asked to perform a motor imagery task of a gesture related to fencing after the *go* signal was provided. They had to kinaesthetically imagine performing a parry IV – attack combo.

In particular, primary motor cortex excitability was tested in:

- **BASELINE session.** Participants were instructed to relax as much as possible and keep their eyes close. No mental task was required.
- **MI no-tool session.** Subjects seated with their eyes closed without any tools. After the *go* signal (PG_{nE}) they had to imagine kinaesthetically the fencing gestures. TMS was delivered 1 second after the *go* sound, during the imagination task. At the end the experimenter asked the athlete whether the TMS was delivered while she/he was still involved in the imagery task. Trials acquired after the end of the imagery period were discarded.
- **REST sessions.** Subjects seated with their eyes closed, handling and lifting either cE or pE in a natural position. TMS was either delivered 1 second after the *go* signal ($REST_{cE}$, $REST_{pE}$). No mental task was required in these sessions.
- **MI tools sessions:** Subjects seated with their eyes closed handling and lifting either cE or pE in a natural position. TMS was either delivered 1 second after the *warning* sound (PW_{cE}; PW_{pE}) and 1 second after the *go* signal (PG_{cE}; PG_{pE}). Subjects were instructed to wait until the second sound, then to kinaesthetically imagine the parry – attack. At the end the experimenter asked the athlete whether the TMS was delivered while she/he was still involved in the

imagery task. Trials acquired after after the end of the imagery period were discarded.

During REST sessions and MI tools sessions subjects held the tools. To have a comparable facilitation along the experiment, an estimate of maximum voluntary isometric contraction (MVIC) was obtained from APB and ECR muscles with both objects before the TMS measurement. Participants learnt to hold the swords under the 10% of their MVIC. Trials with background EMG activity higher than 10% of MVIC were excluded from analysis.

