

Exploring the neural basis of cognition: multi-modal links between human fMRI and macaque neurophysiology

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Although functional magnetic resonance imaging (fMRI) with sophisticated behavioral paradigms has enabled the investigation of increasingly higher-level cognitive functions in humans, these studies seem to lose touch with neurophysiological studies in macaque monkeys. The application of fMRI and other MRI-based techniques to macaque brains allows studies in the two species to be linked. fMRI in human and macaque subjects using equivalent cognitive tasks enables direct comparisons of the functional brain architecture, even for high-level cognitive functions. Combinations of functional or structural MRI and microelectrode techniques provide ways to explore functional brain networks at multiple spatio-temporal scales. These approaches would illuminate the neurophysiological underpinnings of human cognitive functions by integrating human functional neuroimaging with macaque single-unit recordings.

Introduction

The macaque monkey is one of the most valuable animal models for neurophysiological investigations of human cognitive functions. Many neurophysiological and anatomical findings in the study of macaque brains have been discovered using invasive techniques, such as microelectrode recordings, tracer injections, microstimulations, drug injections and experimental lesions [1–4]. By contrast, the development of functional brain imaging methods has enabled the mapping of human brain activation as it relates to specific cognitive functions. In particular, functional magnetic resonance imaging (fMRI) [5], which uses blood oxygenation level-dependent (BOLD) signals as an indirect measure of neuronal activity, has become a major technique in the investigation of human cognitive functions in terms of brain activation. fMRI studies in humans began with the investigation of sensory and motor functions and, with the application of sophisticated behavioral paradigms based on experimental psychology, they have since been used to explore internal states and high-level cognitive functions, such as learning, memory, attention, emotion, language and even social cognition [6–9].

In this context, it seems that research on human brain function is losing touch with macaque neurophysiological studies, which are essential models for elucidating the electrophysiological underpinnings of human cognitive functions. Since the late 1990s, neurophysiologists have begun to apply fMRI and other MRI-based techniques to animal models, including rats, cats, rabbits and monkeys [10–17]. In particular, fMRI in macaque monkeys has the potential to link findings from human fMRI to those from macaque neurophysiology. Using the same measure, this approach enables direct comparisons of human and macaque brain activation, not only in sensory systems but also in high-level cognitive functions. Furthermore, combined with microelectrode techniques, fMRI and other MRI-based techniques used in the study of macaques enable multi-dimensional investigations of brain functions that are impossible in humans. The aim of this article is to review recent attempts to apply fMRI and other MRI methods to macaque brains and discuss how these methods can advance our understanding of the neuronal mechanisms that are involved in cognitive functions.

Comparative fMRI in humans and macaques

Comparison and integration of the findings from human fMRI studies with findings from macaque studies with traditional invasive methods are important for obtaining a more precise view of the neuronal mechanisms that underlie human cognitive functions. However, attempts to compare and integrate such findings have not always been straightforward because of species and methodological differences. One direct way to bridge this gap is to conduct ‘comparative fMRI’ experiments, which can be achieved by performing fMRI scans in human and monkey subjects under the same experimental paradigms (Table 1). The visual system is an ideal target for functional comparative studies because studies of macaque visual cortices are more advanced than those of other macaque brain systems. Furthermore, there are remarkable similarities between human and macaque visual systems. Indeed, most comparative fMRI studies of humans and macaques have focused on the visual system [18–26] (Table 1). These studies have indicated that the functional

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Table 1. Comparative fMRI and DT-MRI tractography studies in humans and macaques^a

Studies	Major activated and compared brain regions		Task condition
	Humans	Macaques	
fMRI studies			
Dubowitz <i>et al.</i> [18]	Striate and extrastriate visual areas	Striate and extrastriate visual areas	Passive viewing of movies
Nakahara <i>et al.</i> [36]	Posterior IFG	Anterior bank of the irAS	Cognitive set-shifting
Vanduffel <i>et al.</i> [19]	V2/V3, V3A, LOS, hMT/V5, IPS	V2, V3, V4, MT, FST	3D perception
Kourtzi <i>et al.</i> [20]	V1, V2, V3A, V4V, LOC	V1, V2/V3 (in anesthetized macaques)	Integration of local image features
Tsao <i>et al.</i> [21]	V3A, V7, V4d, caudal parietal	V3, V3A, CIPS	Stereopsis
Tsao <i>et al.</i> [67]	Fusiform gyrus	STS	Face perception
Koyama <i>et al.</i> [51]	PrCS, PrCS/SFS, SPL	FEF, PMv, PMd, LIP, DP	Visually guided saccade
Denys <i>et al.</i> [22]	LOC, V3A, IPS	V3d, V4, STS, TEO, TE, IPS, LIP	Perception of symmetric shape
Denys <i>et al.</i> [23]	IFG	irAS, principal sulcus	Visual responses
Sasaki <i>et al.</i> [24]	V3A, V4, V7, LO	V3A, V4d	Perception of symmetric shape
Sawamura <i>et al.</i> [25]	LOC, IPS	Inferior temporal cortex, IPS	Adaptation and size invariance of shape processing
Sasaki <i>et al.</i> [26]	Early visual areas	Early visual areas	Visual-orientation sensitivity
DT-MRI tractography			
Parker <i>et al.</i> [54]	Cerebral peduncle, optic radiations		
Croxson <i>et al.</i> [55]	Prefrontal cortex		
Ramnani <i>et al.</i> [56]	Prefrontal inputs to the cortico-pontine system		

