UNIVERSITA' DEGLI STUDI DI PARMA

DOTTORATO DI RICERCA IN NEUROSCIENZE

XXII CICLO

The representation of tool use in the monkey cerebral cortex. An fMRI study.

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1.Introduction

1.1 Tool use in monkeys

Classically, tool use has been defined as 'the external employment of an unattached environmental object to alter more efficiently the form, position, or condition of another object, another organism, or the user itself when the user holds or carries the tool during or just prior to use and is responsible for the proper and effective orientation of the tool' (Beck 1980). Tools are mechanical implements that allow individuals to achieve goals that otherwise would be difficult or impossible to reach.

Before Köhler (Köhler 1927) findings in captive chimpanzee, tool-use was considered a uniquely human characteristic. In his studies Kohler suspended bananas out of the reach of chimpanzee, proving that they were capable of piling up boxes to reach the bananas.

Sixty years later Goodal described the ability of chimpanzee in assembling tools for fishing termites (Goodal 1986). She observed chimpanzee picking leafy twigs then stripping the leaves so that the twig was a suitable tool for poking into the termites mound. Even if it was identified as 'proto-tool-use': a behavior in which a target is reached by means of an object or substance that it is not definable as a tool, this behaviour was the demonstration that chimpanzees are able not only to use but also to modify an object for a specific purpose.

Now it is known that except for Cebus monkeys (Visalberghi and Trinca, 1989; Moura and Lee, 2004), most monkeys, including macaques, vervets, tamarins, marmosets, and lemurs, use tools only after training (Natale et al., 1988; Hauser, 1997; Santos et al., 2005, 2006; Spaulding and Hauser, 2005). So tool use in lower primates seems to be rather fragmented and

this is so even in great apes other than in chimpanzee. Old world macaque monkeys rarely use tool. In particular the spontaneous use of tool is uncertain, whereas this is more frequent in New world monkeys (Tomasello and Call 1997).

These studies stand out primates among mammals as the most frequent tool user. Recently most works have focused their attention on what are the limits of the primates' cognitive abilities underlying tool use. To clarify this point a series of experiments were carried out to establish whether animals have the cognitive capacity to recognize the relationship between different tools' action sequences in which are present one final goal and different sub goals. Santos and colleagues required to cotton-top tamarins to perform a hierarchically organized tools action sequence in order to gain access to out of reach reward. Their results show that, although tamarins do not solve correctly *means-means* problem, they can be trained to solve simple problems involving the use of two tools. The authors suggest that the animal capacity with tools may derive from more general problem solving abilities (Santos et al 2006)

In an other series of studies, Santos and colleagues (Santos et al.,2006) have examined whether primates tool-users understand the properties that are relevant for the function a of a tool. Their results show that even though tamarins can recognize the functional properties of many features (shape, size, orientation), they do not use this information when solving some problems such as determining the tool's correct three-dimensional orientation. They proposed that the abilities exhibited by primates tool-users may not rely on a specialized neural substrate for tool-use, as a domain-specific view in humans would suggest, but it seems that non-human primates reason about the functional properties of tools using more domain-general mechanisms. Such domain general mechanisms

are likely to include a sensitivity to simple object mechanics (Spelke 1991), including an understanding of solidity (Hauser 2001; Santos and Hauser 2002), material transformations, and possibly causality (Santos et al 2006). Domain-specific account of human tool understanding stands in contrast to a domain-general view, in which our understanding of tools emerges as a result of our more general knowledge of physics, objects motion, and causality (Mandler 2002).

Indeed further works, carried out on capuchin monkeys and chimpanzee, confirm the results described above. They show that despite their natural tendency to use tools in the wild and in captivity, their comprehension of tools is limited to perceptually salient features and lack they ability to establish the causal relationships between tool use and the results obtained by using it (Povinelli 2000).

1.2 Neural mechanism underlying tool use in trained monkeys

Recently many studies have focused their attention on the neural mechanism that underlie tool use. As described in the previous section, monkeys in the wild do not spontaneously exhibit tool use behaviour. However it has been observed that they can pick leafy twigs then strip the leaves in order to use the twig as tool for fishing termites. Even though this proto-tool use behavior represents an exception more than a rule, it highlights monkey's ability to manipulate an object in order to retrieve a distant target. Based on this evidence many experiments used trained monkeys in order to assess behavioral and neural change following tool use. Monkeys are trained to use proficiently tools to gain access to out of reach objects, then when they become dexterous in these human-like

cognitive abilities behavioral, and neurological changes are evaluated. Anatomical and functional studies carried out on monkeys have discovered different parieto-frontal networks constituted of premotor and posterior parietal cortices. Parietal posterior regions display a rich variety of passive (somatosensory and visual) and motor properties, that often are integrated at the single neuron level (Rozzi et al 2008). This integration is crucial for sensorimotor transformations and for the online control of actions. In addition, it has been demonstrated that, besides their motor role, neurons of ventral premotor (Pmv) and inferior parietal lobule (IPL) cortices are also involved in cognitive processes. In fact, a typical characteristic of these neurons is that of coding the goal of the motor act (grasping) regardless of the motor effector used for achieving it (Rizzolatti et al. 1988, Ferrari et al. 2003, Rozzi et al. 2008), and, in some cases, also when the goal is achieved by using a tool (Ferrari et al. 2005, Umiltà et al. 2008)

The manual interactions with external objects in the environment represents a challenge for the primate sensorimotor system. Functional studies on non human primates indicate that such problems are solved by the above mentioned parieto-frontal circuit that transforms sensory representation of the body and the surrounding environment into motor plans for prehension (reaching, grasping and manipulation of object). During tool use the physical characteristic of the tool influence the relationship between the body and the surrounding environment. The posterior parietal cortex include different areas, each of which involved in the analysis of particular aspects of sensory information. Different circuits involving IPL areas are committed to visuomotor transformation for grasping (AIP-F5), or to coding of arm/face peripersonal space for

reaching/avoiding stimuli (VIP-F4) or devoted to visual transformation for guiding eye movements (FEF-LIP) (Rizzolatti et al 1998).

Iriki and co-workers (Iriki et al 1996) recorded a group of bimodal neurons from the anterior bank of intraparietal sulcus. This area, corresponding to PEip (Luppino, 2005), is located in the anteromedial bank of the IPS, posteriorly to the shoulder-to-forearm representation of the postcentral somatosensory cortex. These neurons respond both to tactile stimulation on the hand (a neuron's tactile receptive field) and to visual stimuli presented in the same spatial vicinity as the tactile receptive field [the same neuron visual receptive field (R.F.)]. These visual R.F.s were not confined to any region of the retina, but followed the hand around everywhere it was moved in the three-dimensional space. The authors interpreted these neuronal properties as involved in coding the image of the hand in space (Maravita & Iriki 2004). Recording the same neurons when monkeys were using a rake, resulted in a dynamically altered visual R.F. in accordance with the characteristics of the tool used. Infact the visual R.F.s extended outwards along the axis of the tool to include the rake's head.

This phenomenon was observed only when the monkeys were grabbing the tool for using it, while visual R.Fs shrank to cover the original body space, that is that around the hand, when the monkeys were passively grasping the rake. In conclusion a wielded tool may become incorporated into the 'body schema'.

The concept and term of body schema has recently been used to describe cognitive or high order representation of the body that continuously update the position of our limbs while we are moving. The body schema serves to spatially organize our action (Witt et al., 2005).

Thus when we use a tool, this is incorporated, in the brain, as a part of our body representation.

Indeed during tool use learning phase it has been detected an increased expression of immediate early genes, neurotrophic factor (BDNF, NT-3) and receptor *trk* B. This training dependent genetic expression was found in monkeys inferior intraparietal sulcus where bimodal neurons, with an expanded visual R.F after tool training, were recorded. This genetic expression could represent an indicator of neural reorganization induced from tool use learning that finally produce the novel bimodal somatosensory visual response properties in IPS neurons (Ishibashi et al 2002). This could be the substrate of coding a modified body representation following tool use. A further evidence supporting cortical reorganization following tool use behavior was provided by a work of Hihara et al 2006. The authors injected a retrograde tracer (Fast Blue) into intraparietal area of two groups of monkeys: one naïve and the other trained to use a rake (Hihara et al. 2006). Comparing the two groups, they found that ventral prefrontal cortex and the temporoparietal junction region were labelled only in the trained monkey brains. Afterwards, they injected an anterograde tracer (BDA) into temporoparietal junction. Comparing control monkeys, where axons arising from temporoparietal junction were relegated to the deep layers at the fundus of IPS, in trained monkeys additional axons were discovered extending farther, into superficial layers of a shallower portion of the bank IPS. These new connections between far cortical areas could set up a novel type of multimodal integration in the intraparietal cortex, which in turn would endows the monkeys with the capacity to use tools as extensions of their innate body parts. A further experiment carried out by PET imaging confirmed this result (Obayashi et al 2001). The results of this

experiment show an activation of IPS during tool use. Indeed comparing tool-use activities with the control condition (stick manipulation, eliciting the same tool use somatosensory processes but without motor involvement) resulted in an enhanced cerebral blood flow in basal ganglia, pre-supplementary motor area, premotor cortex, and cerebellum. In particular, premotor cortex could have a role in maintain and update the new body image necessary for tool use. It has been already shown that motor neurons in this area encode motor acts (goal-related movements, such as grasping) rather than movements (Umiltà et al 2008). This study show single-neuron activity recorded in ventral permotor cortex from monkeys' trained to grasp objects by means two different kind of pliers: a normal pliers, requiring a typical grasping hand movements, and 'reverse' pliers, which require fingers opening in order to grasp an object with that pliers. Area F5 neurons discharged during the same phase of grasping in both conditions, regardless of whether this involved opening or closing of the hand. The tool, after learning, is coded in the motor system as if it were an artificial hand and this is observed not only when the mechanics of pliers is congruent with that of the hand (normal pliers), but also when the mechanics is opposite. In area F5 it has been also discovered a group of neurons that discharge both during the execution and the observation of actions done by others (mirror neurons; Gallese et al 1996). It has been suggested that the activation of F5 mirror neurons during the observation of motor acts allows the observer to understand the goal of the observed action (Rizzolatti et al 2001). Furthermore, recording mirror neurons activity during observation of action made by tools, with which the monkeys were previously trained, Rochat et al., 2010 showed that neurons normally responding to the observation of hand grasping actions also responded to the observation of grasping

performed by using pliers. and, many of them to the observation of actions done by using a stick to pick up a peace of food, action that was never performed by the observer monkeys. The authors show that the discharge the neurons exhibit during the observation of different motor actions can be different in term of intensity and temporal pattern of the response , in particular observation of hand grasping led to the earliest and the strongest response, while pliers grasping and spearing observation determined weaker and later responses. They conclude that F5 grasping mirror neurons respond to the observation of a class of actions sharing the same goal. However, the response depends on how similar is the observed action respect to that performed by the hand, that represents the natural motor template. Indeed it has been shown that after long tool use visual exposure a sub-class of neurons can exhibit a particular behaviour (Ferrari et al 2005). These authors recorded, in ventral premotor cortex (F5), a new type of visuomotor neurons (*tool-responding mirror neurons*) discharging when monkeys were observing an action made by a tool. The authors suggest that tool-responding mirror neurons could allow to extend the comprehension of the goal of an action that do not strictly correspond to the motor representations of the observer.

1.3 Neural mechanism underlying tool use in humans

Although several animal species use simple tools to extend their physical capabilities, humans are unique in using complex tools as well as in their level of understanding of the physical relationship between own body and surrounding objects, and in their grasp of the causal relationships between the use of a tool and the goal that can be achieved by using it.

In contrast with simple tools used and sometimes built by other species, we create complex artefacts that reflect such knowledges and the unique demands of the external environment in which we live (Povinelli,2000).

Much of what we know about the neural substrates of tool use human abilities comes from investigations of patients with acquired brain injuries. Clinical studies of human brain lesion specify that two elements of human tool use are distinguishable 1) abilities in handling tools and 2) conceptual knowledge, associating functional knowledge of the action with functional knowledge of the tool (Liepmann 1905, Leiguarda Marsden 2000). For example in a right handed patient, a lesion on left hemisphere can affect tool use abilities or the capacity to imitate tool use without affecting the ability to describe the same tool or to explain how it should be correctly used (Geschwind and Damasio 1985). On the other hand, brain lesions involving other regions, again typically in the left hemisphere, can compromise the concept of actions appropriate for a right use of a given tool. These different behavioural patterns led to the definition of *apraxia* as an impairment in the representation of acquired skills that cannot be attributed to difficulties in linguistic, sensory or lower-level motor functions (Geschwind and Kaplan 1962; Heilman and Rothi 1997). This term is used to describe a broad series of higher order movement disorders (differently from limb paralysis) involving reaching or grasping movements (Koski et al., 2002). With respect to the tool use abilities exhibited from the patient, two different kinds of apraxia have been defined. One is the ideomotor apraxia, that is characterized by the patient knowing what he/she has to do with the tool but without knowing how to do it. They show impairments in timing or action sequencing especially when involving tools (Leiguarda Marsden 2000). The second is

ideational or conceptual apraxia (De Renzi and Lucchelli 1988), in this case the patient does not know which kind of movement he/she has to perform with a tool in order to achieve a goal (Goldenberg and Hagmann 1988). Classically, apraxic patients display difficulties when they are required of pantomiming the uses of familiar tools or other handle objects (i.e., transitive actions) in response to verbal commands and/or imitation (Leiguarda and Marsden 2000). The ability to pantomime such transitive actions to verbal command is considered a critical test for apraxia because it isolates the retrieval of stored action representations in response to minimally informative stimuli (Goldenberg 2003). Thanks to advances in neuroimaging over the past decades, numerous studies have contributed to a more detailed understanding of which cortical brain regions are involved in the use of tools and some of the specific functional roles they cover.

All the results of functional neuroimaging on tool use studies have shown that, although the precise regions implicated vary somewhat depending on the tasks used, left parietal and/or frontal mechanisms are always active in the representation of skills involving objects. Preparation and/or execution of tool use actions is consistently associated with increases in neural activity in the left parietal cortex within and along IPS, and in left premotor and/or prefrontal cortex (Moll et al. 2000; Choi et al. 2001; Johnson-Frey et al., 2005). This evidences have led to postulate that left parietal cortex may be the critical node for the integration of conceptual and sensory-motor representations into contextually appropriate action plans (Johnson-Frey 2004; Johnson-Frey et al. 2005; Frey 2007).

These studies indicate that tool use capabilities rely on a circuit more generally involved in hand action. Furthermore, several studies have led to the evidence for homologies among primates in parietofrontal circuits

that control prehension. Grasping actions, for instance, in both monkeys and humans are coded within a circuit involving the inferior parietal lobule (IPL) and ventral premotor cortex (PMv). In monkeys, this network comprise the two areas that are also anatomically connected: anterior intraparietal area (AIP) and premotor area F5 (Pmv)., This circuit is devoted to transforming objects' intrinsic spatial properties into motor programs for grasping (Jeannerod et al., 1995; Rizzolatti and Luppino, 2001). Functional neuroimaging studies in humans suggest that the anterior IPL, within and along the intraparietal sulcus (aIPS), and ventral precentral gyrus (putative Pmv) may constitute a homolog of the monkey parieto-frontal grasping circuit (Binkofski et al., 1999; Ehrsson et al., 2000; Frey et al., 2005). Human IPL regions play a role in different aspects of both the planning of motor skills and motor imagery of object use. In right-handers, the left IPL is activated when making judgements about the manipulability of objects whether viewed or heard (Rumiati et al., 2004; Boronat et al., 2005; Lewis et al., 2005),thus it has a role in visuomotor and audiomotor integration. The left IPL could code the representations of moving limbs, selecting which limb to use for the task at hand, or preparing motor actions with that limb (Goldenberg and Hagmann 1998; Chaminade et al., 2005). Furthermore it may store engrams of known hand movement gestures (Buxbaum et al., 2003, Buxbaum et al.,2005).