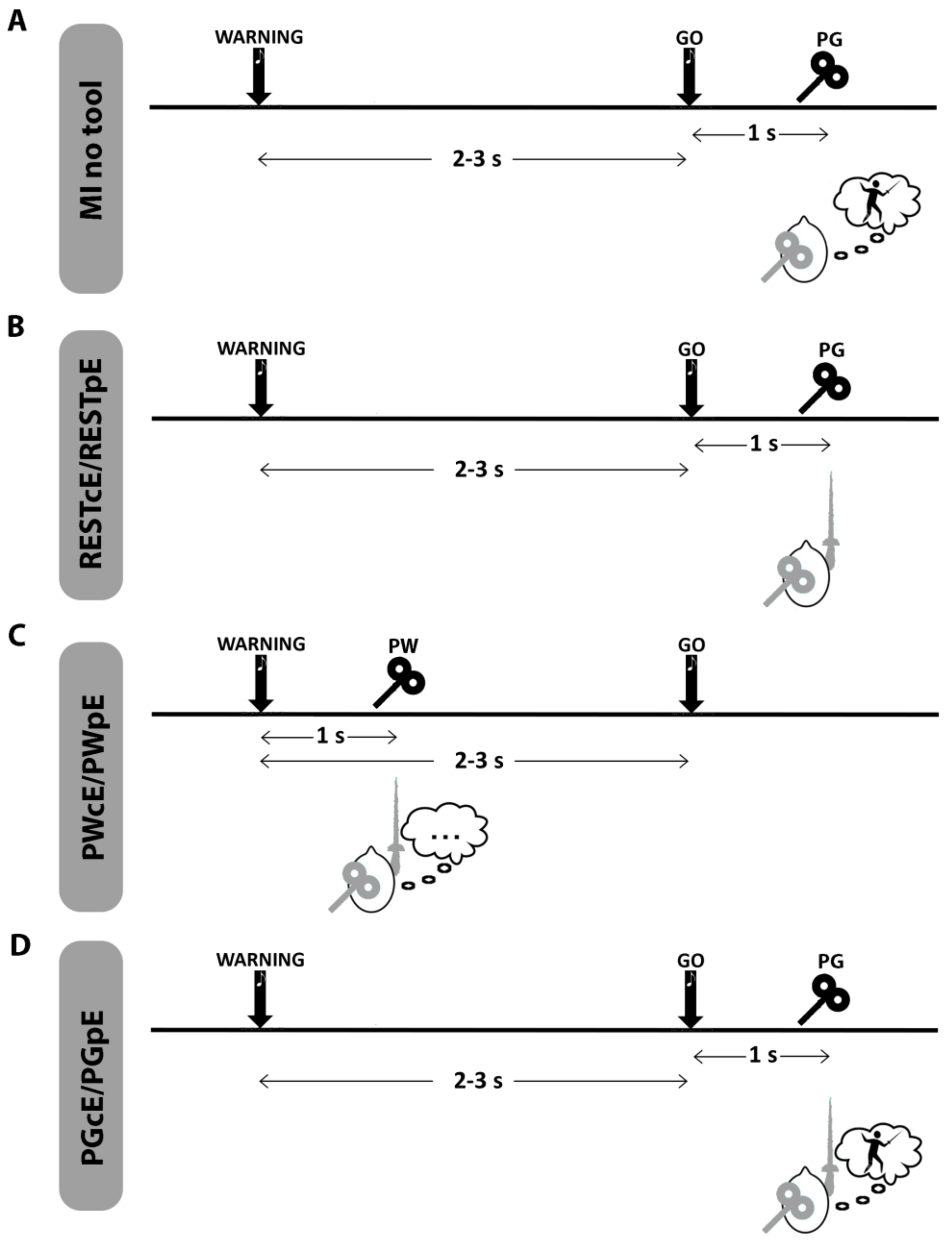

Figure 2. Warned reaction time task. *The "warning" and "go" represent the auditory 'warning' and 'go' cue, respectively. The horizontal arrows represent the timing between sounds or between sound and TMS delivery. Panel A represent 'MI no-tool' session. Subjects seated with their eyes closed without any tool. They had to imagine a parry IV-attack combo after the 'go' sound. TMS was delivered 1 second after the sound, during the MI. Panel B represent REST sessions. Subjects had to held common èpèe (cE) or personal èpèe (pE) without any mental task. TMS was delivered 1 second after the sound. Panels C and D represent different trials of 'MI tools' session. Subjects had to held common èpèe (cE) or personal èpèe (pE) and were instructed to get ready (after the 'warning' cue) to imagine (as soon as possible after the 'go' sound) a parry IV-attack combo. TMS were delivered during preparation (PW – panel C) or during MI (PG – panel D).*

Participants’ general motor imagery ability was evaluated by means of the Italian version of the Movement Imagery Questionnaire (MIQ-R). The MIQ-R is an 8-item self-report questionnaire, in which participants rated the vividness of their mental representations using two 7-point scales, associated to kinaesthetic and visual imagery: the score “7” means “really easy to feel/see”, whereas the score “1” corresponds to “really difficult to feel/see” (best score = 56, worst score = 8). All participants showed good motor imagery abilities (mean ± SE: 20.83 ± 2.00).

Data processing and statistical analysis

MEP amplitude was measured peak-to-peak. A paired t-test was adopted to evaluate the increasing of MI no-tool with respect to BASELINE.

Other data were analyzed with a repeated measure analysis of variance (ANOVA) with OBJECT (cE vs. pE) and CONDITION (REST, PW, PG) as within subjects factor. This analysis was repeated separately for APB muscle and ECR muscle with the same factors.

Newmann-Keuls post hoc analysis was used to interpret significant interactions. Values are presented as mean ± standard errors.

3.2.3. Results

Tool embodiment evaluation

Figure 3 shows the reaction time values in NEAR and FAR conditions when the participants handled the common and the personal èpèe.

ANOVA showed an effect of the factor POSITION ($F(1,11) = 18.91$, $p = 0.001$): the reaction times associated to Far audio stimuli ($RT_{Far} = 332 \pm 12.93$ *ms*) were significantly higher than reaction times related to Near stimuli ($RT_{Near} = 322 \pm 12.45$ *ms*) (Figure 3). Furthermore, a significant interaction OBJECT*POSITION was found ($F(1,11)=13.27$, $p=0.004$). Post hoc test showed that, when subjects handled cE $RT_{Far} > RT_{Near}$ (*343.46 ms > 327.89 ms*; $p = 0.001$), whilst there was no difference between reaction times when subjects handled their personal èpèe ($p = 0.08$). cE RT are always higher of pE RT (cE RT_{Near} > pE RT_{Near} *327.89 ms > 316.33 ms;* cE RT_{Near} > pE RT_{Far} *327.89 ms >320.59 ms;* cE RT_{Far} > pE RT_{Near} *343.46 ms > 316.33 ms;* cE RT_{Far} > pE RT_{Far} *343.46 ms >320.59 ms.* p *always* < 0.001*).*