Abbreviations: CIPS, caudal intraparietal sulcus; DP, dorsal prelunate; DT-MRI, diffusion tensor magnetic resonance imaging; FEF, frontal eye field; FST, fundus of superior temporal; IFG, inferior frontal gyrus; IPS, intraparietal sulcus; irAS, inferior ramus of the arcuate sulcus; LIP, lateral intraparietal; LO, lateral occipital; LOC, lateral occipital complex; LOS, lateral occipital sulcus; PMd, premotor dorsal; PMv, premotor ventral; PrCS, precentral sulcus; SFS, superior frontal sulcus; SPL, superior parietal lobe; STS, superior temporal sulcus; TE, inferior temporal area; TEO, inferior temporal area.

^aOnly studies that applied fMRI to both humans and macaques using equivalent task conditions are listed.

architecture of the two species is generally similar in early visual areas, but there are significant discrepancies in the higher visual areas (for reviews of these studies, see Refs [27–29]).

Beyond the sensory areas, direct functional comparisons can also be made between humans and macaques in higher-order association areas [4,16] by conducting fMRI scans in human and macaque subjects while they perform the same high-level cognitive tasks (Box 1). This approach is particularly promising for investigations of the prefrontal and posterior parietal cortices, where inter-species comparisons have relied mainly on cytoarchitectonics or sulcal landmarks. Below we review some recent comparative fMRI studies that focus on these brain areas.

Comparative brain imaging of prefrontal functions

The prefrontal cortex (PFC) is an association area that is evolutionarily more highly developed in primates than in other species. Neurophysiological studies in macaques and imaging studies in humans have shown that the PFC is involved in various executive functions such as working memory, action planning, response inhibition and retrieval of long-term memory [30–35]. However, correspondences in the functional architecture of the PFC in humans and macaques are poorly understood. In a functional comparison of the PFC in humans and macaques, Nakahara *et al.* [36] used fMRI to focus on one of the central functions of the PFC – flexible adaptation to changing circumstances (Figure 1). An essential cognitive component that is required in flexible adaptation is ‘cognitive set-shifting’, which can be efficiently examined using the Wisconsin card-sorting test (WCST), a sensitive detector of dysfunctions in patients who have damage in the PFC. In this test, subjects are required to shift intermittently from one behavioral pattern (cognitive set) to another, to adapt to changing circumstances. The performance of cognitive set-shifting in the WCST is often impaired in both humans and macaques that have damage in the PFC [37–39]. Nakahara

et al. conducted event-related fMRI in human and macaque subjects while they performed a modified WCST [36] (Figure 1a). fMRI scans that were conducted while the subjects performed the task revealed brain activation related to cognitive set-shifting in the rostral bank of

Box 1. fMRI and awake, behaving macaques

Functional magnetic resonance imaging (fMRI) is the most widely used non-invasive brain imaging method for investigating brain activation in human subjects, using blood oxygenation level-dependent (BOLD) contrast as an indirect measure of neuronal responses. There are some unique technical challenges that apply to awake macaque subjects, compared with human subjects [12,13,16,17]:

- (i) All devices that are necessary for awake macaque experiments (such as head holders, reward delivery systems and response devices) must be made from MR-compatible materials, and their effects on fMRI images must be evaluated before the experiment.
- (ii) Because the volume of macaque brains is much smaller than human brains, higher static magnetic field (3 Tesla or higher) is desirable to obtain good spatial resolution and signal-to-noise (S/N) ratios. Intravenous injections of iron-oxide contrast agents, such as monocrySTALLINE iron-oxide nanoparticle (MION), enhance the S/N ratio and improve signal localization in functional imaging, even in the conventional (1.5 Tesla) magnetic field [13].
- (iii) Susceptibility artifacts in gradient-echo echo-planar imaging (GE-EPI), a popular MRI sequence used in functional imaging, are more severe in studies of macaque brains, presumably because macaque brains are smaller in volume and larger in curvature compared with human brains. Strong gradient power and excellent shimming are required in studies of macaque brains to obtain GE-EPI images of satisfactory quality.
- (iv) Unwanted excess body movements by macaque subjects can cause motion artifacts in MR images. Behavioral shaping is required to keep macaque subjects motionless during fMRI scans. For example, macaque subjects can be trained to place their hands and feet on ‘home positions’ during an fMRI scan. Some researchers use specially designed motion-detection devices to abort trials that are contaminated with unwanted limb or jaw movements [17].