Injury involving the left IPL can lead to "ideational apraxia," in which patients have impaired tool use and pantomimic ability (De Renzi and Lucchelli 1988; Liepmann 1908; Buxbaum and Saffran 2002; Buxbaum et al., 2003). However, such patients could maintain the capacity to use tools in a simple way with ability based on the "feel" of the object and other perceptual features (Sirigu et al 1995; Goldenberg and Hagmann 1998). They also have a tendency to keep an understanding of the

function of a tool, and be able to name it (Buxbaum and Saffran 2002; Fukutake 2003). The other region belonging to the previous mentioned network is represented by human ventral premotor cortex. This regions has a somatotopic organization, including hand and mouth representations (Buccino et al 2001). Dorsal portions of the left human ventral premotor cortex have a role in planning and preparing arm and hand movements, especially when involving implements such as tool use (Binkofski et al., 1999; Rumiati et al., 2004; Fridman et al., 2006). These regions are also involved in more cognitive functions including spatial perception, imitation, and action understanding, especially when involving handle objects (Gerlach et al 2002; Rizzolatti et al 2002). The most anterior and ventral portions of the left VPmC are relatively more involved in motor execution (Fridman et al.,2006). This region classically defined Broca's area, has a role in controlling oro-laryngeal movements for speech production. This part of the left ventral premotor cortex is activated when viewing tools and when naming tools or naming actions (Chao and Martin 2000, Gerlach et al 2002).

Moreover recent neuropsychological studies on patients with parietal lesions display behavioral effects consistent with single unit data from macaques. For instance, Berti and Frassinetti (Berti and Frassinetti, 2000) evaluated the effect of tool-use in a right parietal damaged patient with unilateral left-neglect, who showed neglect on line bisection in near but not far space. When requested to show the mid point of a distant drawn on a wall line by using a laser point, the bisection was perfect. By contrast, when a long stick was used for the same far-line bisection, left-neglect was again evident.

Similar results were obtained from Maravita (Maravita et al., 2002) by using a crossmodal (somatosensory–visual) interference task. In this task, visual

distractors (LED flashes at the fingertips usually interfere when required to localize vibrotactile targets presented concurrently. This interference is stronger for the hand located ipsilateral to the visual distractors than for the contralateral hand. Nevertheless, when the hands are crossed to contact contralateral distractors, the effect reverses: visual distractors interfere more with localization of somatosensory targets administered to the contralateral hand. In their experiments Maravita and colleagues found that, when the tactual stimulated hands are handling tools and visual distractors are administered at the tool tip, a similar reversal of crossmodal interference occurred. In other words, reaching for a visual stimulus with the hand or with the tip of the tool seems to produce similar crossmodal interference effects. The present results are consistent with such tool-based modulation of visual–tactile interactions, similar to the expansions of visuotactile RFs in IPS neurons as shown in Iriki et al .,1996. Use of a tool causes distant space to be remapped as "within reach". These are further evidences that highlight the involvement of parietofrontal circuits, implicated in reach, grasp, and object manipulation, in complex tool use behaviors.

1.4 The representation of tool use in Humans and Monkeys: common and uniquely human features.

While it is clear that the use of tools by humans reflects an understanding of the causal relationship between the tool and the action goals (Johnson-Frey, 2003), this is far less true for apes. The available evidence (Visalberghi and Limongelli, 1994; Povinelli, 2000; Mulcahy and Call, 2006; Martin-Ordas et al., 2008) indicates that chimpanzees may have some causal understanding of a trap, for instance (Visalberghi and Limongelli

1994), but lack the ability to establish analogical relationships between perceptually disparate but functionally equivalent tasks (Martin-Ordas et al., 2008; Penn et al., 2008).

An important question is whether such difference between human and monkey can be due to an evolutionary specialization of a human brain region devoted to tool use, which may be involved in the coding of the causal relationships between the use of the tool and the outcome of its use, or simply reflects the fact that humans have extensive knowledge and practice with tools, while monkeys lack any similar experience. This is an important issue, since spirited debate is currently going on in the field of cognitive neuroscience as to whether the responses in certain brain regions, apparently specialized in processing and recognition of a particular class of visual stimuli (for instance faces or body parts), are the result of an innate domain specific brain system (Kanwisher et al., 1997) or result from experience and training with any class of visual stimuli (Gauthier et al., 2000).

Since primates in general exhibit the same level of understanding of tool use, whether they use them spontaneously or only after training, it has been suggested that primates rely on domain-general rather than domain-specific knowledge (Santos et al., 2006). Only humans should consequently have specialized neuronal mechanisms allowing them to understand the functional properties of tools, a species difference that should apply to all tools, both simple and complex. In a recent comparative functional imaging Orban and collagues (Orban et al., Soc Neurosci abstr., Oct 2006, 114.2), found that the observation of videos showing actions done with the hand activates homologous regions in both humans and monkeys (grasping circuit), while observation of actions done with tools activated a specific part of the rostral inferior parietal

lobule (aSMG) only in humans, and not in monkeys. Interestingly, a lesion of this aSMG sector causes ideomotor apraxia, a major aspect of which is the inability to use tools (Heilman KM, 1979). These data suggest that an area devoted specifically to tool use may have evolved in human parietal cortex.

On the other hand, the finding that the training induces the appearance of bimodal visuo-tactile properties in parietal neurons (Iriki et al., 1996), has led to the suggestion that the use of simple tools might rely on similar mechanisms in both species (Maravita and Iriki, 2004). According to this hypothesis only the use of more complex tools requires special neuronal mechanisms typical of humans (Johnson-Frey, 2004; Frey, 2008). Thus it is unclear whether or not the neuronal mechanisms involved in the use of simple tools, such as a rake, are similar in humans and monkeys.

1.5 Aims of the study

The present series of experiments was designed to compare the cortical regions activated during the observation of action done with simple tools in humans and in monkeys. To do this, it is important to compare monkeys' brain responses to the observation of tool-use actions, before and after the monkeys have been extensively trained to use the same tools as viewed in the videos. We used functional magnetic resonance imaging (fMRI) in the awake monkey (Vanduffel et al., 2001; Nelissen et al.,2005). The development of awake monkey fMRI has been a major advance in the field of neuroscience, providing the missing link between human fMRI and monkey single cell studies. This monkey fMRI technique allows direct comparisons with human fMRI results and permits the investigation of

possible functional homologies or differences between the human and monkey brain (Vanduffel et al., 2002). A considerable advantage of functional imaging over single cell recording in monkeys is the fact that fMRI allows investigating stimulus induced brain responses throughout the brain, rather than within a small restricted area.

In order to compare monkeys brain response with that of humans, parallel experiments were carried out in both species. Even though human experiments are not part of this thesis, their results will be briefly presented and discussed for allowing a comparison with monkey results.

2. Brain imaging technique - Methodology

2.1 Realignment

One of the major sources of artefacts during fMRI acquisition are created by motion of the head or the brain alone (pulsations associated with respiratory and cardiac cycles). The aim of motion correction is to adjust the series of images so that the brain is always in the same position. For motion correction successive image volumes in the time series are coregistered to a single reference volume (first volume). The rigid body transformation used assumes that brain's size and shape of two subsequent volumes do not change so that one can be superimposed exactly on the other by a combination of three translations and three rotations. The six parameters are calculated using the least-square approach and incorporated in the general linear model (GLM) as variables of no interest to remove motion related signal.

2.2 Matching of the functional images in the anatomical template

Whereas the anatomical boundaries of functional images are difficult to identify, such boundaries and region of interest can be easily located in structural images. Because the size, shape and sulcal pattern of the brain are much more distinct on structural images, it is advantageous to use

information from structural images to guide the normalization of functional images (coregistration).

The normalization is the transformation of MRI data of an individual subject to match the spatial properties of a standardized image, such as an averaged brain derived from a sample of many individuals.

Using rigid and non-rigid matching software (MATCH), functional images were matched to an anatomical high resolution template of the monkeys own brain or a standard monkey brain (M3).

2.3 Spatial smoothing

In a subsequent step, the functional images are smoothed (convoluted with a Gaussian kernel). This leads to an improved signal to noise ratio. Furthermore, smoothing prior to group statistic eliminates residual interindividual anatomical differences after spatial normalization, suppressing noise and effects due to differences in anatomy by averaging over neighboring voxels. This allows to achieve better spatial overlap, enhanced sensitivity, and greater validity of statistical assumptions.

Finally smoothing the data makes the data more conform to the Gaussian field theory which relies on statistical inference. When a Gaussian spatial filter is applied, it spreads the intensity at each voxel in the image over nearby voxel. The width of the filters refers to the distance of this effect. Spatial filter width for fMRI is generally expressed in millimeters at half of the maximum value (FWHM full-width-half-maximum).

2.4 Statistical analysis

The basic objective of the analysis of functional MRI data is to identify voxels showing signal change that varies with the changing brain states of interest across the serially acquired images. The analysis consists of two successive step: a t-statistic is calculated for each voxel and each contrast, using the general linear model, and the significance of the results in term of p-value is estimated (statistical inference).

The results of the statistical analysis are displayed as statistical parametric maps (SPMs), three- dimensional spatial distributions of colour-code voxels with significance values higher than the specified threshold and overlayed on the anatomical image of the brain.

2.5 T statistic and General Linear Model

Data analysis is based on the timing and duration of the evoked neuronal activity on which the General Linear Model (GLM) can generate a predicted hemodynamic response. These models contain predicted time courses for the entire session, and different predictions (regressors) that correspond to different hypothesized processes (visual processes, motor response). The relative contribution of each of these regressors to the measured data, within each voxel, is then statistically evaluated using a technique know as linear multiple regression analysis (evaluation of the relative contribution of several independent variables to a dependent variable).

GLM describes the signal $x_{i,j}$ in each voxel j of each image i, in term of a linear combination of k explanatory or independent variables $g_{i,j}$ plus an error term $\varepsilon_{i,j}$ expressing the residual variability:

$$x_{i,j} = g_{i,1}\beta_{1,j} + g_{i,2}\beta_{2,j} + \ldots + g_{i,k}\beta_{k,j} + \varepsilon_{i,j}$$

$\beta_{k,j}$ is the unknown parameter for each voxel j that represents the relative contribution of each of the explanatory variables and is estimated using a standard least-square method for each voxel.

We identify a regionally specific effect in term of a difference between parameter estimates for separate conditions. Such differences between parameters estimates are specified using linear contrast between conditions. The significance of the linear contrast is assessed by converting the this linear contrast to a statistical variable according to a known distribution (Student's t-test).


2.6 Analysis of eye movements

During fMRI scans, monkeys were required to maintain fixation within a 2 x 2 degree window centered around a small red dot, while visual stimuli were presented on the background. Eye movements were recorded using a infrared pupil/corneal reflection tracking system (RK-726 PCI, Iscan, Inc., Cambridge, MA). Horizontal and vertical components of the eye traces were included in the General Linear Model used for statistical analysis as covariate of no interest. This enables to separate eye movements related MR response from stimulus linked MR signals.

We selected runs for statistical analysis based on several criteria: overall performance of the monkey (% above 85 for the whole run, no significant differences in % fixation between different conditions tested within a single

run; no significant difference in standard deviation of the horizontal and vertical eye traces between conditions).

Overview of SPM Analysis
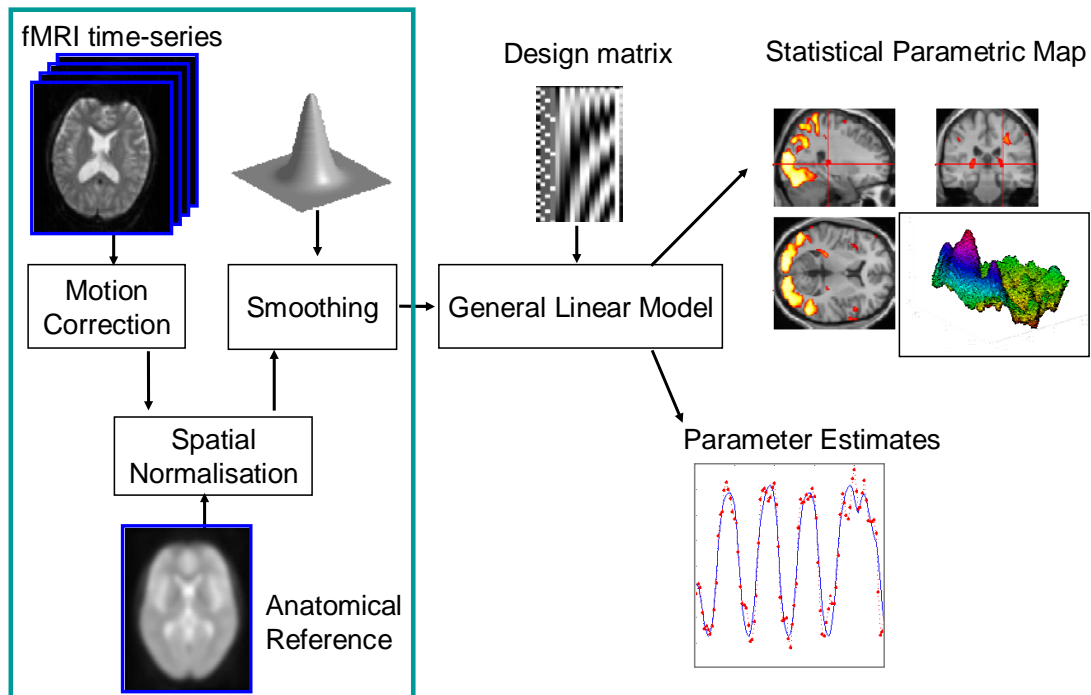
3 Materials and Methods

3.1 Surgery and training

Five (M1, M5, M6, M13, M14, one female) rhesus monkeys (3–6 kg, 4–7 years of age) were scanned. Two monkeys participated in experiment 1 (M1, M5), three in experiments 2-3-4 (M6, M13, M14) and two in experiment 5 (M13, M14). They were trained on the fixation task and the high acuity task (Vanduffel et al., 2001) used to calibrate the eye movement recordings. All animal care and experimental procedures met the national and European guidelines and were approved by the ethical committee of the K. U. Leuven Medical School.

Prior to MR scanning, each monkey was implanted with an MR-compatible plastic headset attached to the skull by plastic T-like devices, plastic, and ceramic screws. The plastic headpost was attached to the skull using C&B Metabond adhesive cement (Parkell) together with Palacos R+G bone cement and 15 ceramic screws (Thomas Recording). All operations were performed under isoflurane (1.5%)/N 2 O (50%)/O 2 (50%) or ketamine anesthesia (10 mg/kg, Ketalar, i.m., Parke-Davis, Zaventem, Belgium) supplemented with xylazine (0.5 mg/kg, Rompun , Bayer, Leverkusen, Germany). Antibiotics (50 mg/kg i.m., Kefzol , Lilly, Brussels) and analgesics (4 mg/kg, i.m., Dolzam , Zambon, Brussels) were given daily for 3–7 days following each surgery. The surgical procedures were conformed to national, European, and National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.. After recovery, the monkeys were adapted to physical restraint in a small plastic box (see Figure 1), then habituated to the sounds of MR scanning

in a "mock" MR bore. The monkeys were seated comfortably on their haunches, in the so-called "sphinx" position. The monkeys were water deprived during the period of testing. The monkeys were trained to optimal performance on a high-acuity orientation discrimination task, used to accurately calibrate a pupil/corneal reflection tracking system (RK-726PCI, in the macaque can be largely Iscan, Inc., Cambridge, MA).. The monkey had to interrupt a light path with its hand to indicate when a bar target changed its orientation from horizontal to vertical. Each correct response was rewarded with apple juice, delivered through a magnet-compatible juice delivery system (Figure 1). To ensure that the monkeys viewed the bar foveally, the bar was gradually reduced in size during training to 5 x 18 min arc. Then the monkeys were trained on a fixation task. After the eye tracking system was calibrated, we presented a fixation spot instead of a fixation bar. The monkey was rewarded for maintaining fixation within a square-shaped central fixation window (2° on a side). The interval between rewards was systematically decreased (from 2500 to 500 ms) as the monkey maintained his fixation within the window. After 20 to 50 training sessions, each monkey (in its plastic restraint box) was placed into a horizontal bore, 1.5T Siemens Vision scanner, equipped with echoplanar imaging. A radial surface coil (10 cm diameter) was placed over the head (Figure 1). This coil covered sufficiently the whole monkey brain, even though with an 30% signal intensity decrease along the dorsoventral axis of the brain. Before the scanning, MION (4–7 mg/kg) diluted in an isotonic saline buffer or sodium citrate (pH 8.0) was injected intravenously into the femoral vein.

