RESEARCH HIGHLIGHT

EM-fMRI: A Promising Method for Mapping the Brain Functional Connectome

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Electrical microstimulation (EM) can be used to locally stimulate the cerebral cortex or subcortical nuclei. Meanwhile, functional magnetic resonance imaging (fMRI) can non-invasively visualize the activity of the whole brain. When EM is combined with fMRI (EM-fMRI), it is possible to measure the changes of the whole-brain neural activity using fMRI while applying electrical stimulation to a specific brain site, and accordingly infer the causal links between the stimulated site and the activated brain areas. Figure 1 illustrates the general principle of EM-fMRI. Recently, Xu *et al.* used this method to map the functional connectome of the lateral prefrontal cortex (LPFC) in macaques [1]. The authors stimulated ~200 sites in the LPFC, and found reliable fMRI activation in multiple regions of the cerebral cortex. The authors clustered the activated regions into different cortical domains, including temporal cortex (TEMP), posterior parietal cortex (PPC), orbitofrontal and insular cortices, dorsomedial prefrontal cortex, and posteromedial cortex, and then calculated the connectivity between each of the cortical domains and the stimulation sites in the LPFC. Thus, the authors found substantial differences between the connectivity patterns of stimulation sites in the LPFC. More importantly, these differences revealed a degree of topography between the LPFC and the cortical domains. Specifically, when stimulation sites were moved from the rostroventral to the caudodorsal surface of the LPFC, the TEMP connections shifted from the most rostral to progressively more caudal regions in the superior temporal sulcus, while the PPC connections progressed from rostral to caudal in the intraparietal sulcus. Therefore, based on the findings of such orderly progressions of connections between the LPFC and the cortical domains, the authors suggested that there may be layout-preserving, surface-to-surface mappings between the LPFC and other major association cortices in macaques, coarsely resembling how the retina maps onto the visual cortex. These findings provide a millimeter-scale functional connectome sketch for how the LPFC interacts with different cortices.

Interestingly, to confirm the validity of connectivity mapping using EM-fMRI, this study compared the EM-fMRI results to studies using neuronal tracing and diffusion MRI, respectively. On one hand, the authors found a strong correlation between the inter-areal functional connectivity mapped by EM-fMRI and the anatomical connectivity from the neuronal tracing study. Further, the connections mapped by EM-fMRI showed several major regularities that were comparable to the neuronal tracing studies, for example, the strength of the connections declined exponentially as inter-areal distance increased. Therefore, the authors suggested that EM-fMRI is able to not only map brain connections with both nearby and at long-distance, but also indicate the relative strength of these connections. On the other hand, the inter-areal neural tracts between the LPFC and other cortical

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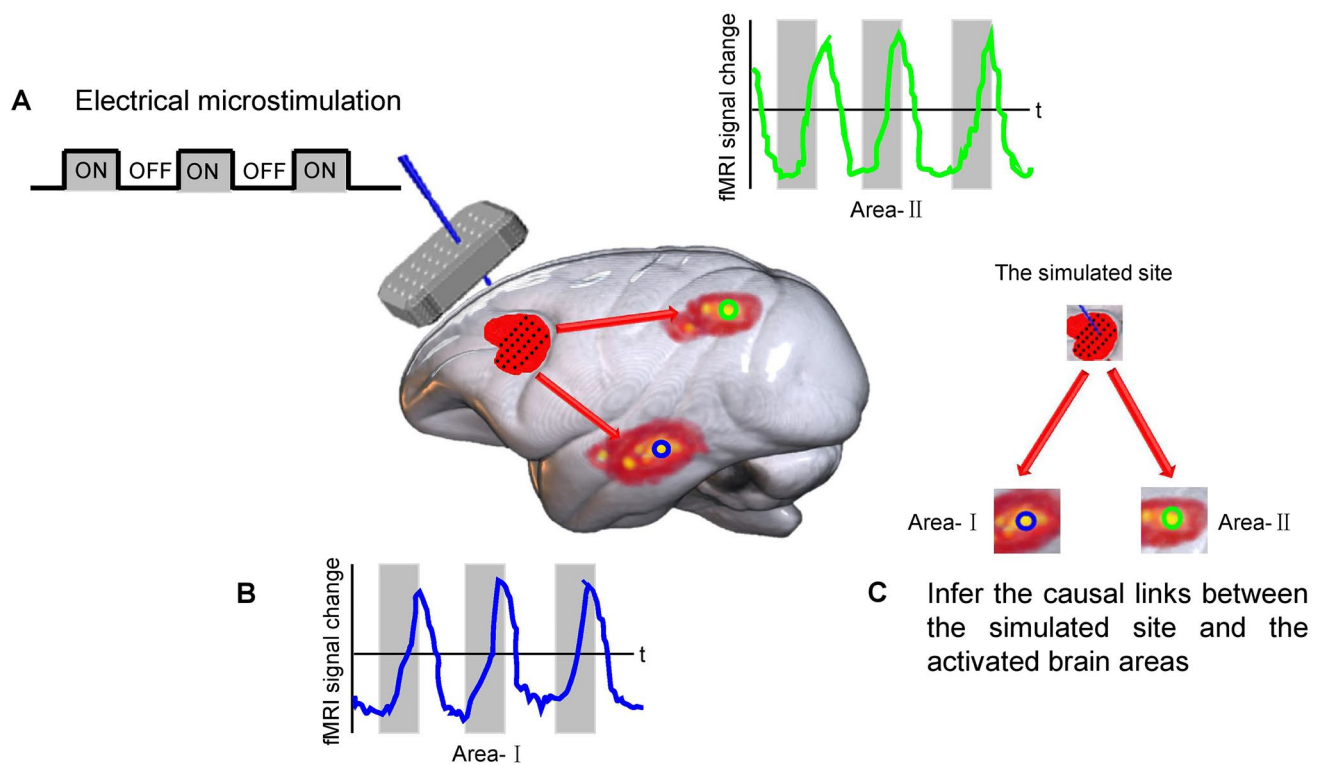6   Research Center for Augmented Intelligence, Zhejiang Lab,
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Fig. 1 The general principles of EM-fMRI. **A** Electrical microstimulation is delivered to a specific brain site. **B** Whole-brain activation is mapped using fMRI during electrical microstimulation. **C** Causal links between the stimulated site and the activated brain areas are inferred.