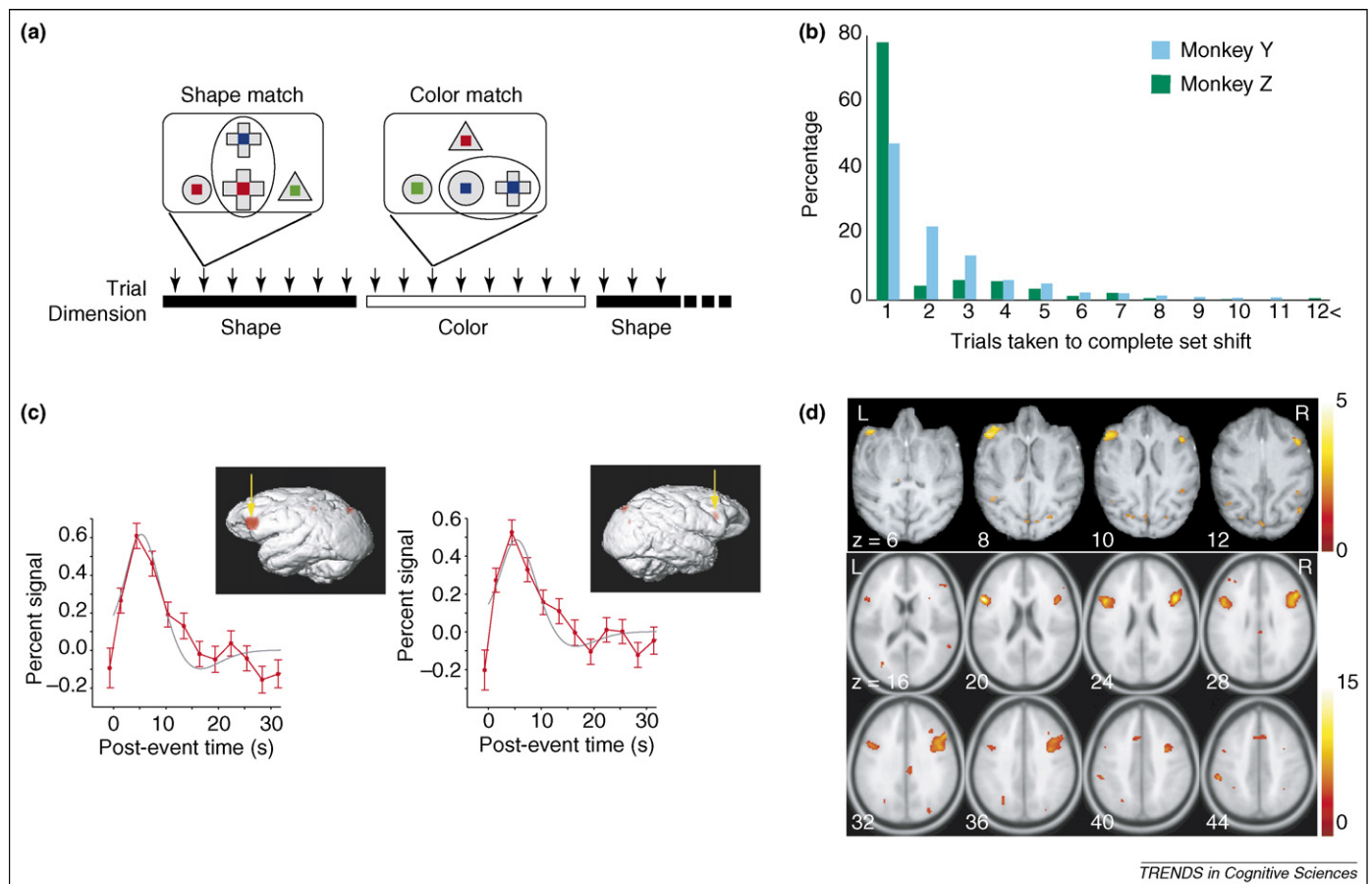


Figure 1. Comparative fMRI of prefrontal function in humans and macaques [36]. **(a)** Macaque monkeys were trained on a modified version of the Wisconsin card-sorting task (WCST). On each trial, subjects were presented with a center cue stimulus and three surrounding choice stimuli. Each stimulus consisted of a colored square that was superimposed onto a gray shape and exhibited two stimulus dimensions: color (red, blue or green) and shape (circle, triangle or cross). Subjects were required to respond to one of the three choice stimuli by matching the attributes of the cue stimulus. However, only one dimension (color or shape) was considered relevant when determining the correct match at a given time during the task. At the end of each trial, visual feedback was delivered to the subjects that indicated whether the matching was correct. After six to eight successive correct matches, the currently relevant dimension was changed to the other dimension without notice (dimensional change). The subjects had to shift their cognitive set to resume correct matching, based on the feedback stimuli, and adapt to the other dimension (cognitive set-shifting). Event-related fMRI scans were conducted in human and macaque subjects while they performed the modified WCST. **(b)** Behavioral performances of macaque subjects. The histogram shows the percentage of set-shiftings sorted by the number of trials taken to complete set-shifting. More than 80% of set-shiftings were completed within three trials. **(c)** Time course of BOLD activation in the macaque bilateral ventral prefrontal cortex related to cognitive set-shifting. **(d)** Direct comparison of brain regions that showed activation related to cognitive set-shifting in humans and macaques. The upper row shows activation in macaque monkeys. The middle and bottom rows show activation in humans. The z coordinate in bicommissural space is indicated at the bottom of each image (see Refs [36,51]). Panels (a), (c) and (d) were adapted, with permission, from Ref. [36].

the inferior ramus of the arcuate sulcus in macaques (Figure 1c,d). In humans, prominent activation related to cognitive set-shifting was found in the posterior part of the bilateral inferior frontal sulcus, which is consistent with previous imaging studies [40] (Figure 1d). Thus, this comparative fMRI experiment revealed a common set-shifting-related activation in the caudal part of the inferior PFC in both humans and macaques (Figure 1d). These activation foci largely correspond to areas 45/posterior 12 of Walker's area or areas 45B/45A/44 of the Petrides and Pandya areas in macaques. In humans, these activation foci correspond to Brodmann's area (BA) 44/45. These results suggest that cytoarchitecturally equivalent regions in the two species are also possible functional equivalents with regards to cognitive set-shifting.