During scanning sessions monkeys sat in the sphinx position in the plastic monkey chair directly facing the screen. Throughout the training and testing sessions, the monkey's head was restrained by attaching the

implanted headpost to the magnet compatible monkey chair. Thus, during the tests, the monkeys were able to move all body parts except their head. It is important to note, however, that body movements are usually unfrequent when the monkeys perform a task during scanning. A receive-only surface coil was positioned just above the head. During fixation training the monkeys were required to maintain fixation within a 2 x 2° window centered on a red dot (0.35 x 0.35°) in the center of the screen. Eye position was monitored at 60 Hz through pupil position and corneal reflection. During scanning the fixation window was slightly elongated in the vertical direction to 3°, to accommodate an occasional artifact on the vertical eye trace induced by the scanning sequence. The monkeys were rewarded (fruit juice) for maintaining their gaze within the fixation window for long periods (up to 6 s), while stimuli were projected in the background. With this strategy monkeys made 7–20 saccades per minute, each monkey exhibiting a relatively stable number of saccades over the different sessions/runs: 7/min for M13, 9/min for M6, 11/min for M14, 13/min for M1 and 20/min for M5. In no experiment was the number of saccades made by individual subjects significantly different between the experimental conditions.

Before each scanning session, a contrast agent, monocrystalline iron oxide nanoparticle (MION), was injected into the monkey femoral/saphenous vein (4–11 mg/kg). In later experiments the same contrast agent, produced under a different name (Sinerem) was used. Use of the contrast agent improved both the contrast-noise ratio (by approximately fivefold) and the spatial selectivity of the magnetic resonance (MR) signal changes, compared with blood oxygenation level-dependent (BOLD) measurements (Vanduffel et al., 2001; Leite et al., 2002). While BOLD measurements depend on blood volume, blood flow,

and oxygen extraction, MION measurements depend only on blood volume (Mandeville and Marota, 1999). For the sake of clarity, the polarity of the MION MR signal changes, which are negative for increased blood volumes, was inverted.
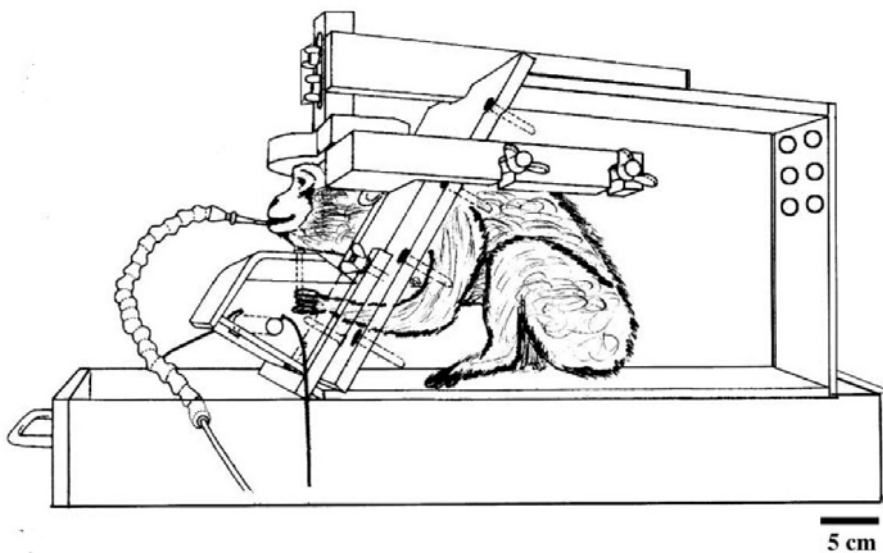

Figure 1. MR compatible monkey chair (from Vanduffel 2001)

3.2 Visual stimuli.

Visual stimuli were projected onto a transparent screen in front of the subject using a 6300 liquid crystal display projector (1024 x 768 pixels; 60 Hz). Optical path length between the eyes and the stimulus measured 54 cm. All tests included a simple fixation condition as a baseline condition in which only the fixation target (red dot) was shown on an empty screen.

Two monkeys (M1, M5) participated in experiment 1. During this experiment, we displayed videos (16° by 13°) showing either a human hand grasping objects ("hand action") or a mechanical hand grasping the same objects ("mechanical hand action"). A male, female or a mechanical hand grasped and picked up a candy (precision grip) or a ball (whole hand grasp)(Fig.2 E,F). The mechanical hand (three fingers, Fig.2 A), was moved toward to object by a human operator (invisible), while the grasping was computer controlled. This mechanical hand action video is thus clearly different from that of (Tai et al., 2004). The same videos were described as robot hand action in Nelissen et al., 2005. One action cycle (grasping and picking up) lasted 11 s, randomly selected cycles were presented in a block of 36 s . Static single frames (36 s) and scrambled video sequences, obtained by phase scrambling each of the frames of the sequence, were used as controls.

Human hand and mechanical hand runs were tested separately and typically included action, static, scramble and fixation repeated twice, with 3 different runs/orders of conditions.

Three monkeys (M6, M13, M14) were scanned in experiment 2, 3 and 4.

In experiment 2 we presented within the same run videos (same size and duration as in experiment 1) showing either a human hand grasping objects (same as in experiment 1, goal-directed action) or a screwdriver held by a human hand and used to pick up objects (Fig.2 B). As a control, a static (refreshed each 3.3 s) single frame of the action videos was used. A typical hand and tool action run included actions performed with a screwdriver, goal-directed hand actions, other actions irrelevant to the present experiment, their respective static controls, and fixation (Nelissen et al., 2005), with the same sequence repeated once and 5 different runs/order of conditions.

In experiments 3 and 4 we used the same 2 X 2 factorial design (tool action, hand action and their static controls) as in experiment 2, but replacing the screwdriver by a rake (experiment 3) (Fig.2 C) or pliers (Fig.2 D) (experiment 4) and the corresponding hand actions consisted of dragging (experiment 3) or grasping actions (experiment 4).

Finally, two monkeys (M13, M14), which had participated in experiments 1–4, were trained (experiment 5, see below) to use the rake and, after a series of scanning sessions, were also trained to use pliers and scanned a third time. In these scanning sessions runs with different tools were again interleaved.
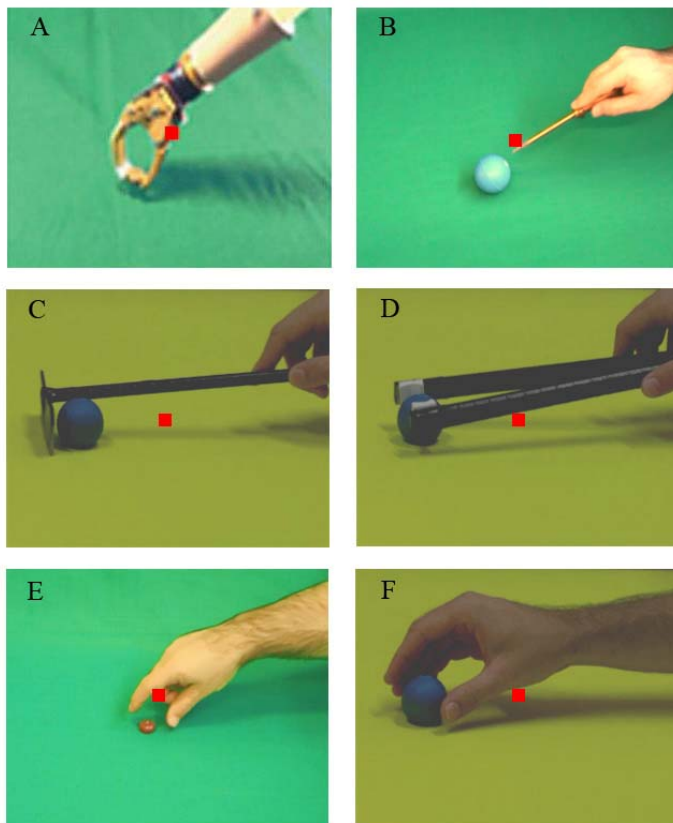
Figure 2. Visual stimuli presented in experiment 1 (A,E,F), experiment 2 (B,E,F), experiment 3 (C,E,F) and in experiment 4 (,D,E,F,). The same stimuli were presented to the monkeys after tool training.

3.3 Training of monkeys to use tools.

Monkeys were trained to use two tools: rake and pliers. Both tools were custom built. Both were 25 cm in length; the rake-head was 10 cm wide and the pliers opened to 5 cm.

For the training sessions monkey subjects sat in an MR-compatible primate chair laid on the table on which the tools and pieces of food were presented. These pieces were located 34 – 40 cm from the shoulders of the monkey, out of reach. The monkeys were trained to use the tools with their right hands.

The training sessions started with a familiarization period during which the monkeys acquired familiarity with training set up. After this phase the training proceeded in steps: touching the tool, lifting the tool, directing the tool toward the food position and retrieving the food by retraction of the arm. The first stage consisted in training the monkeys to correctly handle the tools (rake or pliers). This session began with the tool placed in front of the monkey; the experimenter first led the monkeys hand toward the tool handle. The monkeys obtained food when their hand was correctly, even if passively, handling the tool. When the monkeys associated reward obtaining with the correct motor action, they were able to perform it without experimenter intervention.

During the following phase both monkeys were trained to lift the tool and to direct it toward the different food positions. Finally in the last tools training phase, the monkeys learned to retrieve the food by their arm retraction.

The two different tools trainings differed in few phases, because the correct pliers and rake use require different motor plan acts. While training the monkey to use the rake was relatively easy, the use of pliers required

more training, largely because the pressure needed to close the pliers had to be applied at the right moment and maintained until the food was retrieved.

A correct pliers' handle is very important for the task outcome. The monkeys had to grab both pliers stick. In the following part of the task the monkeys had to learn to not apply more pressure on the pliers, otherwise the pliers would be closed before food approaching. At the end of the food approaching phase the pliers had to be placed in a way to have the food between the two pliers' extremities. In the last training part the monkeys had to learn to keep the pliers closed duting arm backward movement.

In the final part all these single motor acts were linked together. Tool use can be considered as a behavioural chain. A behavior chain is a series of related motor acts each of which provides the cue for the next one in the series, with the last motor act producing reinforcement.

Chaining is the reinforcement of successive elements of a behavior chain. Forward chaining is a chaining procedure that begins with the first element in the chain and progresses to the last element (Tool handle to obtain reward). In forward chaining, the experimenter starts with the first task in the chain (Tool handle). Once the monkey could perform that element satisfactorily, the experimenter trained the monkeys to perform the first and second elements (Tool handle and lifting) and reinforce this effort. When the monkeys mastered the motor act, the experimenter could move to the next element of the chain.

3.4 Analysis and evaluation of behavior

To assess the level of skill reached by the monkeys to use the rake and the pliers, a series of behavioural tests and an analysis of the movement kinematics were performed at the end of each of these two training phases.

The monkeys were tested in three consecutive sessions of 75 trials. Each trial started with the food pellet placed on the table out of monkey's reach while the tools were placed within the animal's reach. Differently from the training sessions, where the food pellets were placed only in front of the monkeys, during these tests the food pellets were located in five different positions. One of these positions coincide with that used in training session, so the food was directly in front of the monkey. In the other four positions there was an angle of 45° or 22,5° between the location of the food and the axis of the tool. The tools were placed in two different positions: in front of the monkey when the food was on its right or left side and on the monkey's right side when the food was in front of it. The trials corresponding to the five food positions were presented in a random order, and 15 trials of each type were administered. In each of these different food positions, the monkey had to move its elbow and forearm in a lateral direction, to obtain the food reward (Ishibashi et al., 2000).

This set-up allowed us to asses if monkeys retrieved the food in a stereotyped manner, as shown in the early phase of tool-use learning and to examine whether macaque monkeys could use the tool in an environment different from which they were originally trained. Each trial started when the monkeys came in contact with the tool and finished either when the food was retrieved, when the monkeys stopped trying to

retrieve the food or when the food was displaced by the monkeys to a point beyond the reach with the tool. After food and tool placement, the monkeys had 30 seconds to act on the tool, otherwise the trial was considered incorrect.

Although after training the monkeys completed these standard trials in much shorter time, a constant time criterion was maintained during training, testing in standard trials, and testing in special trials (see below). The training lasted 3– 4 weeks for the rake and 4 (M13) to 6 (M14) weeks for the pliers. The interval between the two training epochs lasted 1 month. Monkeys reached ~ 95% correct in the standard trials, except M14 for the pliers (76%).

3.5 Behavioral tests

In addition to these standard trials, two other types of trials were administered during testing. First, to estimate the monkeys' comprehension of the functional features such as shape of the used tools, useless object (irrelevant ring or spherically shaped objects) were presented at random 10 times in the course. Second, in 30 trials, interspersed among the standard trials, the tool was presented to the monkey not in its standard upright position but rotated 90° in either direction or turned 180°.

3.6 Kinematics analysis

To analyze the kinematics of the monkeys action when using tools, all trials of the three testing sessions were videorecorded by a digital video

camera and markers were attached to the tools: one at middle of the rake-head and two on the arms of the pliers 3 cm from the tip. These markers were considered as reference points to obtain the representative path of the tools head. The recorded images were sent to a P.C, where a 2-D motion analysis of the trajectories and the speed along these trajectories was performed.

This analysis allowed the evaluation of the tool-use skill development. Infact it has been shown that during the early stage of tool-use learning, the velocity profile of the tool consists in two different segments: pushing the rake toward the target position and then pulling-in toward the reaching space. This indicates that the two actions are independently performed. Further training in tools-use results in a smoother profile indicating that the two actions are integrated in a single smooth movement.

3.7 fMRI scanning.

The monkey MRI images were acquired in a 1.5-T Sonata MR scanner (Siemens, Erlangen, Germany) using a surface coil. Each functional time series consisted of gradient-echo echoplanar whole-brain images (1.5 T; TR, 2.4 s; TE, 27 ms; 32 sagittal slices, 2 X 2 X 2 mm voxels). In total, 2160 volumes/condition were analyzed in experiment 1 and 1440 volumes/condition in experiments 2–4. For each subject, a T1-weighted anatomical (three-dimensional magnetization prepared rapid acquisition gradient echo, MPRAGE) volume (1 X 1 X 1 mm voxels) was acquired under anesthesia in a separate session.

3.8 Analysis of monkey data.

Data were analyzed using statistical parametric map (SPM5) and Match software. Only those runs in which the monkeys maintained fixation within the window for ~ 85% of the time and in which no significant differences in the number of saccades between conditions occurred were included in the analysis. In these analyses, realignment parameters, as well as eye movement traces, were included as covariates of no interest to remove eye movement and brain motion artifacts. The fMRI data of the monkeys were realigned and nonrigidly coregistered with the anatomical volumes of the template brain [M12, same as subject MM1 in the study by Ekstrom et al. (2008)] using the Match software (Chef d'Hotel et al., 2002). The algorithm computes a dense deformation field by composition of small displacements minimizing a local correlation criterion. Regularization of the deformation field is obtained by low-pass filtering. The quality of the registration can be appreciated in the study by Nelissen et al.(2005). The functional volumes were then subsampled to 1 mm^3 and smoothed with an isotropic Gaussian kernel [full-width at half-height, 1.5 mm].

Group analyses were performed with an equal number of volumes per monkey, supplemented with single subject analysis. The level of significance was set for the interactions at $p < 0.001$ uncorrected for multiple comparisons, as for humans. For all experiments a fixed effect analysis was performed, except for experiment 5, in which we emulated a random effect analysis by calculating first level contrast images for each set of two runs for a single monkey. The single effects of hand action observation were thresholded at $p < 0.05$ corrected.