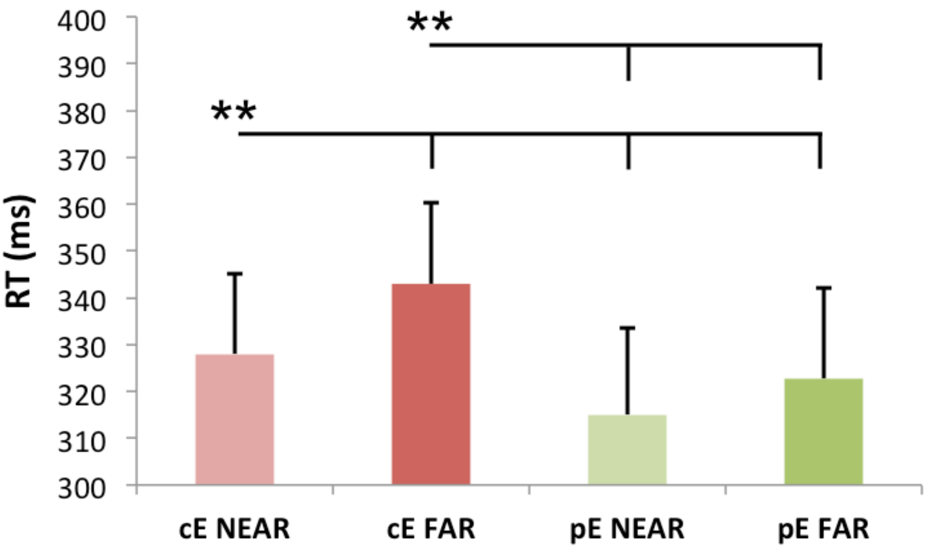

Figure 3. *Averaged reaction times (RT, ms) recorded in Near and Far conditions in common èpèe (cE) and personal èpèe (pE) sessions. The error bars refer to the standard error of the mean. **refers to p<0.01.*

Cortical excitability evaluation

The results of the paired t-test comparing BASELINE session and MI no tool session showed that when fencers imagine executing a gesture related to their sport without any tool their cortical excitability did not increased, nor in APB ($p = 0.35$) neither in ECR ($p = 0.20$).

ANOVA on APB comparing REST sessions and MI tool sessions with both personal and common èpèe showed a significant interaction between OBJECT and CONDITION ($F(1,11) = 4.61$, $p = 0.02$).

Post hoc analysis (Figure 4) revealed that during imagination while handling the personal tool cortical excitability increased significantly with respect to its REST condition (PG_{pE} *vs.* $REST_{pE}$: $3.71 > 3.16$ *mv;* $p= 0.02$). PG_{pE} was also significantly higher with respect to all the cE conditions (PG_{pE} *vs.* $REST_{cE}$: $3.71 > 3.27$ *mv;* PG_{pE} *vs.* PW_{cE}: $3.71 > 3.20$ *mV;* PG_{pE}

vs. PG_{cE}: *3.71 > 3.17; p always < 0.03*). Similarly, PW with personal èpèe was significantly higher with respect to its REST condition and to all cE conditions (*PW_{pE} vs. $REST_{pE}$: 3.61 > 3.16 mv; PW_{pE} vs. $REST_{cE}$: 3.61 > 3.27 mv; PW_{pE} vs. PW_{cE}: 3.61 > 3.20 mV; PW_{pE} vs. PG_{cE}: 3.61 > 3.17; p always < 0.05).* PW_{pE} and PG_{pE} did not differ significantly (p = 0.54). Notably, $REST_{pE}$ did not differ significantly from $REST_{cE}$ (*3.16 vs. 3.26; p=0.90*).

As regard ECR, ANOVA comparing REST sessions and MI tool sessions with both personal and common èpèe did not show any significant differences.