Macaque fMRI has revealed another aspect of functional organization in the PFC. Previous single-unit studies have shown that a class of neurons in the macaque ventral premotor cortex (F5c) discharges during execution of action (e.g. grasping) and during observation of the same action when performed by other individuals [41,42]. These

neurons are known as 'mirror neurons' and their activities probably represent observed actions and internal motor representations [41]. Human fMRI studies have shown that observation of the actions of another individual activates wider frontal regions than seen in macaques, including not only premotor area 6 but also prefrontal areas 44 and 45 [41]. In a recent macaque fMRI study, Nelissen *et al.* [43] tried to obtain a more detailed view of how the actions of others are represented in the macaque frontal cortex across premotor area F5 and adjacent prefrontal regions. They showed that macaque prefrontal areas 44 and 45, as well as area F5, were activated by observations of the actions of others. Further analyses indicated that the degree of abstraction in the representation of action observation progressed from premotor area F5c to prefrontal area 45B. For example, area F5c responded only to the observation of actions when there was a full view of the subject who was grasping something but not to the observation of a grasping hand alone. By contrast, area F5a responded to the observation of a grasping hand alone. Furthermore, F5a responded to the observation of grasping

by a robot arm and action mimicking, whereas area F5c did not. In the PFC, area 45B was activated not only by the observation of actions but also by the observation of graspable objects. In humans, BA 44 and 45 show activation that is associated with the observation of another individual's actions, and this area seems to correspond to Broca's area. This apparent overlap leads to an attractive but debatable hypothesis that the linkage between action execution and observation might be a basis for the function of communication and, hence, might provide a possible explanation of the evolution of language [42].

Comparisons of oculomotor-control brain networks

The brain areas that are responsible for controlling saccadic eye movements constitute a characteristic network across frontal and posterior parietal parts of primate brains [44–50]. However, controversy surrounds the functional correspondence of these networks in humans and macaques. For example, the frontal eye field (FEF), a frontal center that is responsible for saccadic eye movement, has been assigned to BA 8 using neurophysiological and microstimulation studies [45,47], whereas, in humans, FEF has been assigned to BA 6 using functional imaging studies [48,49]. In the posterior parietal cortex, oculomotor functions have been implicated in lateral intraparietal areas in macaques [44,46] and multiple areas in the superior parietal lobule (SPL) in humans [50]. Koyama *et al.* [51] conducted a comparative fMRI study while human and macaque subjects performed identical visually guided eye-movement tasks to elucidate functional correspondences of oculomotor cortical networks between the two species. As expected from previous findings, multiple-activation foci that are related to saccadic eye movement were found in the frontal and posterior parietal cortices in both species. In the macaque frontal cortex, activation peaks were observed in the FEF, premotor areas (BA 6) and the posterior end of the principal sulcus (BA 45/8). In humans, activation peaks were found along the precentral sulcus (PrCS, BA 6), including the intersection with the superior frontal sulcus (SFS, border of BA 6 and 8). Koyama *et al.* indexed the preference for contralateral saccades at each activation focus by calculating the differences in percent BOLD signal change between ipsilateral and contralateral saccades, thereby inferring the functional correspondence between the two species. Based on these indices and other findings [45,47–52], it was proposed that the human PrCS/SFS is functionally equivalent to the macaque FEF. The saccade-related activation area extended from the FEF to the premotor area (BA 6) in macaques; thus, the area that showed saccade-related activation was larger than has been found in previous single-unit studies. Therefore, viewed using fMRI, brain activation that is associated with saccadic eye movement overlaps in BA 6 in both species, which implies inter-species similarity.

In the posterior parietal cortex of macaques, activation foci for saccadic eye movement were found in the lateral bank of the intraparietal sulcus (LIP), areas 7a/7b, the anterior intraparietal and the dorsal preunate. In humans, activation peaks were found in the posterior and anterior parts of the SPL and at the junction of the intraparietal sulcus (IPS) and the transverse occipital

sulcus. Based on the preference for contralateral saccades at activation foci, it was suggested that the dorsal part of the LIP in macaques and the posterior part of the SPL in humans were functionally equivalent [50–53]. A recent study that used contrast-enhanced fMRI (C. Wardak *et al.*, unpublished) found that macaque area 7a shows equivalent activation under saccadic conditions and visual-control conditions. Under the visual-control condition, macaques fixated a central spot while peripheral dots were flashed to mimic the visual aspect of the saccadic task. The findings suggest that this region has a role in attention rather than in saccadic eye movements. Further improvements to task designs, such as distinguishing between saccade-related activation and activation induced by retinal slip, will enable more detailed functional mappings of the IPS of humans and macaques.