From the activity profiles, we derived the magnitude of the interaction given by the equation: percentage signal change (SC) in tool action

minus percentage SC in static tool minus the difference between the percentage SC in hand action and percentage SC in static hand. The SEs of the individual conditions were relatively similar. Since the SEs are indicated only for illustrative purposes, we therefore assumed equal SEs in the four conditions and can then set the SE of the interaction magnitude to twice the average of the SEs in the four conditions. To test the selectivity of an activity profile for tool actions two paired t tests were performed. One compared the difference between tool action and its static control to the difference between hand actions and their static control. The second directly compared the tool actions to the hand actions. Comparisons were made across runs for the individual monkey subjects. While the first test ensured that a significant interaction was present in the ROI, the second ensured that this interaction was due to a difference in the experimental conditions, rather than a difference between the static control conditions. For both tests, the level of significance was set at $p < 0.05$ corrected for multiple comparisons.

fMRI data, registered onto the anatomy of M12, were mapped onto the macaque M12 atlas (Durand et al., 2007) using the same tool in Caret.

3.9 R.O.I analysis

In addition, four ROIs (Region Of Interest) were defined directly onto the MR anatomical images of M12, corresponding to the four architectonic subdivisions of IPL: PF, PFG, PG and Opt, as defined by (Gregoriou et al., 2006). Of these four IPL regions, mirror neurons are most prevalent in area PFG (Fogassi et al., 2005; Rozzi et al., 2008). The ROIs included 89 voxels in PF, 70 in PFG, 137 in PG, and 84 in Opt of the left hemisphere. Numbers

were very similar for the right hemisphere. Percentage MR signal changes, relative to fixation baseline, in these ROIs were calculated from the group or single subject data. The SE is calculated across runs in single subject profiles and in group profiles.

The data of the first two scans of a block were omitted to take into account the hemodynamic delay.

3.10 Human experiments

One of the aims of the present work is to compare the cortical regions activated during the observation of action done with simple tools in humans and in monkeys. To this aim parallel experiments were carried out in humans. Even if these experiments are not directly part of this thesis, their results will be briefly presented and discussed for allowing a comparison with monkey results, thus, for sake of clarity, the corresponding methods will be briefly described in this paragraph.

In Experiment 6 on humans (similar to monkey experiment 1)hand action and mechanical arm videos were presented to 20 volunteers. Twenty-one volunteers took part in experiment 7, in which videos were presented showing either a human hand goal-directed action or a screwdriver used to pick up objects (as in monkeys experiment 2). Experiment 8 ,in which the subjects observed actions done by a rake or a hand dragging an object (see monkeys experiment 3), was performed on 8 volunteers. Finally eight volunteers participated to experiment 9 in which they observed a pliers seizing an object or an hand grasping the same object.

In the first two experiments, (experiments 6 and 7) the first level contrasts for the different action types (human hand, mechanical hand and tool)

versus their respective controls were calculated for every individual subject. These contrast images were subjected to a second level, random-effects ANOVA analysis. The interactions were investigated by subtracting hand action minus its control from mechanical hand action minus its control or from action with screwdriver minus its control. These interactions were conjoined with the main effect of actions compared with their static controls. In the other experiments (experiments 8, 9) a fixed-effects group analysis was performed. For each contrast, significant MR signal changes were assessed using T-score maps. Thresholds were set at $p < 0.001$ uncorrected for the interaction effects. As a final analysis we performed a conjunction of the interaction effects in the four different experiments (each at $p < 0.001$ or $p < 0.005$ level). This conjunction was itself conjoined with the single effects of mechanical hand or tool action in the same experiments. A threshold of $p < 0.05$ family wise error corrected for multiple comparisons was used for the single effects of hand actions compared with their static or scrambled controls, and for the shape and motion localizers. For descriptive purposes in figures we use a lower level: $p < 0.001$ uncorrected.

Activity profiles plotting the MR percentage signal changes compared with fixation were obtained for the various experiments. These profiles were calculated for the different regions of interest (ROIs) defined by conjunctions of contrasts. The profiles were calculated from the single subject data by averaging all the voxels in a ROI. The first two data points of a run were not taken into account to compensate for the hemodynamic delay of the BOLD response.

4 Results

In order to compare the cortical regions activated during the observation of action done with simple tools in humans and in monkeys, a series of preliminary experiments were conducted in humans. Even if the present work is mainly focused on the monkey study, in order to allow a comparison between species, in the following section the results of human experiments will be first briefly presented, followed by monkey results.

4.1 Human fMRI results

The results of experiment 6 showed that during the observation of human hand actions, compared with their static controls, fMRI signal increased in occipitotemporal visual areas, in the anterior part of the intraparietal sulcus (IPS) and the adjacent inferior parietal lobule (IPL), and in premotor cortex plus the adjacent inferior frontal gyrus, bilaterally as well as in the cerebellum. Observation of actions performed by a mechanical hand, compared with their static control, activated a circuit similar to that activated during hand action observation, although the parietal activation in the left hemisphere appears to be more extensive than that for the biological hand (Fig. 3A). Any difference between the two contrasts is revealed directly by the interaction between observation of tool action compared with its static control versus observation of hand action compared with its static control. These interactions show a significant signal increase in the occipitotemporal region bilaterally and in the rostroventral part of left IPL convexity, extending into the posterior bank of postcentral sulcus [left anterior supramarginal gyrus (aSMG)]

In experiment 7, 21 volunteers observed video-clips showing hand actions and actions in which a tool (a screwdriver) was used to pick up an object.

Confirming experiment 6, the contrast tool use versus hand grasping, both relative to their static controls, revealed a signal increase in the left parietal lobe (Fig. 3B). Figure 4A uses the projection of the activation pattern onto a flattened hemisphere to show the overlap of the interactions for mechanical hand (blue outlines) and tool actions (red outlines) within the aSMG.

In experiments 8 and 9 we assessed the generality of these findings, using the same experimental design of experiment 7, but replacing the screwdriver with two other tools (rake and pliers). The results confirmed those of experiment 7: interaction sites included left aSMG (Fig. 3C,D). Since a significant tool versus hand action interaction was observed in aSMG in all four experiments, we performed a conjunction analysis of these interactions. This analysis yielded the aSMG site and two inferior occipital gyrus (IOG) sites (Fig. 3E). Figure 4B illustrates the location on a flat-map of the 75 (black) voxels yielded by the conjunction analysis which define the core of the aSMG region. This core activation is located (59% overlap) in cytoarchitectonically defined area PFt of Caspers et al. (2006). Its activity profiles (Fig. 4C–F) clearly indicate that in each of the four experiments the interaction was significant and was due to a significant difference between the action conditions, rather than between the control conditions. While the mechanical arm interaction site overlapped the interaction sites for the 3 tools (screwdriver, rake and pliers) in left aSMG (Fig. 4B), this was not the case in the two IOG conjunction regions (Fig. 5 A). In IOG the interaction sites common to the 3 tools (Fig. 5B,C, white voxels) were located anterior to the mechanical arm interaction sites (blue outlines), with a few intervening voxels in common, corresponding to the conjunction regions (black voxels). This explains the functional heterogeneity of these conjunction regions

indicated by their activity profiles (Fig. 5, compare D,E with F–K). A further difference between the aSMG and IOG conjunction sites is that the latter are clearly visual in nature being both shape and motion sensitive, while the aSMG lacks these simple sensitivities. This analysis of the IOG conjunction sites shows that they differ considerably from the left aSMG core.
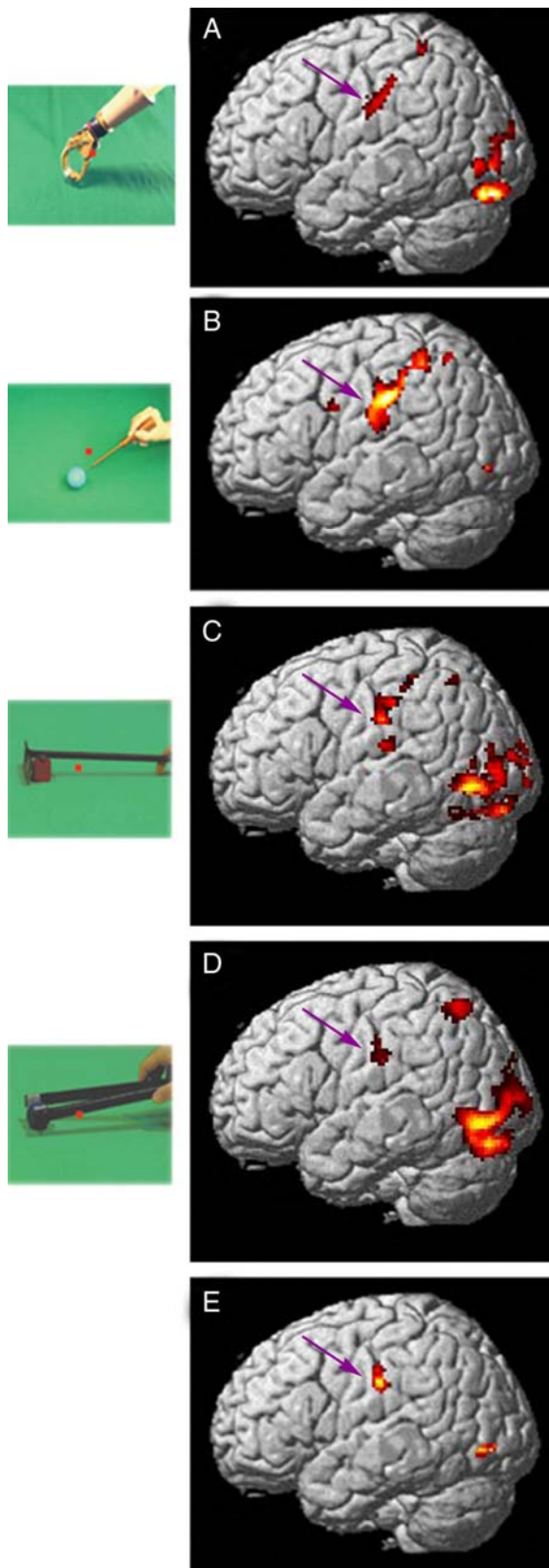
Figure 3. The left hemisphere interaction sites in the four experiments.

A. Cortical regions more active (colored red to yellow) during the observation of grasping performed with a mechanical implement than during the observation of the same action done by hand, each relative to its static control, rendered on lateral views of the left hemisphere of standard (MNI) human brain (experiments 6, 20 subjects, random effect analysis).

B. Cortical regions active in the contrast observation of screwdriver versus hand action relative to their static controls (experiment 7, 21 subjects, random effect analysis).

C. SPMs showing significant voxels in the interaction observation of rake vs hand actions (experiment 8, 8 subjects, fixed effect analysis relative to their static control).

D. Results of experiment 9 (observation of action with pliers , 8 subjects, fixed effect).

In all four interactions threshold is $p<0.001$uncorrected.

E. Conjunction of the interactions in the four experiments; threshold for each interaction $p< 0.005$ uncorrected, yelding the core of aSMG. Purple arrows indicate left aSMG.
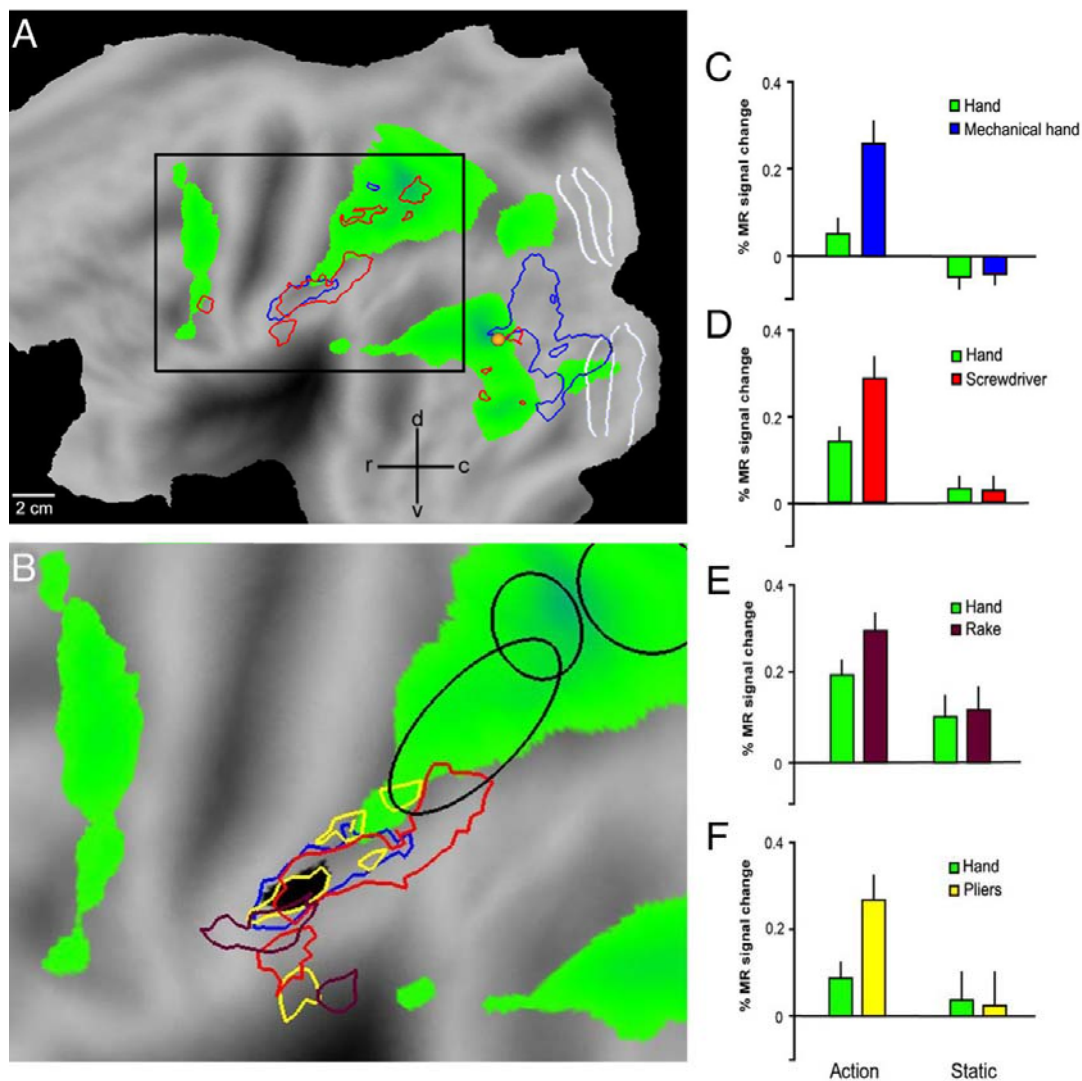
Figure 4. The anterior supramarginal gyrus interaction site. A, Flat map of the left hemisphere.Green areas show activations in the contrast hand action minus static control (p<0.001, experiment 6, same data as Fig. 3A).Blue and red outlines show the regions where the interactions tool versus hand, relative to their controls, were significant (p<0.001) in experiments 6 and 7 (as Fig.3 A,B). White lines indicate areas V1-3. **B,** Enlarged view of the data shown inside the black square in A. Brown and yellow outlines show the regions where the interactions tool actions versus hand actions, relative to their controls, were significant (p<0.001) in experiments 8 and 9, respectively. Blue and red outlines as in A. Black voxels indicate the conjunction of the four interactions (each p<0.005). Blue ellipses indicate from left to right putative human AIP (phAIP), the dorsal IPS anterior (DIPSA) region and the dorsal IPS medial (DIPSM) region. **C–F,** Activity profiles of the core of the left aSMG region (black voxels in B) in experiments 6– 9.All eight paired t-tests were significant. p < 0.0001 for both tests in C (interaction and direct comparison of actions),p<0.001 for both tests in D, p<0.01 for both tests in E and p<0.05 for both tests in F.
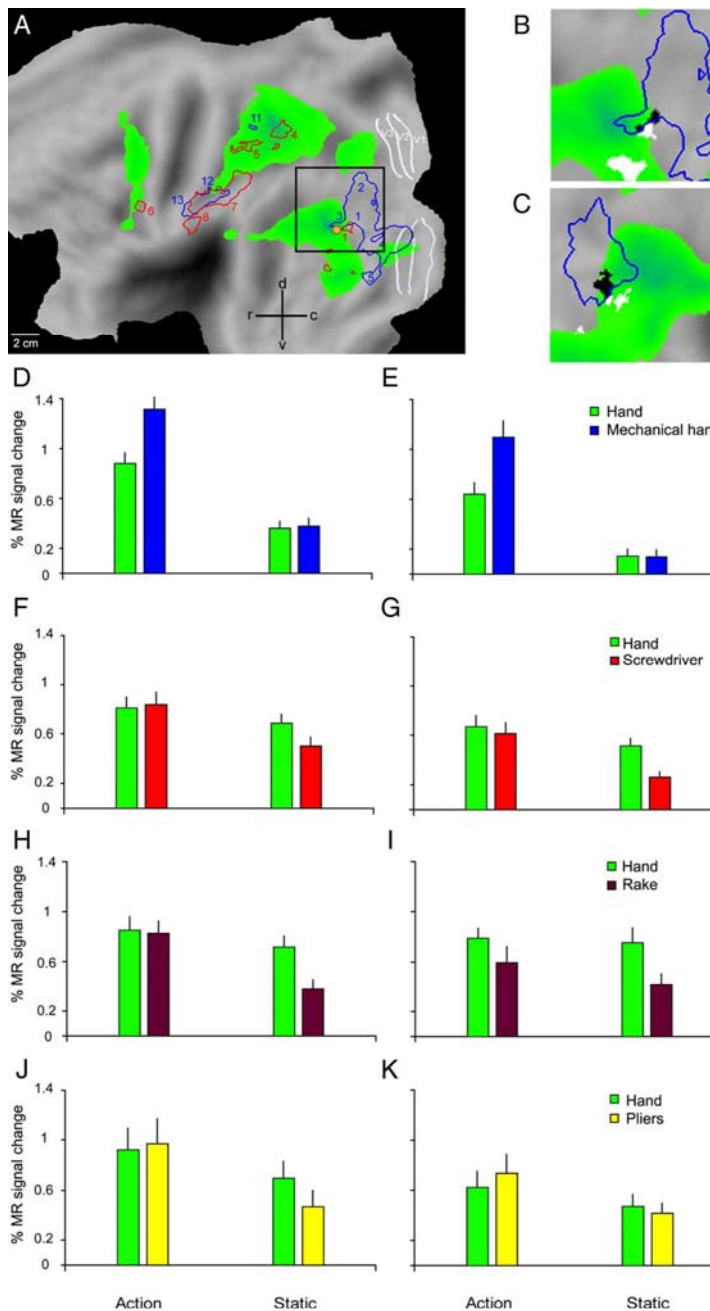
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Figure 5. Human IOG interaction sites.