regions using diffusion MRI showed much lower consistency with neuronal tracing studies in comparison to EM-fMRI, especially for long-distance connections. To summarize, the authors concluded that the inter-areal connections from EM-fMRI are consistent with those from neuronal tracing, whereas diffusion MRI is relatively less consistent.

Although neuronal tracing is almost considered to be the gold standard for studying inter-areal anatomical connections, this technique requires elaborate procedures, including injecting tracers, brain sectioning, tissue staining, slice imaging, and 3D reconstruction [2]. Thus, it is always necessary to kill the animals. Conversely, EM-fMRI is an *in vivo* imaging technique. For example, Xu *et al*. inserted microelectrodes into the cortex in the LPFC during the experiment. In principle, the macaques could be used for other research, for example, mapping functional connectomes for other brain regions. In addition, EM-fMRI enables finer stimulation sites, corresponding to more explicit functional regions or networks, and allows finer capture of connectivity differences between adjacent sites.

In fact, since EM-fMRI was developed in the first decade of the 21st century, many studies have successfully used this technique. EM-fMRI is able to demonstrate causal links between neural activity and cognitive functions. For example, Tolias *et al*. used EM-fMRI in the primary visual cortex

(area V1) of the macaque to characterize the activity patterns that were generated locally at the site of stimulation and distally in brain regions innervated by the site of stimulation [3]. Amemori *et al*. combined EM-fMRI with viral tracing and found the role of the corticostriatal pathway in regulating affective judgment and decision-making [4]. Recently, Han *et al*. used EM-fMRI to map the functional gradients of the striatal circuit in non-human primates [5]. In addition, EM-fMRI has great potential in the management of brain diseases [6] and the development of cognitive neuroprosthetics [7].

Some caution is required when using EM-fMRI. First, electrical stimulation parameters need to be carefully considered, such as current pulse duration and current amplitude. Low current cannot saturate or inhibit the network, while high current can cause brain injury. As a valuable reference, Xu *et al*. used 200-ms, 500-mA cathodal current pulses to stimulate the LPFC in the macaques. Second, fMRI signals have a low signal-to-noise ratio and change slowly, especially for blood oxygen level-dependent signals, thus EM-fMRI cannot precisely detect brain activation and distinguish between direct transmission and indirect trans-synaptic transmission. Fortunately, the use of ultrahigh-field ($\geq$7 T) MRI scanners and iron oxide contrast agents can improve the signal-to-noise ratio of fMRI [5]. On the other hand,

combined with reversible inactivation techniques to temporarily inactivate the relevant brain areas, EM-fMRI can elucidate details of causal links and functional interactions, and potentially shed light on how information flows between brain networks [8]. Third, it is almost impossible for electrical microstimulation to stimulate a particular type of neuron within a site. In comparison, optogenetics allows specific cell types to be targeted for stimulation by the introduction of opsins. By combining the precision of optogenetic stimulation with fMRI, optogenetic functional magnetic resonance imaging (ofMRI) enables cell type-specific mapping of functional neural circuits and their dynamics across the whole brain [9]. However, ofMRI studies are currently limited to non-human primates [10].

In future, with the development of dense electrical microstimulation and high spatial resolution functional MRI, it may become possible for EM-fMRI to capture the activity patterns of cortical column or laminar neurons, which should lead to a more detailed and finer functional connectome. Moreover, combining EM-fMRI with neuronal tracing and reversible inactivation techniques, researchers can explore the detailed functional connectome of specific neural circuits. Besides, recent studies suggest that the brain is a dynamic control system that switches between different cognitive states [11]. EM-fMRI will provide an important approach to unravel the basic principles of brain state transition and neural trajectory perturbation, which could promote the development of basic and clinical neuroscience, for example, managing epilepsy. In summary, EM-fMRI will play an increasingly important role in brain research.

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