Comparative fMRI studies in humans and macaques have also revealed inter-species differences in the functional architecture of the IPS. Vanduffel *et al.* [19] and Orban *et al.* [53] reported that there are four motion-sensitive regions in the human IPS (ventral IPS, parieto-occipital IPS, dorsal IPS and dorsal IPS anterior). By contrast, the macaque IPS contains only one motion-sensitive area (the ventral intraparietal area: VIP). Moreover, the four motion-sensitive areas in the human IPS are also sensitive to three-dimensional structure from motion (3D-SFM), whereas the one motion-sensitive area in the macaque IPS is not particularly sensitive to 3D-SFM. These dissimilarities suggest that the human IPS contains areas for visuospatial processing, whereas the macaque IPS does not.

As described above, comparative fMRI studies have revealed functional similarities and dissimilarities in anatomically corresponding regions in humans and macaques. These results can predict which brain areas are equivalent in function but not in cytoarchitecture, and vice versa, in the two species. The relationship between cytoarchitectonical and functional equivalences is an important issue for future research. Research should also examine whether functionally equivalent regions are evolutionarily 'homologous', particularly when studying the evolution of human intellect and mind [28,29,53]. Evidence from comparative fMRI studies, together with evidence from cytoarchitectonics and neuronal connections, helps to infer the homology of a given brain area in humans and macaques. Thus, directly comparing neuronal connections in humans and macaques using diffusion tensor MRI (DT-MRI) tractography [54–56] (Table 1) is an important approach and can complement comparative fMRI studies. However, caution should be exercised in concluding homology based on evidence from these studies; it is impossible to prove that functionally and anatomically similar brain regions in humans and macaques have evolved from regions that existed in a common ancestor, which is an essential criterion to conclude homology. Moreover, homologous regions are not necessarily functionally equivalent throughout evolution.

Combination of MRI and microelectrode techniques in macaques

Although comparative fMRI is an intriguing and important method, on its own, it is insufficient to link human imaging

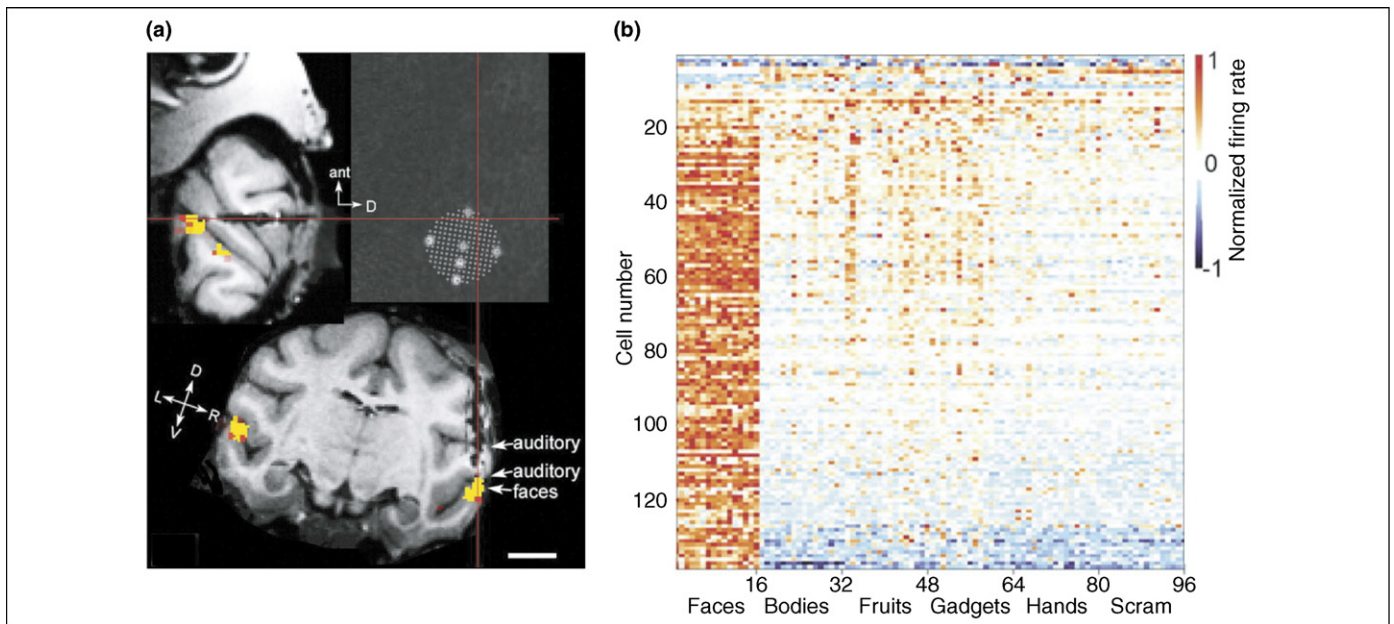


Figure 2. fMRI-guided microelectrode recordings in macaques [63]. **(a)** A recording track coordinate that targeted a brain region in the lower bank of the superior temporal sulcus that showed face-selective fMRI activation. Coronal and parasagittal sections of the face-selective patch and a grid that was inserted into the recording chamber are shown. Microelectrode recordings were performed from the hole in the grid at the intersection of the red lines. **(b)** Normalized responses of all visually responsive neurons (182 cells) to six categories of images [faces, bodies, fruits, gadgets, hands and scrambled patterns (scram)], with 16 images for each category, were recorded from the fMRI-determined face-selective patch in one monkey. Each row and column represents one neuron and one image respectively. Normalized neuronal responses to each image are color-coded. Adapted, with permission, from Ref. [63].