A, Flatmap of the left hemisphere. Green areas show activations in the contrast observation of hand action minus static control (p<0.001, experiment 6). Blue and red outlines show the regions where the interactions tool versus hand, relative to their controls, were significant in experiments 6 and 7 (p < 0.001). White lines indicate areas V1-3; yellow dot, hMT/V5+; black square, part shown in B.

B, C, Part of flat maps of left and right hemispheres showing the regions corresponding to the conjunction of the interactions for the 3 tools (white voxels), to the interaction for the mechanical hand (blue outlines), and the conjunction of the four interactions (black voxels).

These latter voxels, located in IOG, reflect the overlap between two largely distinct regions, probably induced by the smoothing and the averaging across many subjects; green voxels same as in A

D–K, Activity profiles of left (D,F,H,J) and right (E,G,I,K) IOG regions yielded by the conjunction analysis of experiments 6–9 (black voxels in B, C). The larger percentage signal changes in these IOG regions compared with those in IPL (Figs. 4,) underscore their visual nature.

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4.2 Naive monkey fMRI experiments (experiments 1-4)

Experiments 1–4, were performed in five monkeys (M1, M5, M6, M13, M14), using the fMRI technique previously described (Vanduffel et al., 2001; Leite et al., 2002). Cortical regions activated in the contrast observation of hand action compared with its static control, and in the contrast mechanical hand action observation compared with its static control are shown in Figure 6, *A,B* and *C*, respectively. The activation pattern included occipitotemporal regions, premotor and inferior frontal regions, and parietal regions. For both contrasts the activation in parietal cortex was located mainly in the lateral bank of the IPS (Fig6). The strongest activation was located in the anterior, shape-sensitive part of lateral intraparietal area (LIP) (Durand et al., 2007), with extension into posterior AIP and posterior LIP. It is noteworthy that the parietal activation was asymmetric, being stronger in the left than in the right hemisphere, an observation which also applied to the human parietal cortex (Fig. 1*B*). It is likely that this asymmetric activation reflected an asymmetry in the action stimuli, in which most motion occurred in the right visual field. Alternatively, it may reflect the use of the right hand in the videos (Shmuelof and Zohary, 2006). No activation was observed in any of the four areas of monkey IPL: PF, PFG, PG or Opt (Gregoriou et al., 2006). As expected from Figure 6, no significant interaction between the use of mechanical implements and hand actions, relative to their static controls, was observed in monkey IPL in experiment 1 (Figs. 7*A*, 8*A*), or in any of the three other experiments (Fig7*B-C-D*, 8*B-C-D*).Figures 7 and 8 illustrate the tool action versus hand action interaction sites observed in experiments 1–4 on the flattened left and right hemispheres respectively. The voxels more active (fixed effect, p < 0.001) in the contrast mechanical hand versus hand actions relative to their static controls are shown in blue (7,8A), the regions activated in the contrast

screwdriver compared with hand actions relative to their static control are represented in red (7,8 B),while brown indicates the interactions sites for observation of rake (7,8 C), finally the voxels more active in the contrast pliers versus hand actions relative to their static controls are shown in yellow (7,8 D). Significant interactions were, observed in visual areas, predominantly in TEO, sometimes extending into the superior temporal sulcus (STS) or V4, and to a lesser degree in early visual areas, probably reflecting low level visual or shape differences between the videos. No significant interaction was observed in monkey IPL using the voxelbased analysis, with the sole exception of left area PG in experiment 3 (Fig. 7 *C*, rake action).
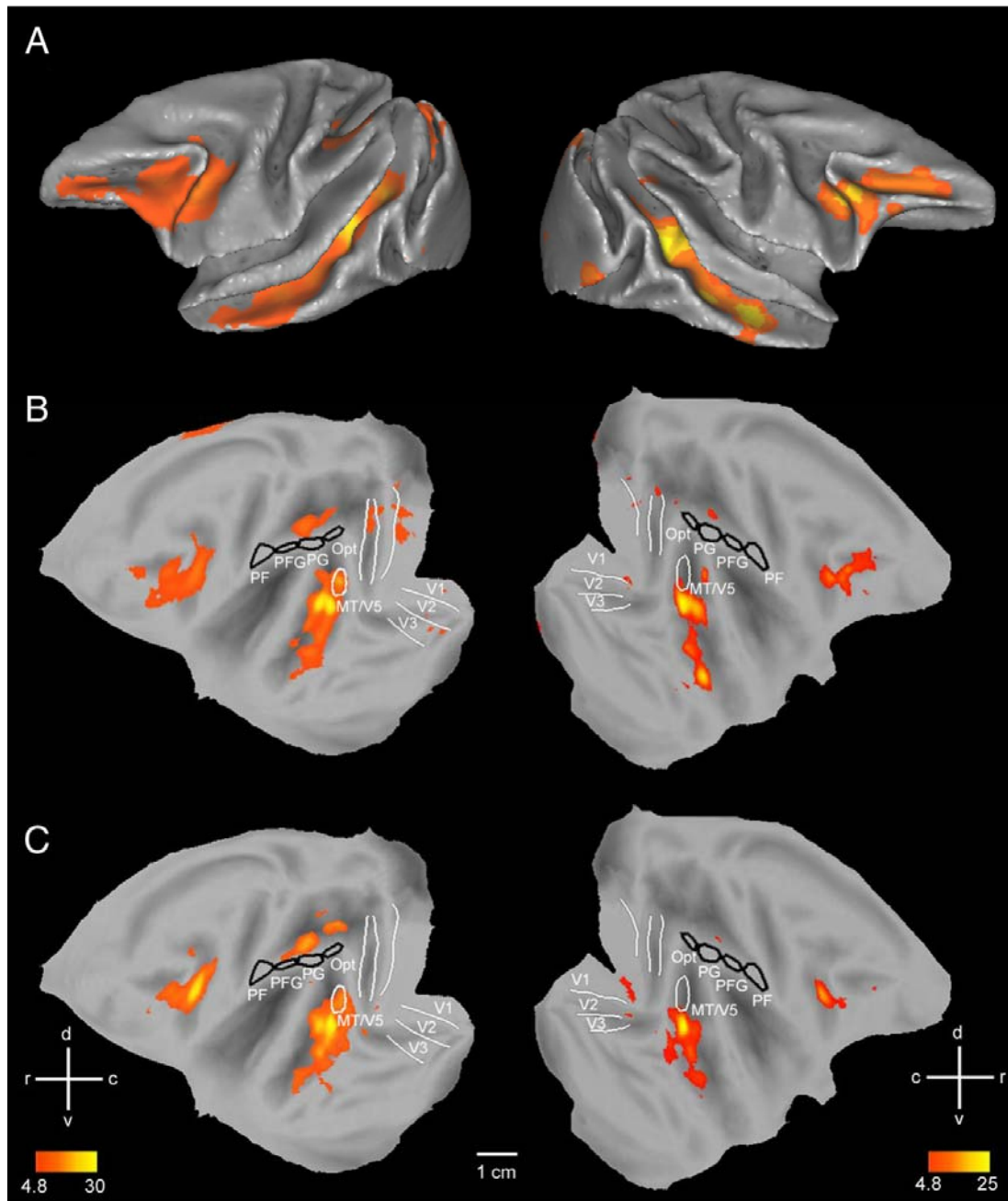
Figure 6. Results of experiment 1. A–C, Folded left and right hemispheres (A) and flatmaps (B, C) of left and right hemisphere of monkey template (M12) brain (Caret software) showing cortical regions activated (2 monkeys, fixed effects, p< 0.05 corrected, experiment 1) in the subtraction observation of hand action minus static hand (A, B) and observation of mechanical hand action minus static mechanical hand (C). Both subtractions were masked inclusively with the contrast action observation minus fixation. Black outlines, PF, PFG, PG and Opt; outlines of visual regions(V1-3,MT/V5) are also indicated. Color bars indicate T-scores.
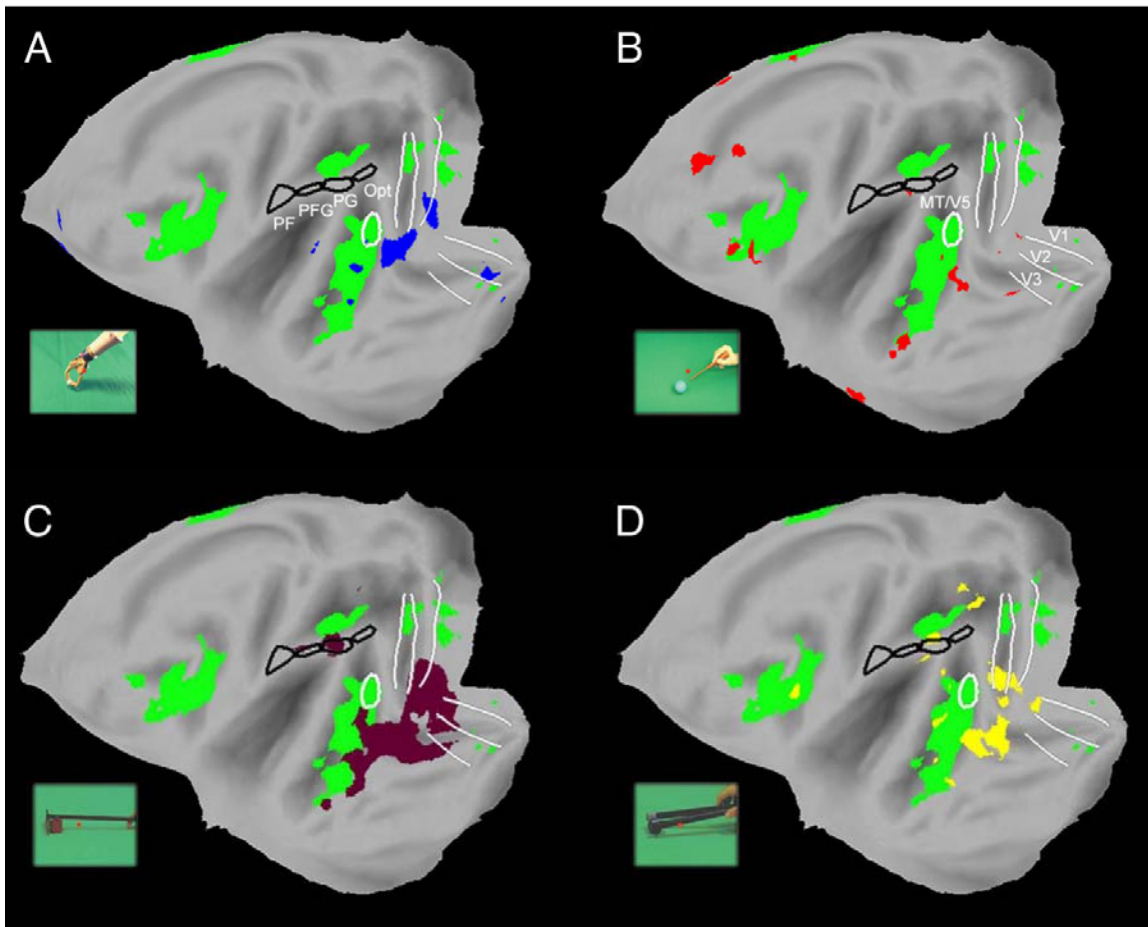
Figure 7. Interaction sites in the monkey. A–D, Monkey cortical regions activated during the observation of hand grasping action relative to their static controls (green voxels, fixed effect, $p < 0.05$ corrected, experiment 1, same data as in Fig. 5B). Data plotted on flatmaps of the M12 left hemisphere. The voxels more active (fixed effect, $p < 0.001$) in the contrast tool versus hand actions relative to their static controls (experiments 1– 4) are shown in blue (A, mechanical and), red (B, screwdriver), brown (C, rake), and yellow (D, pliers). ROIs as in Figure 6.
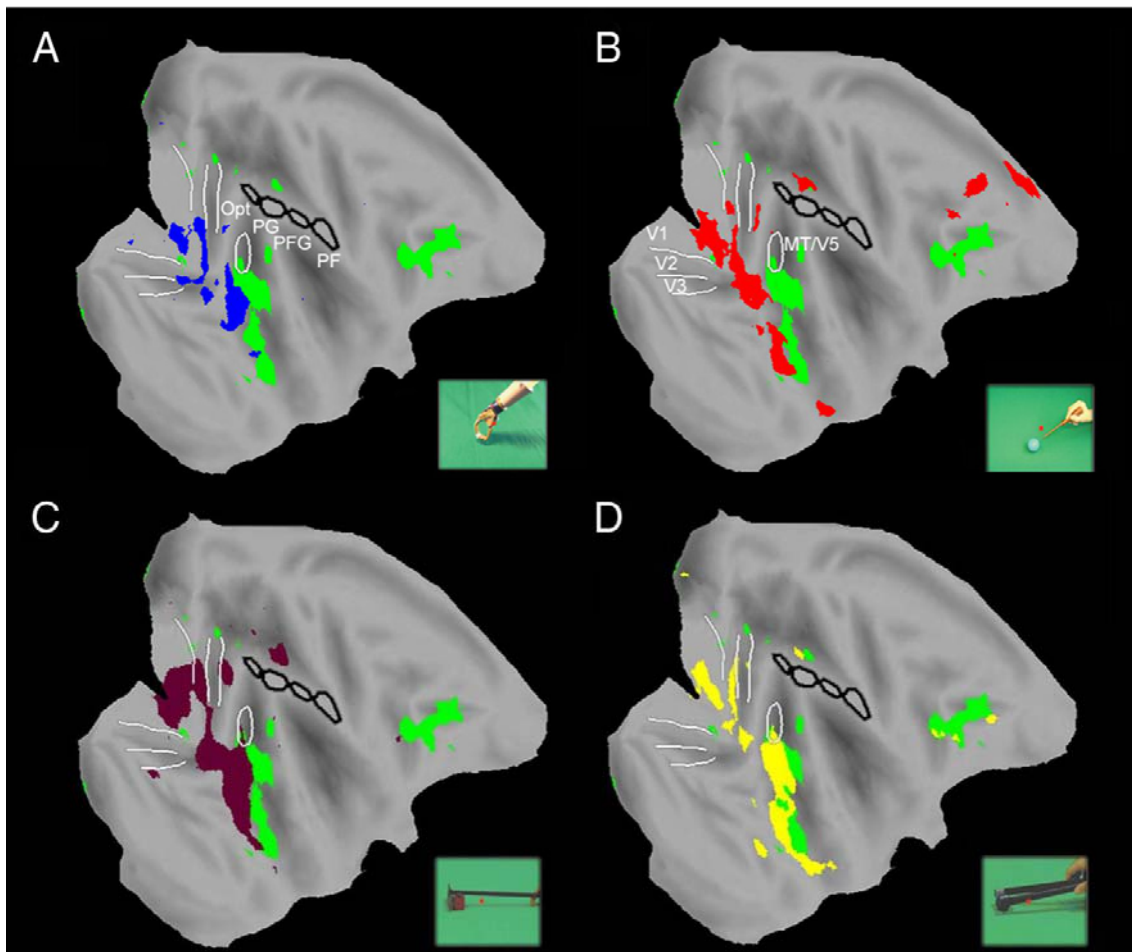
Figure 8. Interaction sites in the monkey. A–D, Flatmaps of the right hemisphere of monkey template (M12) brain (Caret software) showing regions significantly activated (fixed effects, $p < 0.05$ corrected) in the interaction in experiments 1–4 (same color code as in Fig. 7, 2 monkeys in experiments 1 and 3 monkeys in experiments 2 – 4). ROIs as in Figure .6