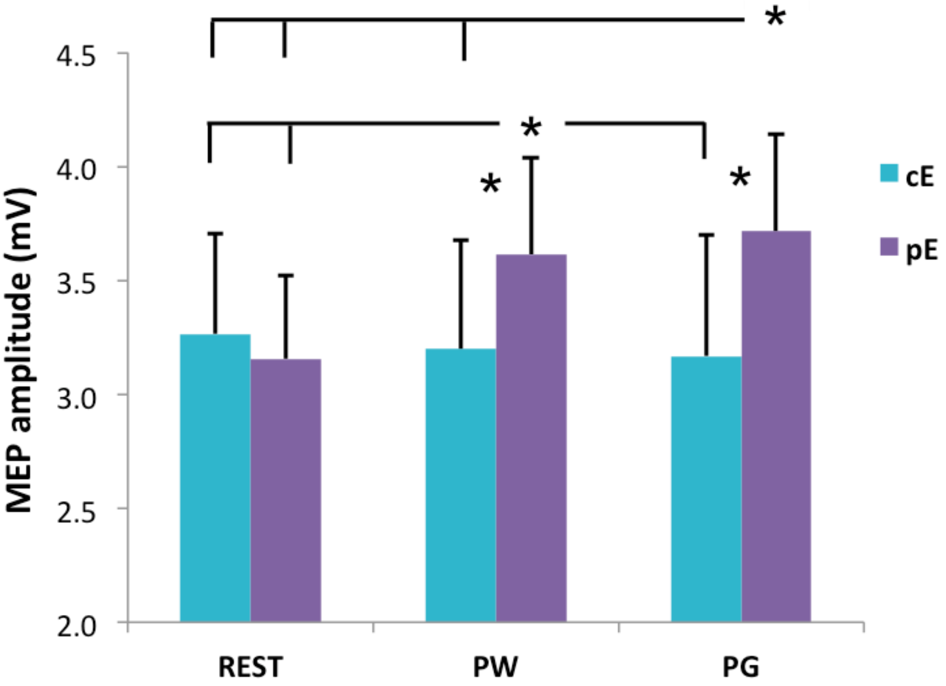

Figure 4. *Motor evoked potential (MEP) amplitudes acquired from APB while participants held their common èpèe (cE, light blue) and personal èpèe (pE, purple) in ‘REST’ sessions and ‘MI-tools’ sessions. PW corresponds to the preparation phase to motor imagery (Post Warning acustic cue), PG correspond to MI phase (Post Go acustic cue). The error bars refer to the standard error of the mean. *refers to p<0.05.*

3.2.4. Discussion

The aim of the present study was to assess whether long-term experience with a specific tool affects motor representation in athletes. It is known that the boundaries of PPS enlarge immediately after a few minutes of practice with an unfamiliar object, but this change does not occur when the object is only passively handled before the test[23]. Different from this short term expansion is a long term PPS enlargement, strictly linked to the motor experience with a tool. This phenomenon has been described in blind subjects, population that uses a cane as tool to explore the surrounding environment, and it was shown that the PPS enlarged to embody the tool, even without being immediately preceded by training[20]. In a previous work we found that tennis players stably embodied in their PPS the tennis racket, but only the specific one they used during their daily training. In this work we replicated this finding in expert fencers.

The main finding of this work is that expert fencers showed facilitation during the preparation and the actual motor imagery task, but only when they held their personal tool. An important aspect of motor preparation is to retrieve and activate the appropriate motor representations required for a particular action[24], and preparation of imagined movement showed activations in cortical motor areas similar to those activated during motor imagery[25–27]. It is known that MI abilities vary as a function of the afferences from the periphery. Skilled performance (e.g., racket sports) involves sensorimotor tasks, requiring a close coupling of actions with sensory inputs[28]. It has been shown that both visual and somatosensory information influences brain activity during motor imagery[29–31]. As an example, corticospinal excitability during imagery with an object is modulated by actually touching an object through the combination of tactile and proprioceptive inputs[32]. This is also in line with a previous behavioral study of our group showing that the isochrony between MI and movement execution in expert tennis players was maintained only when athletes handled the tennis racket[8]. Here we made a step forwards showing that the response of the motor system during MI and its preparation was sensitive to the handled tool, and, in particular, increased with the specific tool athletes used daily. Notably our athletes did not show an increased cortical excitability during the simple MI, but they require to held a tool. Therefore, it can be suggested that a reliable process of motor imagery may be initiated in case of match between the afferent information and the information included in the athletes' internal motor representation of the sport gesture. Moreover, the afferent information could be useful in an early phase, during the retrieving of the correct motor representation. Notably, when subjects held the two swords, no difference appears in MEP's amplitude. This seems to suggest that the differences between tools emerged only with action-oriented purpose, like motor imagery or while detecting stimuli in multisensory integration paradigm. Our data highlighted a modulation only in APB muscles and no effect in ECR. Despite other works found a facilitation during motor imagery of tennis gesture both in hand and in forehand muscles[7], our results is similar to those found from Wang and colleagues in badminton players[13]. Badminton players showed higher motor response than novices during motor imagery when they held a specific implement, but only in FDI muscle, and not in ECR. Authors proposed that, being tennis a sport requiring more power than badminton, which is a sport of precision, muscles could be involved in different ways. Our data may require a different explanation. To distinguish between two swords that have the same characteristics may require a very fine process. Even if fencers usually wear gloves, the body part that is in contact with the tool is the hand. It is possible that afferent properties related to the familiar tool are sensed specifically from distal muscles. This could be the reason why proximal muscle were not influenced by the tool held by fencers.