studies with macaque neurophysiological studies. The next step is to combine fMRI and invasive methods, which is only possible in experimental animals. One application is the combination of (f)MRI and microelectrode techniques. For example, simultaneous microelectrode recordings and fMRI scans will be a powerful tool to elucidate the relationship between the BOLD signal and neural activities. BOLD response does not measure neural activities directly, rather it measures hemodynamic changes, including alterations in blood flow, blood volume and intravascular magnetic susceptibility [5,57]. Furthermore, there remain many unsolved problems that concern the exact relationship between BOLD and neural signals [57]. Attempts to resolve these problems using simultaneous electrophysiological recordings and fMRI [58,59] are underway. The results and future perspectives of these studies are discussed in Ref. [57]. Not only has fMRI been used to help overcome these problems, but it has also impacted on

microelectrode techniques that are used to study neuronal mechanisms of cognitive functions.

Simultaneous microstimulation and fMRI

Microstimulation through a microelectrode can activate restricted populations of neurons in awake animals; in some cases, these experimental stimulations induce particular animal behaviors [3,47]. This technique is useful, not only to establish causal links between neuronal activities and behaviors but also to elucidate brain functional connectivity because microstimulation activates brain areas that are interconnected at a stimulated site. However, detection of the microstimulation-induced activation of remote brain regions has relied on point-by-point microelectrode recordings. Using fMRI, one can detect local and global brain activation patterns that are induced by microstimulation. Tolia *et al.* [60] investigated BOLD activation patterns induced by microstimulations that

Box 2. Precise localization of cortical recording sites using high-resolution MRI

There are several factors that potentially affect the accuracy of localization of microelectrode tips using MRI, such as electrode material, field strength, signal-to-noise ratio, pulse sequence and other MRI parameters [73]. For example, typical microelectrode tips are only 20–30 μm in diameter, at 100 μm from the tip end, which occupies only a few percent of a single-image voxel even with an in-plane resolution of 100 μm . Therefore, precise detection of the microelectrode tip would be difficult because of low signal-to-noise ratio. An MRI method developed by Matsui *et al.* [71] uses the susceptibility-induced effect to enable the precise localization of cortical recording sites; this effect thickens the metal microelectrode appearance on the magnetic resonance image and enables detection of the microelectrode tip, even though the tip volume is extremely small relative to an image voxel. In this method, the angle between

the static magnetic field and a microelectrode axis is set at $>60^\circ$ and frequency-encoding direction is set perpendicular to the microelectrode to optimize the susceptibility-induced effect for precise detection of the microelectrode tips. Matsui *et al.* [71] quantitatively investigated the accuracy of localization of microelectrode tips using this method (Figure 1, next page). They also applied this method to recording microelectrodes that were inserted into the monkey brain at an in-plane resolution of $150 \times 150 \mu\text{m}^2$ and localized recording sites within highly contrasted anatomical images of the cerebral cortex (Figure 1b,e). Furthermore, they compared the locations of microelectrode tips that were determined using MRI with corresponding lesion marks in histological sections. Statistical analysis validated that this MRI-based method could localize microelectrode tips *in vivo* with single-voxel accuracy.