4.3 ROI Analysis

To ascertain whether there is a monkey parietal sector specific to tool action observation, we used a ROI approach which is more sensitive than a whole brain analysis. Based on cytoarchitectonics, anatomical connections and functional properties, we performed a detailed region-of-interest analysis on the inferior parietal lobule (IPL) part of cortex shown to be involved in coding goal-directed hand action. Within left IPL convexity we defined four ROIs, corresponding to areas PF, PFG, where hand mirror neurons have been reported in single neuron studies; (Gallese et al. 2002; Fogassi et al., 2005; Rozzi et al., 2008),PG (Rozzi et al 2008).

The results are shown in Fig. 9 *A*, *B*. Since the interaction differs from subject to subject, we calculated the magnitude of the interaction for each tool-subject combination by the formula (b-a)-(d-c), in which b and a are the MR signals in tool and hand action conditions respectively and d and c the signals in the corresponding static control conditions. The statistical analysis of tool-hand interactions, relative to their static controls, showed that out of 33 parietal interactions calculated, only two, PFG for rake in M14 and PG for pliers in M13, reached significance with the two paired t-tests used in the analysis of human aSMG (Fig. 9 *B*). This is close to chance, which predicts 1/20 false positives. To investigate the similarity between the occipitotemporal interaction sites of the monkey and the human IOG interaction sites, we also investigated the interactions for the tools separately from that for the mechanical arm. In monkeys the interaction site common to the three tools (Fig. 11*A*, white voxels) was located in TEO, in front of the mechanical arm interaction site (blue outlines), which involve predominantly V4, extending into posterior TEO. This arrangement is similar to that observed in humans if one takes into

account that the human data are averages over many more subjects and are smoothed more than the monkey data. In the TEO regions the interaction for the three tools arose mainly from a reduced response to static tools (Fig. 10*B*), as was the case in humans. Thus, the monkey occipitotemporal interaction sites bear close similarity to the interaction sites observed in human IOG.
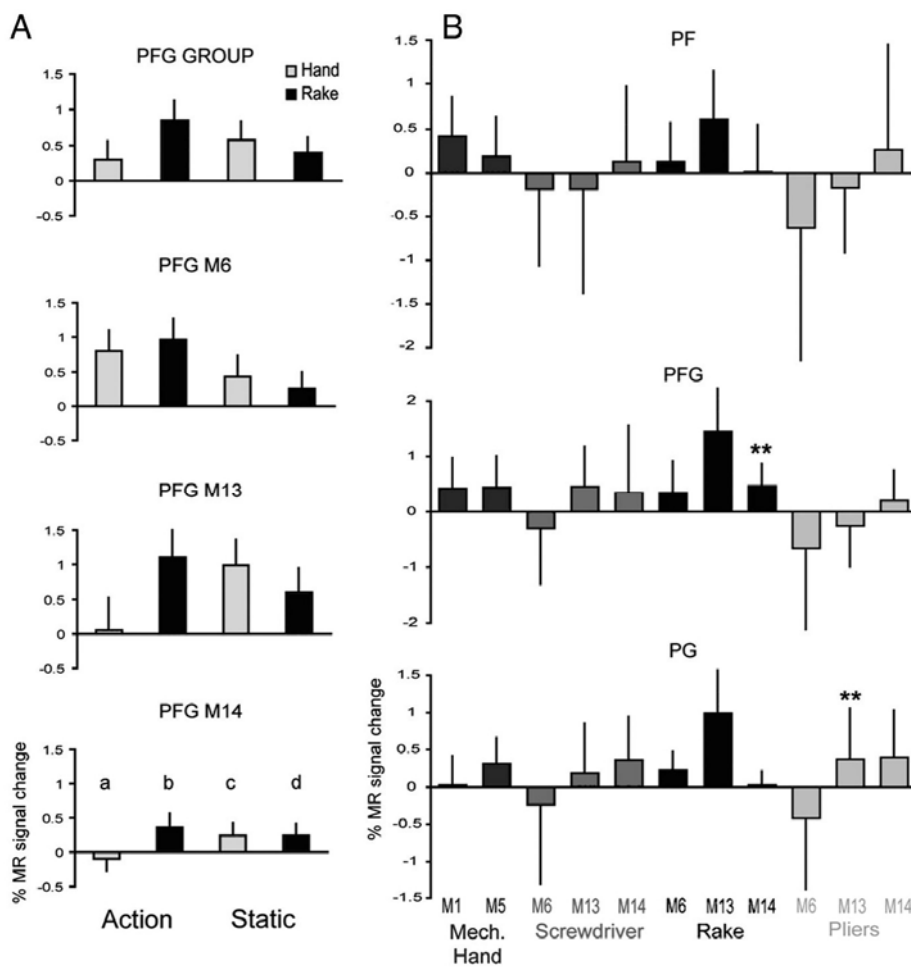

Figure 9. ROI analysis of naïve monkey IPL. A, Activity profiles in PFG (ROI analysis, experiment 3). B, Magnitude of the interaction (b – d)-(a - c) in PF, PFG and PG for different tools and monkeys. Stars indicate significant interactions.
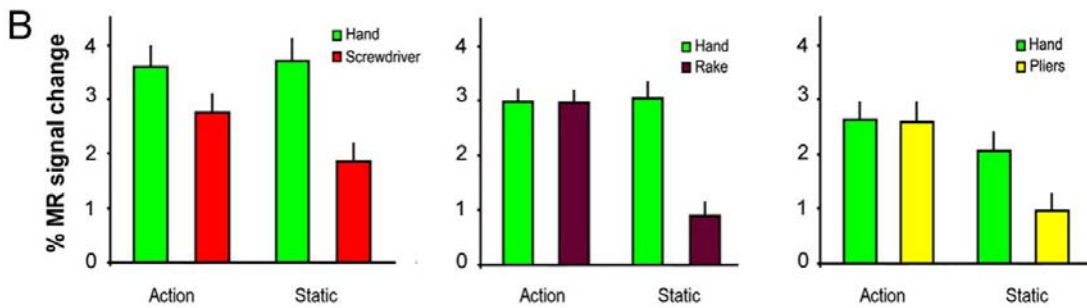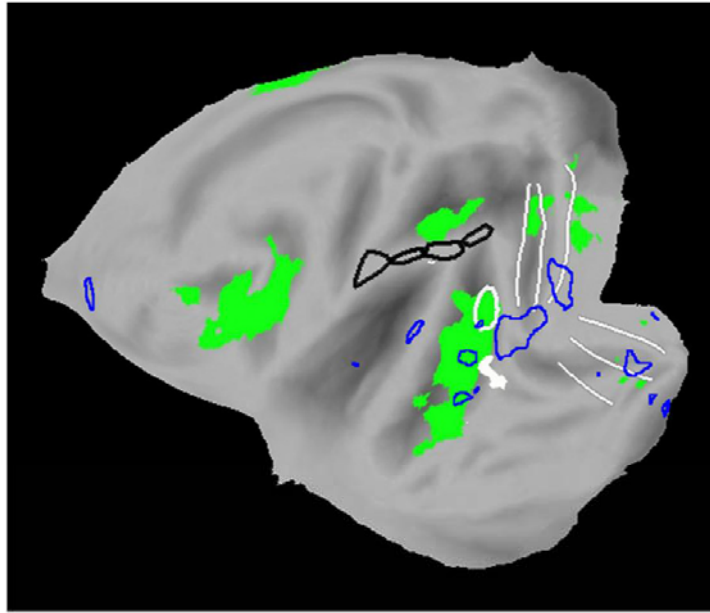
Figure 10. Occipitotemporal interaction sites in monkeys. A, Flatmap of left hemisphere of M12 showing the regions activated (fixed effect, p < 0.001) in the contrast viewing hand action minus viewing static hand (green voxels, experiment 1) and in the interaction for the mechanical hand actions (blue outlines, experiment 1) as well as the voxels common to the interactions for the 3 tools (white voxels, experiments 2–4); **B,** Activity profiles of the local maximum and 6 surrounding voxels in TEO (white area in A) in experiments 2–4.

4.3 Trained monkey fMRI experiments (experiment 5)

The negative results obtained in the five monkeys we tested might reflect the fact that macaque monkeys do not normally use tools or do so only rarely. Thus, to assess whether the activation observed in humans in the

rostral part of IPL might be the neural substrate of the capacity to understand tool use, we trained in experiment 5 two monkeys (M13 and M14) to use a rake, and later pliers, to retrieve food.

4.3.1 Behavioural and kinematics results

Both animals learned to use these tools proficiently (Fig 11 A, B) and were able to retrieve the food positioned in 5 different locations in the working space in front of them. M13 and M14 reached a score of 95% and 94% successful trials when using the rake and 97% and 76% when using the pliers, respectively. They were able to use tools even if these implements were presented rotated 180 or 90° (Fig12 A, B), except for M14 when tested with turned pliers (see Materials and Methods). Finally, the analysis of the action kinematics revealed stable motion trajectories with both tools for all food positions. Figure 13, *A* and *E*, illustrate the trajectories of the rake head when the monkey placed the rake beyond the piece of food and then pulled the rake back to retrieve the piece of food. For the central food position (green olive curves) a different starting position was used, somewhat more to the right than that used for all other food positions. In this way in order to retrieve the out of reach food in all five positions, it was required to the monkeys a movement of the forearm and elbow in a lateral direction. While the peripheral locations required monkeys to move the tool away from their bodies, the required movement in the central position was in the direction of the monkeys. Even though there were differences between monkeys, with M13 always using the left side of the rake to pull the food pellet back and M14 switching sides, the trajectories for both monkeys were extremely stable.

The variance was quite small, especially in the direction directly in front of the monkey. The first peaks of the speed diagrams (Fig. 13*B,F*) represent the higher speed reached during the reaching phase, it is followed from a decreasing of velocity corresponding to the approaching phase of the target. Then the velocities reached their lower value when the shaft of the rake was placed beyond the food or the pliers tip grabbed the food. The following increase of speed and the corresponding peaks represents the final pulling in phase. The speed diagrams also show a very consistent pattern across trials: both monkeys moved more quickly in the first phase (reaching phase) for the more peripheral food position (red curves). Figure 13*C* and *G*, illustrate the trajectories of the two tips of the pliers for the initial phase of movement toward the food. Trajectories are similar in the two animals and again show remarkably little variance. Note that M13, who mastered the use of tools slightly better than M14, consistently moved the tools more slowly than the other monkey: maximum speeds were lower and total duration of the action longer. To evaluate monkeys comprehension of the functional feature of the used tools, some useless objects (ring or spherical object) were presented during the previous described standard test. Since both monkeys immediately stopped trying to use these objects, this testing was performed only in the first session. In addition, when the tool was presented to the monkey not in its standard upright position, in the first session, both monkeys failed to grasp the tool, but from the second session on, they understood that they had to turn the tool to retrieve the food pellet and were able to do so in the 30 s allowed. For the rake, the percent correct was 95% and 85% for M13 and M14 respectively, but for the pliers only M13 reached 95% correct, whereas M14 never learned to use the rotated pliers.