We can conclude that the long-term physical training of athletes with a specific implement not only provoke a stably remap of the PPS representation in athletes, but also induce an integration in motor schema of the personal object with respect to a generic one that has the same purpose.

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PHD ACTIVITIES

Publications during PhD period related to this thesis

- **Biggio M.**, Bisio A., Avanzino L., Ruggeri P., Bove M. (2017). This racket is not mine: the influence of the tool-use on peripersonal space. Neuropsychologia, 103: 54-58.

- Bisio A.*, Garbarini F.*, **Biggio M.**, Fossataro C., Ruggeri P., Bove M. (2017). Dynamic shaping of the defensive peripersonal space through predictive motor mechanisms: when the “near” becomes “far”. Journal of Neuroscience, 0371-16.

Other publications during PhD period

- Garbarini F., Bisio A., **Biggio M.**, Pia L., Bove M. (2018). Motor sequence learning and intermanual transfer with a phantom limb. Cortex, 101: 181-191.

- Bove M., Strassera L., Faelli E., **Biggio M.**, Bisio A., Avanzino L. and Ruggeri P. (2017). Sensorimotor Skills Impact on Temporal Expectation: Evidence from Swimmers. Front. Psychol. 8:1714.

- Bonassi G., **Biggio M.**, Bisio A., Ruggeri P., Bove M., Avanzino L. (2017). Provision of somatosensory inputs during motor imagery enhances learning-induced plasticity in human motor cortex. Scientific Reports, 7(1),9300

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Abstract submitted to national and international conferences during PhD period

- **Biggio M.**, Bisio A., De Alti S., Ruggeri P., Bove M. Tool dependent motor imagery effects in primary motor cortex excitability in expert fencers. II Sport & Exercise Psychology, Pisa, 28-30 Settembre 2017

- **Biggio M.**, Bisio A., Avanzino L., Ruggeri P., Bove M. Cortical modifications following the interaction between motor training and a protocol combining action observation and peripheral nerve stimulation. IV Fresco Conference, Lucca, 12-14 Settembre 2016

- Bonassi G., **Biggio M.**, Bisio A., Bove M., Ruggeri P., Avanzino L. Motor cortical plasticity induced by motor learning through motor imagery combined with peripheral nerve electrical stimulation. IV Fresco Conference, Lucca, 12-14 Settembre 2016

- Garbarini F., Bisio A., **Biggio M.**, Pia L., Bove M. Phantom learning: intermanual transfer of sequence learning in an amputee with phantom limb. XXI Congresso SIPF (Società Italiana di PsicoFisiologia), Firenze, 27-29 Novembre 2014.

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- Strassera L., Avanzino L., **Biggio M.**, Faelli E., Bisio A., Ruggeri P., Bove M.- Temporal Expectation in swimming. VII Congresso nazionale SISMeS (Società Italiana delle Scienze Motorie e Sportive), Padova, 2-4 Ottobre 2015

Participation to summer schools and training

- Partecipation to summer school "Integrazione di metodi e tecniche per la ricerca, la clinica e la riabilitazione in psicofisiologia e neuroscienze", Università cattolica S. Cuore, Milano 2015