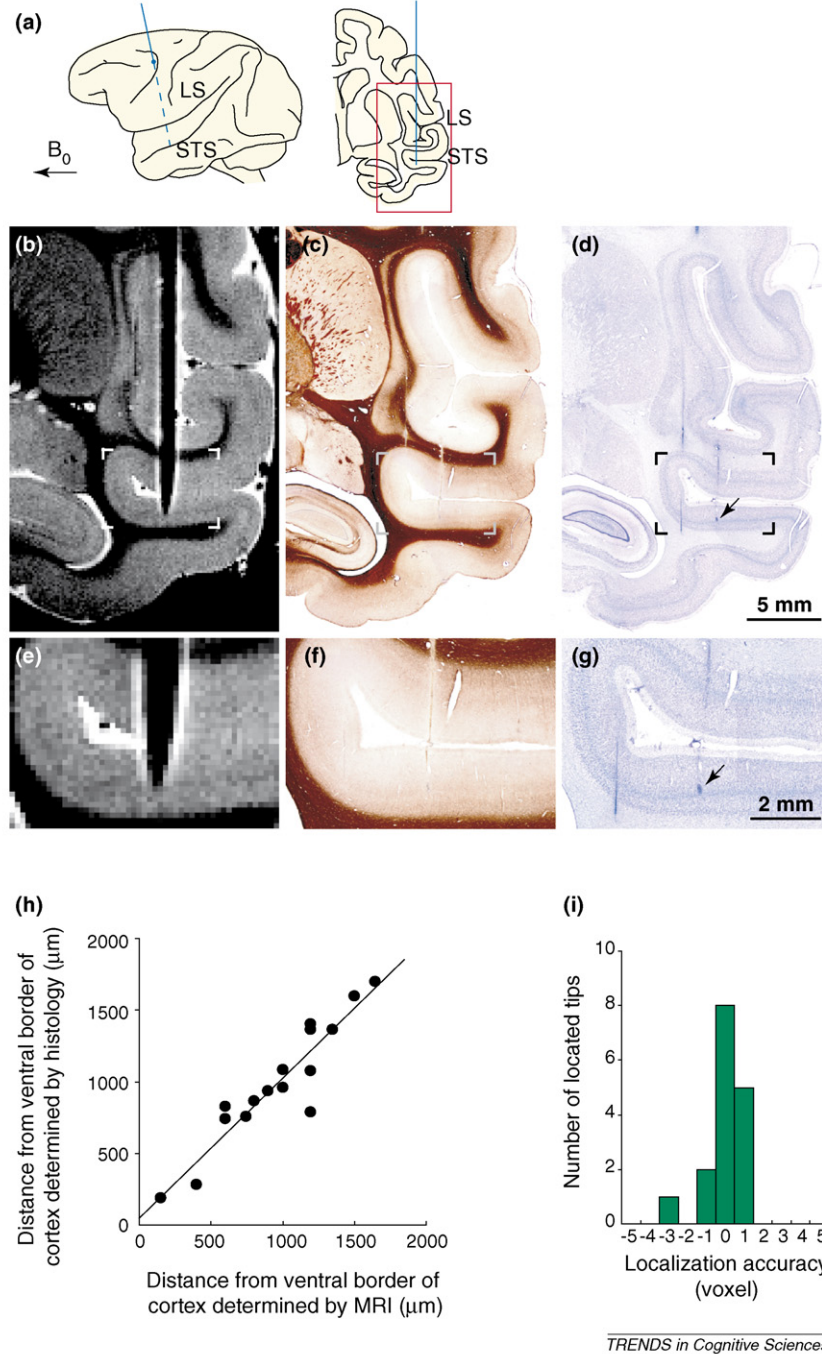


Figure 1 (Box 2). MRI-based localization of cortical recording sites at single-voxel accuracy. (a) Schematic drawings of a macaque brain with a microelectrode insertion. The microelectrode (blue line) was inserted into the superior temporal sulcus (STS) and the angle between the microelectrode and the static magnetic field was set at $>60^\circ$. The red frame indicates the area presented in (b–d). (b) Magnetic resonance image of an inserted microelectrode and the surrounding anatomy of the cortex. Inversion-recovery fast spin-echo sequence (a type of MRI pulse sequence, which is effective in obtaining a good contrast between gray and white matter) was used (in-plane resolution: $150 \times 150 \mu\text{m}$). Corresponding histological sections were stained for myelin (c) and for Nissle (d). (e–g) Magnified images from the framed areas in (b–d). The cortical location of the microelectrode tip is well matched with the corresponding lesion mark on the histological sections [arrowheads in (d) and (g)]. (h) Cortical locations of microelectrode tips determined by histology (y-axis) were plotted against those determined by MRI (x-axis; $N = 16$). (i) Distribution of localization accuracy, which was normalized against the voxel size. Mean \pm SD was 0.13 ± 0.97 voxel, which significantly fell within \pm one voxel (equivalence test, $p < 0.005$). Abbreviation: LS, lateral sulcus. Adapted, with permission, from Ref. [71].

were delivered to the V1 area of macaques. They found that the spatial extent of induced activation around the stimulated site was larger than expected by assuming only a passive spread of current, which implies possible contributions of the horizontal connections in V1. They also detected multiple-activated foci in the extrastriate visual areas.

fMRI-guided electrophysiology

Although microelectrode recordings can detect electrical activities of single or multiple neurons with high spatio-temporal resolution, using this method alone leads to difficulties when investigating how local neuronal activities are involved in brain-wide neuronal networks. Macaque fMRI revealed distributed brain activation at the

whole-brain scale [12,13,17,36,52], which could complement the investigation of local neuronal activities using microelectrode recordings. Moreover, fMRI in macaque monkeys can serve as a navigational tool to target microelectrodes. Using fMRI, researchers can identify multiple responsive brain regions and make appropriate decisions on electrode-penetration sites.

Examples of the combination of fMRI and electrophysiology can be found in studies of the somatosensory system [61,62]. The study by Hayashi *et al.* [11] was the first to investigate somatosensory activation in anesthetized macaque monkeys using fMRI. They demonstrated differential focal activation in primary (SI) and secondary (SII) somatosensory cortices, depending on the stimulated body regions, which indicated the feasibility of somatotopic mapping using fMRI in macaque monkeys. To explore how effectively somatotopic mapping based on fMRI data matches that based on microelectrode recordings, Disbrow *et al.* [61] compared macaque cortical maps of the SI based on 1.5 Tesla fMRI with maps based on microelectrode recordings, using identical somatosensory stimulations. There was a 55% concordance between fMRI-based maps with a voxel size of $1.56 \times 1.56 \times 4.00$ mm and maps based on electrophysiology. The combination of microelectrode recordings and fMRI was also used to investigate a possible information-integration mechanism in the SI. Lipton *et al.* [62] reported that direct electrical stimulation of the median nerve of macaque monkeys elicited fMRI activation in the primary somatosensory cortex (area 3b), contralateral and ipsilateral to the stimulated side. They examined this paradoxical ipsilateral activation in area 3b by performing microelectrode recordings and second derivative analysis of field potentials, and suggested that the ipsilateral response had modulatory effects on contralateral input processing.