Fig 11 (A), Frames showing M13 using the rake to retrieve out of reach food (B). The same monkey using the pliers to seize out of reach food.
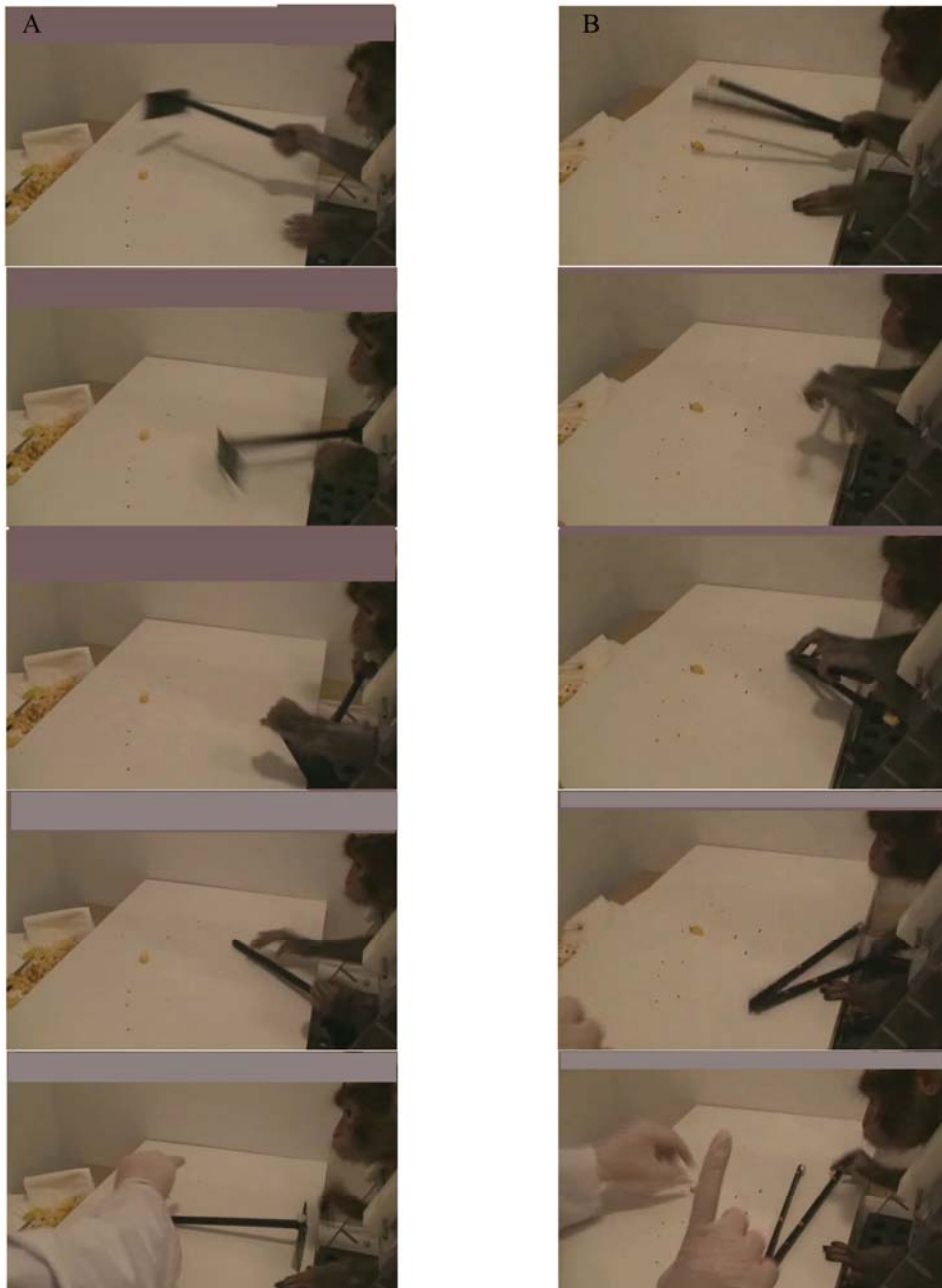
Fig 12 A, B After tools training monkeys were able to use tools even if these implements were presented rotated 180° or 90°. (A) M13 turning rake presented rotated 180°. (B) M13 turning pliers presented rotated 90°
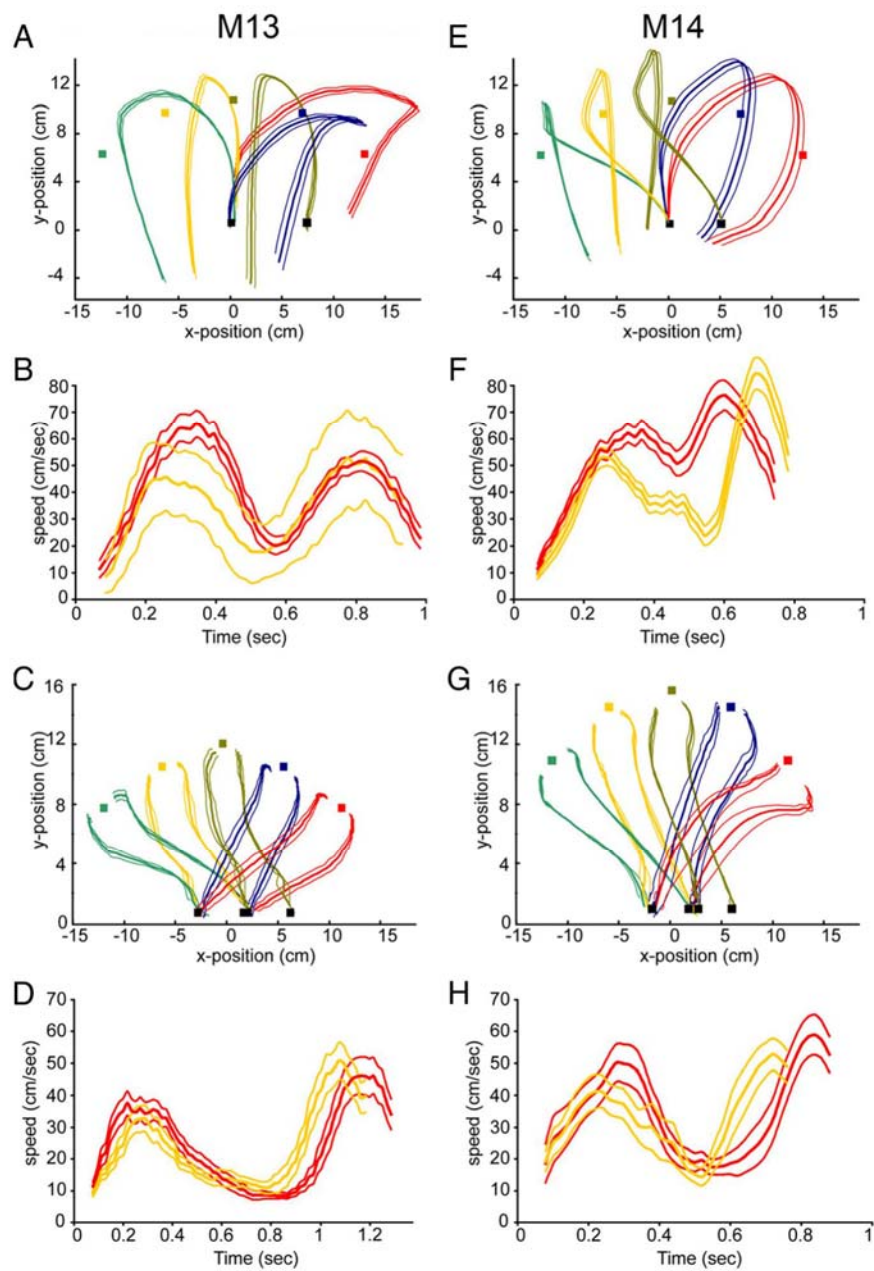
Figure 13. Effects of training in M13 and M14: kinematic analysis. A, C, E, G, Tool trajectories (mean ± 1 SD) for reaching and retrieving food of trained monkeys. Black squares: starting points. Colored squares: food position (5 directions: -45 to +45°). A, E, Trajectories performed by the tool when the rake was used M13 and M14. A marker was attached at the middle of the rake-head. The right starting point was used for all directions except when the food was placed in the central position. C, G, Trajectories performed by the tool when the pliers were used (M14). Markers were attached on the arms of the pliers, 3cm from the tip. B, D, F, H, Mean (1 SD) speed-time diagrams for two trajectories (-22.5° and +45°). Rake, M13 and M14 (B, F); pliers, M13 and M14 (D, H)

Once monkeys 13 and 14, already scanned before tool use training, mastered the use of tools, they were scanned again after rake use training, and after learning to use the pliers, using the same visual stimuli (tools use actions and hand actions) presented to the monkeys when they were naïve.

Comparing the activations, the interaction patterns were remarkably similar before and after training (Fig. 14), except for the presence of a clearly enhanced interaction between the observation of pliers action versus that of hand action after pliers training (Fig 14B). Yet, no voxels with significant interactions were observed in either PF or PFG, the anterior sectors of monkey IPL, for any of the tools after the trainings. This voxel-based analysis was complemented by a ROI analysis of left PF, PFG and PG similar to that performed in the naive animals. This analysis (Fig. 15) failed to reveal any significant post training interaction: in none of the ROIs did the interaction reach significance for the observation of actions with the tool, which the monkey had learned to use in the training just preceding the scanning. Overall, only three out of the 54 (2 X 3 X 3 X 3) tests performed in the two trained animals yielded a statistically significant interaction: two tests before training, already mentioned above, and an interaction for the rake in M13 after training with the pliers. This small proportion (3/54) is again close to the predicted chance occurrence of 1/20 false positives. This analysis clearly indicates that even after prolonged training there is no evidence for a tool-related region in the monkey comparable to human aSMG. Yet Figure 15 indicates that the tool vs hand interaction increases in the IPS, with training, especially that with of pliers, and might include some parts of the hand action observation circuit. Figure 16, shows that these interactions are weak in the anterior part of LIP, the most responsive part of the hand action

observation circuit. In fact the interaction is weaker than that observed in the human DIPSM, which is a plausible homologue of anterior LIP (Durand et al., 2009). Furthermore, factors other than tool-action specific mechanisms might account for whatever weak interaction present in the parietal regions of the monkey active during hand action observation. Given the similarity of grasping with the fingers and with the tip of the pliers, the latter type of grasping might have been more clearly understood after training and became therefore a more efficient stimulus. Alternatively, because of its difficulty, observing the use of pliers might have elicited a stronger attentional modulation of the MR activation compared with hand grasping. However, it is also fair to state that even if no interaction is observed in the fMRI, it remains possible that some parts of the lateral bank of monkey IPS house small proportions of neurons similar to those present in human aSMG. We cannot exclude that the training has induced neuronal changes (Hihara et al., 2006) resulting in the recruitment of additional parietal areas by the observation of tool action and that such neurons would escape detection if the proportions are small, because of the coarseness of fMRI (Joly et al., 2009). The proportion has to be small, because parietal neurons responsive to viewing of hand actions would respond to both hand and tool action observation and the presence of additional tool action specific neurons would create an interaction signal in the fMRI, which was weak .
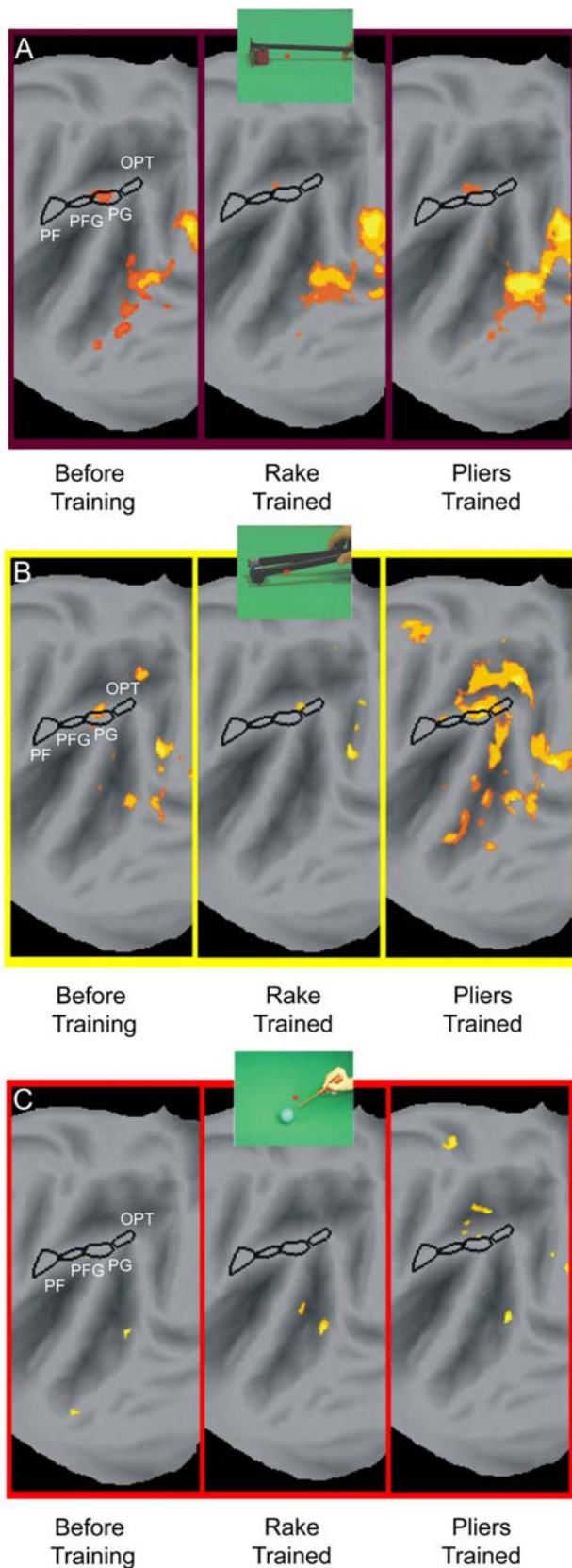
Figure 14. Effects of training: voxel-based analysis. A–C, SPMs showing significant interaction (M13,M14, fixed effects, p<0.001) of tool action versus hand action observation, relative to their static control, for observation of rake (A), pliers (B) and screwdriver (C) observation before training, after rake training, and after pliers training. The significant voxels (orange-yellow) are overlaid on flatmaps of the left hemisphere of monkey M12.
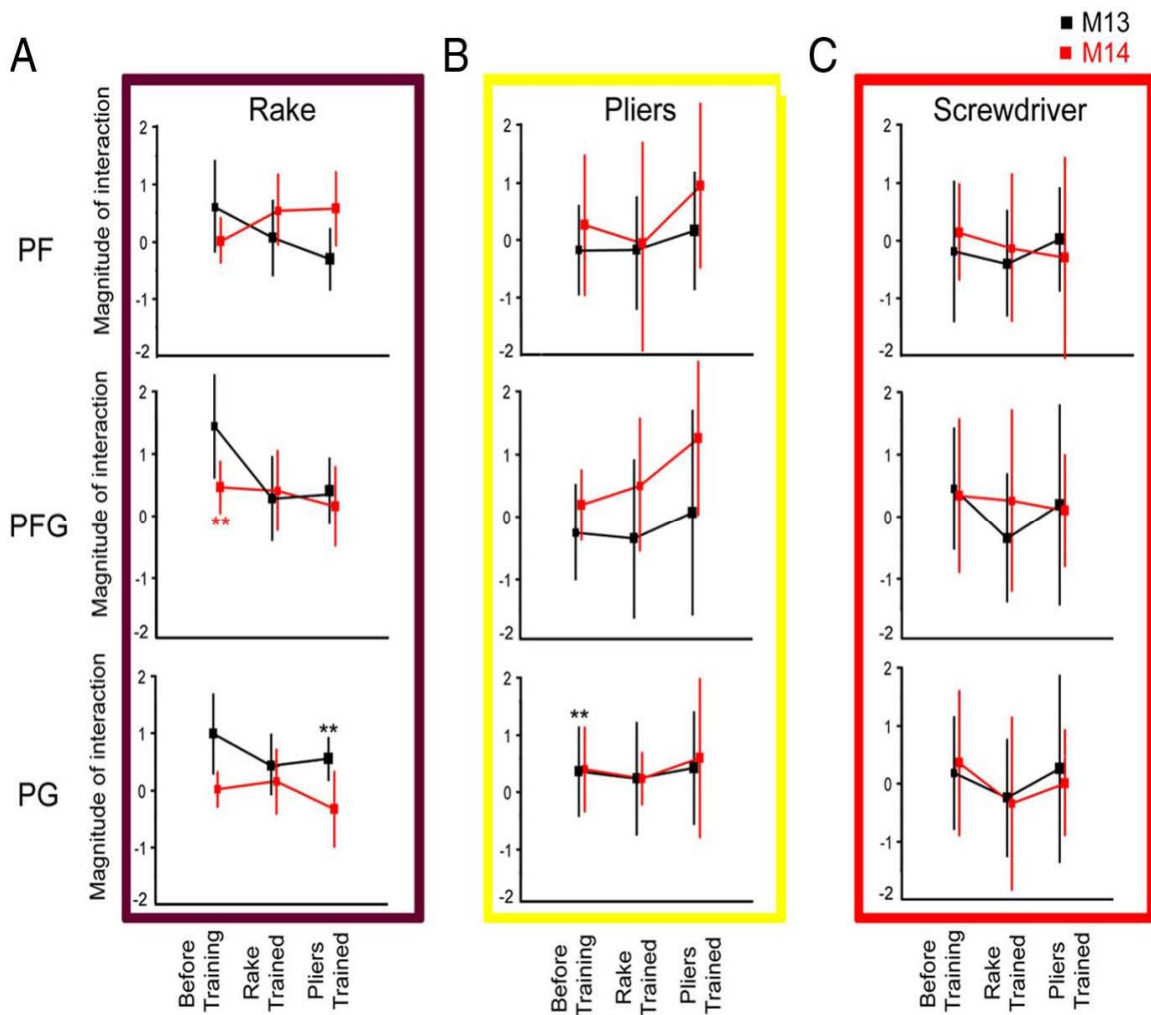
Figure 15. ROI analysis of the magnitude of the interaction (see Fig. 7 for definition) after training. A–C, Interaction magnitude is plotted as a function of epoch for the two subjects (M13, M14) in each of the three ROIs (PF, PFG, and PG) for rake action observation (A), pliers action observation (B) and the screwdriver action observation (C). Significant values (both paired t tests $p < 0.05$ corrected for multiple comparisons) are indicated by stars. Runs from A–C were tested interleaved at each epoch.

Figure 16. Interactions in the hand action observation circuit of humans and monkeys. A-B: Activity profiles of human DIPSM and monkey anterior LIP for the experiments in which observation of mechanical hand actions, screwdriver actions, rake actions and pliers actions are compared to hand action observation. 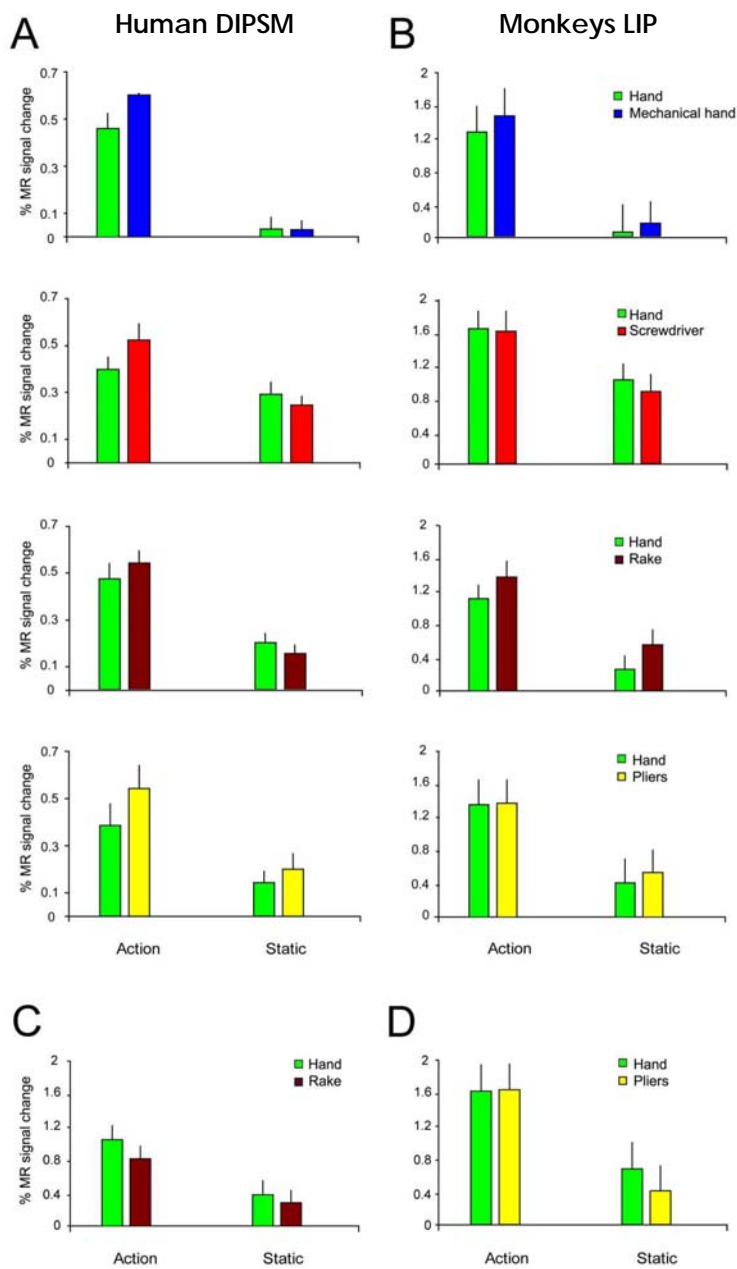C-D: profiles of anterior LIP for the observation of rake actions after rake training (C) and for observation of pliers' actions after pliers training (D).Profiles were calculated for small ROIs (27 voxels in humans and 7 voxels in monkey) centered on the local maxima of the hand action observation (compared to static hand). Small differences in the number of voxels included in the ROIs have little effect on the profiles. Interactions were significant (paired t-test, $p<0.05$) in DIPSM for mechanical hand and screwdriver. Notice that the interaction in anterior LIP after pliers training results from a difference in control condition not experimental condition.

5.Discussion

The first finding of the present study is that in naïve monkeys, the observation of grasping actions performed with simple tools activates a parieto-frontal circuit also active during the observation and execution of hand grasping movements (Bonini et al 2010, see Rizzolatti and Craighero, 2004). In the present experiments, we used a contrast agent increasing the contrast-to-noise ratio fivefold (Vanduffel et al., 2001). Furthermore, we used a ROI approach which is more sensitive than a whole-brain analysis (Nelissen et al.,2005), and we had precise indications about the ROI locations (Fogassi et al., 2005; Rozzi et al., 2008). The similarity between the interaction in human IOG and monkey TEO/V4 regions indicates that our fMRI technique in the monkey is sensitive enough. The activation of the parieto-frontal circuit is consistent with the neural circuit described by Obayashi during active tool use (Obayashi et al., 2001). The author reported a significant task-related activation in the controlateral IPS, in the controlateral ventral premotor cortex (F5) and bilateral TEO. Indeed further neuroimaging studies carried out in the macaque showed that the observation of goal-directed motor-acts activate, the premotor, cortex and part of prefrontal area 45 (Nelissen et al., 2005, Nelissen et al., in press). Our premotor activation was mainly located in the posterior bank of the inferior arcuate sulcus, the absence of activation of the convexity of F5 is in accord with Nelissen et al 2005, confirming that this sector of the ventral premoror cortex required a more contestualized visual information (i.e. needs that the whole agent performing the action is visible, and not only his/her hand). The absence of activation in all four IPL areas (PF, PFG, PG and Opt) during hand action observation could apparently be at odds with single cell studies showing

that area PFG contains mirror neurons (Gallese et al, 2002; Fogassi et al 2005, Rozzi et al 2008; Bonini et al 2010). However a recent monkey fMRI study (Nelissen et al., in press) carried out by using 1.5 T scanner shows that PFG was significantly activated during the observation of video-clips showing a full view of a person performing a grasping action but not when monkeys observed hand and forearm performing the action (corresponding to the stimuli used in the present study). The same authors, using a higher field scanner (3 T), were able to detect in PFG a significant activation for hand action observation even if weaker than that recorded during the observation of action performed by full person view. The second results of the present study indicate that the activation of the above mentioned circuit occurs regardless of how the observed action is performed. In particular no activation for a given tool-action was observed in the IPL of naïve monkey. Two monkeys were scanned after tools use training; the interaction patterns were remarkably similar before and after training. The performance levels and the kinematic analyses clearly indicate that the two monkeys were well trained to use the rake and the pliers. In fact, as reported by Ishibashi and colleagues (Ishibashi et al 2000), in the early stage of tool-use training the corresponding tool-head path was accomplished with two different movements, pushing and pulling in, that were independently performed in a step-like fashion. Further training resulted in an integration of the two movements in a single fluent smooth trajectory, as it was observed in the late phase of training in Ishibashi experiment. Even if, the evidence for a species difference related to IPL activation by observation of a tool action appears to be conclusive, it must be stated that the training of our monkeys was relatively short when compared with the extensive human experience with tools. It is not known whether longer training, with more tools, would

alter the results, even though the new cortical connections, reported after tool use training, were observed in Hihara work (Hihara et al., 2006) by injection carried out after 3 weeks of training when monkeys became over-trained for the task. This period corresponds to the time of training of our monkeys. Note also that since monkey intraparietal regions responded to observation of both hand and tool actions, we cannot exclude that these regions house small numbers of tool-action-specific neurons that escape detection by fMRI. The results of the comparative studies carried out on volunteers indicate that actions observation activates a parieto-frontal circuit (Binkofski et al., 1999; Ehrsson et al., 2000; Buccino et al., 2001; Grèzes et al.,2003; Manthey et al., 2003; Gazzola et al., 2007). More importantly, however, in humans the observation of actions performed with these simple mechanical devices also activates a specific sector of the IPL, the aSMG (Peeters et al.,2009). No equivalent tool-action specific activation was observed in monkey IPL, even after extensive training. The human aSMG activation found in the comparative study is distinct from the parietal activation sites observed during the static presentation of tool images (Chao and Martin, 2000; Kellenbach et al., 2003; Creem-Regehr and Lee, 2005) in posterior IPL, i.e., in the regions active during the observation of hand grasping. A possible explanation is that static pictures of tools, or even their translational motion (Beauchamp et al., 2002), activated the representations of how those tools are grasped as objects rather than the cognitive aspects related to their actual use. This view is supported by the study of Valyear et al. (2007) that describes an area, just posterior to left AIP, selectively activated during tool naming. In agreement with our findings, these authors explicitly posit that two left parietal regions are specialized for tool use: a region they describe behind AIP, involved in the planning of skilful grasping of tools, and a more

anterior region in the SMG related to the association of hand actions with the functional use of the tool. There is no direct evidence concerning the motor properties of aSMG. However, it has been reported that SMG is activated during preparation for (Johnson-Frey et al., 2005) and pantomimes of tool use (Moll et al., 2000; Rumiati et al., 2004; Johnson-Frey et al., 2005) (for review see Lewis, 2006; Króliczak and Frey, 2009) as well as during the manipulation of virtual tools (Lewis et al., 2005). It is, therefore, plausible that aSMG constitutes a node where the observation of tool use is matched to their use. Thus, as hand action observation triggers the parieto-frontal hand grasping circuit, in humans the particular type of movements required to operate tools appears to activate in addition aSMG which codes specific motor programs for tool use. This view is supported by the activation of aSMG by the sounds of tool use (Lewis et al., 2005). The homology between human and monkey parietal lobe is under dispute. Brodmann (1909) suggested that the homologues of the two main cytoarchitectonics subdivisions of monkey posterior parietal lobe (areas 5 and 7) are both located in the human superior parietal lobule and that human IPL (areas 40 and 39) is an evolutionary new region. However, this view is difficult to accept given that the intraparietal sulcus is an ancient sulcus, already present in prosimians (see Foerster, 1936). Hence, recent literature has adopted also for the monkey the nomenclature of (Von Economo, 1929), naming the monkey IPL areas with the same terms as in humans: PF and PG (Von Bonin and Bailey, 1947; Pandya and Seltzer, 1982). The present findings suggest that while the IPL sector around the intraparietal sulcus is functionally similar (as far as hand manipulation is concerned) in monkeys and humans, the rostral part of IPL could be a new human brain area that may not exist in monkeys. We propose that this region proper to humans underlies a specific way for

understanding tool actions. While the grasping circuit treats actions done with a tool as equivalent to hands grasping objects, aSMG codes the tool actions in terms of causal relationships between the intended use of the tool and the results obtained by using it. This type of coding, whether specific to tools or more general, represents a fundamental evolutionary cognitive leap that greatly enlarged the motor repertoire of humans and, therefore, their capacity to interact with the environment. The fact that monkeys learned to use simple tools such as a rake or pliers (Iriki et al., 1996; Ishibashi et al., 2000; Umilta` et al., 2008) does not necessarily imply an understanding of the abstract relationship between tools and the goal that can be achieved by using them. Indeed it has been shown that even if chimpanzee, considered the most capable tool users among primates, can perform well a task in which they have to chose between an intact versus a broken rake handle, nevertheless they fail when effortless variation are introduces in this or in other paradigms (Povinelli 2000). Furthermore their performance is not much better than chance when they are required to chose between pulling a rake in normal conditions versus one condition in which the food could fall in a well without being reached (Povinelli 2000). If given a choice between a functional rake and a modified rake with a rubber made shaft, that make the tool ineffective, they perform not better than at chance (Povinelli 2000). This evidences would suggest that they do not understand the causal relationship between self, tool and goal object. Taking into account the homologies between the parieto-frontal system in human and monkeys this seems to be incongruent. Povinelli argues that the common features exhibited in human and primate behaviour, including also tool use, may be due to the fact that both species rely on similar sensorimotor abilities. However the behavioural results mentioned above would indicate that chimpanzee,

differently from human, lack the ability to understand the abstract causal variables that rule objects and their relationships with the external environment; such an understanding appears during early infancy in human (Spelke et al., 1992). As a consequence, chimpanzee often fail at task, demanding the use of an internal model of causal relationship to predict which of the two options will permit to reach the desired effect. Actually there are no more data concerning the neural mechanism that could be involved in the comprehension of physical causality for tool use. In human it has been shown that patients with frontal/prefrontal injuries show deficit when are required to plan and execute goal oriented actions (Shallice 1982). Following neuroimaging studies demonstrated the role of frontal/prefrontal areas in this processes (Fincham et al 2002). On the other hand, further data suggest that these regions, even if involved in physical causality processes, may not be critical for it. Instead, internal models of objects' physical characteristics and causal relations could be assembled in temporal and/or parietal cortex associated with higher-level perceptual processing. It has been proposed that temporal cortex of monkeys could be involved in coding of the causal relationships between hands and other objects. Perret and colleagues showed that cells within the lower bank of the superior temporal sulcus (STS, area TEa) respond selectively to the observation of movements of objects caused by hands (Perrett et al., 1990). Responses are reduced when objects and hands move congruently but without making physical contact. Furthermore, neuropsychological studies have produced data indicating a left parietal and/or temporal cortex involvement in processes critical to reasoning about causal relationships concerning tools and goal objects. Goldenberg and Hagmann (1998) showed that left brain-injured patients (parietal lesion were significantly more frequent) make errors when

required to select a new tool to perform a particular task choosing on the basis of the tool physical properties, while patients with right brain-injuries could perform correctly the task. Crucially, frontal lobe lesions did not affect this performance.

Rake, or the pliers use in monkeys, might simply become, with training, a prolongation of the arm, as shown by the response properties of neurons recorded from the medial wall of the IPS of trained monkeys (Iriki et al., 1996; Hihara et al., 2006). Hence, monkeys can rely on the hand grasping circuit to handle the tool (Obayashi et al., 2001), although this circuit may include some neuronal elements providing some primitive representation of causal relationships. Indeed it has been hypothesized that animate and inanimate conceptual categories correspond to evolutionarily adapted domain-specific knowledge systems that are subserved by different neural mechanisms. This idea is based on the concurrence of deficits for specific discrete categories (e.g. animals or plants) in brain damaged subjects. Alltogether these findings lead to the assumption that the meanings of the elements of these categories are distinguished primarily by their visual properties, whereas the members of other categories (e.g., tools, etc.) are recognized primarily by their functional properties. This is supported by three PET studies that directly compared the performance for living things and artefacts (Damasio et al., 1996; Martin et al., 1996; Perani et al., 1995). these authors mapped brain activity in normal volunteers (Martin et al., 1996; Perani et al., 1995) and in patients with brain lesions during the visual recognition of living (animals) and non-living (artefacts) stimuli The results of these studies demonstrate a segregation between categories. Even if the specific areas implicated vary across studies, there is some agreement among them. The inferior temporal lobe is activated in processing animals; the posterior middle temporal area may be more important for

tools. Our results suggest that the capacities displayed by monkeys tool-users may not be the results of a specialized structures for tool-use, as a domain-specific view of these capacities might predict. Instead, it seems that non-human primates reason about the functional properties of tools using more domain-general mechanisms. Macaques separated from the ancestors of humans about 25 million years ago. Recent findings concerning the development of tool use during hominid evolution allow us to speculate about the moment at which the parietal region related to tool use emerged. The earliest evidence for hominid tool technology are the sharp-edged flakes of the Oldowan industrial complex [2.5 Million years ago (Susman, 1994; Roche et al., 1999)]. During the Acheulian industrial complex, dating back ~1.5 Ma (Asfaw et al., 1992), large cutting tools were manufactured by Homo erectus and possibly Homo ergaster. Their diversity (cleavers, picks) suggest that these early humans had the ability to represent the causal relationship between tool use and the results obtained with it. Thus the emergence of a new functional area in rostral IPL may have occurred at least 1.5 million years ago (Ambrose, 2001). It may have emerged even earlier, during the Oldowan industrial complex or when apes diverged from monkeys. Apes use tools readily (Whiten et al., 1999) and modify herb stems to make them a more efficient tool, which implies that they have a template of the tool form (Sanz et al., 2009). Causal understanding of tool use, however, may require more than a template as it implies the integration of visual information into specialized motor schemata (Povinelli, 2000; Martin-Ordas et al., 2008). In conclusion, the description of a region in the human brain, the aSMG, specifically related to tool use sheds new light on the neural basis of an evolutionary new function typical of Homo sapiens. Neurons specifically related to tool use might already be present in monkeys

dispersed in the hand action circuit and hence not be detectable by the fMRI. The appearance, however, in humans of a new functional area, in which neurons with similar properties may interact, could have enabled the appearance of new cognitive functions that a less structured organization could hardly mediate.

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