More recently, fMRI-guided electrophysiology was used to explore the neuronal mechanisms that are involved in high-level vision in face perception (Figure 2) [63]. Previous single-unit recordings in macaques have reported the presence of neurons that show selective visual responses to face stimuli [64]. This type of neuron can be found in the bank of the superior temporal sulcus (STS) and the distribution of neurons constitutes clump-like structures [65]. Macaque fMRI studies have clearly identified multiple face-selective brain foci (patches) in the STS [12,66,67]. Tsao and colleagues [63] inserted microelectrodes aimed at one of the face-selective patches in macaques and recorded single-unit activities from neurons located in a restricted portion of this fMRI patch (Figure 2a). They found that a high proportion of visually responsive neurons (302 out of 310) showed a greater response to face stimuli than to objects of other categories (Figure 2b). These results support the notion of a highly specialized functional module for high-level vision, at least for certain types of visual categories, such as faces [68] (but see also Ref. [69]).

High-resolution structural MRI: a precise localizer of cortical recording sites

In microelectrode recordings of neurons in the cerebral cortex, establishing the anatomical locations of recorded neurons in the cortical layers is important for analyzing

the recorded neuronal activities in relation to underlying neuronal connections; layer structures of the cerebral cortex characterize input and output connections and local neuronal circuits in a given cortical region. In conventional methods for localizing cortical microelectrode-recording sites, positions of the tip of the recording microelectrode are marked in brain tissue in several ways, such as electrolytic lesions, and these marks are detected in post-mortem histological sections. Although these procedures provide definite anatomical locations of recording sites within cortical layers, they are usually inefficient in chronic recordings of neuronal responses in behaving primates. Methods for localizing cortical recording sites *in vivo*, during or just after a recording session, would be strong complements to existing localization methods that require post-mortem histology. MRI has been proposed as a promising approach to localize relatively thick electrode and intracranial devices [70]. However, the application of MRI to precise localization of microelectrode tips is technically problematic for several reasons (Box 2). Recently, Matsui *et al.* have developed an MRI method for the precise localization of cortical recording sites [71] (Box 2). Using a 4.7 Tesla MRI scanner, the tip localization accuracy in this method reaches a single-image voxel, at a spatial resolution of $50 \mu\text{m}$ (the highest resolution tested so far) in phantom (a sample microelectrode sunk into a copper sulfate solution). Compared with previous approaches to localizing microelectrode tips in monkey brains [72], this method provides higher spatial resolution and enables the use of a wider range of parameters that are related to radio-frequency pulse timing; this enables a microelectrode tip to be detected precisely and the well-contrasted anatomy of the surrounding cortex to be visualized simultaneously (Figure 1b,e in Box 2). This MRI-based method would help to infer the relationship between recorded neuronal activities and cortical neuronal connections in monkeys.

Concluding remarks

fMRI and other MRI-based techniques are expected to bridge the gap between human imaging studies and macaque neurophysiological studies. Comparative fMRI studies in human and macaque subjects facilitate direct comparisons of functional brain architecture, from sensory systems to high-level cognitive functions in association areas. The combination of fMRI and microstimulation techniques offers multi-dimensional analyses of functional connectivity in living macaque brains. fMRI can help to navigate microelectrodes to correct recording sites in the macaque cortex and to link functional activation in humans and neuronal firing in macaques (fMRI-guided electrophysiology). High-resolution structural MRI is also useful to determine cortical recording sites *in vivo*, which helps to infer relationships between recorded neuronal activities and underlying neuronal connections.

Although the potential of these methods is evident, studies using fMRI and other MRI-based techniques in macaque monkeys have yet to make a significant contribution to our understanding of the neurophysiological basis of high-level cognitive functions because there is no systematic investigation that focuses on particular cognitive functions or that is based on specific hypotheses. Moreover, no

Box 3. Questions for future research

- To what extent is the macaque brain similar to the human brain? Although it is highly unlikely that all human brain regions have functional counterparts in the macaque brain, this question is essential for elucidating the structure of our intellect. Which functional domains have specifically evolved in the human brain? For example, are Broca's and Wernicke's areas only found in human brains or can prototypes be found in macaques or other related primates?
- Whereas strategies for inter-species comparisons in unimodal sensory areas seem straightforward, comparisons in multimodal and association areas are more difficult. In particular, inter-species similarities and differences in the prefrontal and parietal cortices are poorly understood. Future comparative fMRI studies might settle these issues.
- Simultaneous electrode recordings and fMRI in macaque monkeys are powerful investigative tools that are used to identify the relationship between BOLD signal and underlying neuronal electrical activity [57–59]. The same might be true of the neuronal underpinnings of temporal correlation of BOLD signal changes across different brain regions. Such investigative tools are also useful to validate methods to infer causal relationships across multiple brain regions, such as dynamic causal modeling or Granger causality [74,75], which have recently been introduced to human fMRI studies.

studies have completed the comparison between the two species and the linking of fMRI and electrophysiology in a particular behavioral paradigm. However, future enterprising studies using systematic strategies, such as comparative fMRI followed by fMRI-guided electrophysiology, should enable investigations of high-level cognitive functions at the level of neuronal circuits (Box 3).

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