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Functional magnetic resonance imaging of awake behaving macaques

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ABSTRACT

In recent years, more and more laboratories have developed functional Magnetic Resonance Imaging (fMRI) for awake non-human primates. This research is essential to provide a link between non-invasive hemodynamic signals recorded in the human brain and the vast body of knowledge gained from invasive electrophysiological studies in monkeys. Given that their brain structure is so closely related to that of humans and that monkeys can be trained to perform complicated behavioral tasks, results obtained with monkey fMRI and electrophysiology can be compared to fMRI results obtained in humans, and provide information crucial to a better understanding of the mechanisms by which different cortical areas perform their functions in the human brain. However, despite that the first publications on fMRI in awake behaving macaques appeared ~10 years ago (Logothetis et al. (1999) [1], Stefanacci et al. (1998) [2], Dubowitz et al. (1998) [3]), relatively few laboratories perform such experiments routinely, a sign of the significant technical difficulties that must be overcome. The higher spatial resolution required because of the animal's smaller brain results in poorer signal-to-noise ratios than in human fMRI, which is further compounded by problems due to animal motion. Here, we discuss the specific challenges and benefits of fMRI in the awake monkey and review the methodologies and strategies for scanning behaving macaques.

1. Introduction

An important motivation for pursuing fMRI in monkeys is that it allows us to compare data gathered in an extensively studied animal model to measures of human brain function obtained using similar techniques and behavioral paradigms. Humans and monkeys are closely related in evolutionary terms, and both have relatively large and highly developed brains. Because monkeys can be trained to perform complex tasks, brain function of both species can be compared while they are performing the same task [4,5] or viewing the same stimulus [6–14]. Hence, fMRI in awake monkeys is particularly suited for studying the neural basis of higher cognitive functions such as perception, memory, learning, decision making, etc. The macaque, which is typically used for fMRI, has been used in neurophysiology for over 50 years and has been extensively studied with electrophysiology, tracers, lesions and histological techniques. Many of the above-mentioned cognitive processes have been studied extensively with electrophysiology in macaques [15–17], but although they provide very high temporal resolution, with invasive microelectrode recordings one can only sample a single neuron or small population of neurons at any given time. fMRI on the other hand, is particularly well suited for the study of large areas or whole-brain networks. It can also serve as a guide for the placement of electrodes [18], or it can be combined with techniques that alter or interrupt normal function of the system, such as microstimulation [19,20], local pharmacological injections [21] or local cooling [22].

The combination of fMRI with invasive techniques can also help us better understand and interpret the blood oxygen level dependent (BOLD) signal. fMRI has been in use since the early 1990s, and while the BOLD signal is obviously a marker of brain function, it is still not clear what exactly it represents. For example, it is not known whether all neural processes elicit a BOLD response (e.g. synaptic input vs. spiking activity, stimulus-driven and neuromodulatory activity, feedforward and feedback processes, inhibitory and excitatory potentials) or whether these processes are all equally represented in the BOLD signal [23]. For instance, there is evidence that synaptic activity drives the hemodynamic responses better than spiking activity [24,25] and that different receptors contribute differently to the hemodynamic response [26], although the exact contributions are still unknown for most processes. Moreover, it is unclear whether the BOLD response differs across brain regions (e.g. cortical vs. subcortical areas). fMRI in monkeys, and particularly in awake monkeys in combination with invasive techniques is therefore of paramount importance in aiding

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neuroscientists in elucidating some of these issues. Monkeys are supremely suited for such experiments because they can be trained and their brain is large enough that sensitivity issues are not as problematic as in fMRI of rodents.

Despite all these advantages, the rise in the number of monkey fMRI publications has been slow compared to the first 10 years of human fMRI, where there was an exponential increase in the number of published fMRI articles [23]. Only recently has the number of research groups working on fMRI in macaques begun to grow. The lower number of publications obviously reflects the smaller number of labs that work with monkeys, but it is also a consequence of the challenges in implementing the technology.

In essence, fMRI is an insensitive technique, given that the changes in image intensity due to the BOLD signal are only a few percent of the baseline intensity and are similar in magnitude to the physiological variability. While the signal-to-noise ratio (SNR) can be increased in human fMRI by using relatively large voxels or large regions of interest (ROIs), there are limitations to using this strategy in monkeys because of their smaller brains. In addition to requiring higher spatial resolution, and thus a higher SNR, fMRI in awake monkeys is inherently more variable due to animal movement. If one chooses to avoid motion problems by performing fMRI in anesthetized monkeys, there are the difficulties of maintaining anesthesia and the detrimental effects of anesthesia on the BOLD signal.

1.1. Anesthetized or awake monkeys?

The anesthetized monkey preparation is favored by several groups [1,24,27–31]. Although the BOLD signal is typically lower due to the anesthesia and although the effect of the anesthetic on the BOLD signal is not precisely known (and likely varies with the type of anesthesia), the anesthetized monkey preparation offers several advantages. It allows experiments that would be impractical in awake monkeys, for instance ultra high resolution fMRI [23,32,33], very long experiments, or certain combinations of fMRI with invasive electrophysiology [21].

Fig. 1 shows an example of a functional activation map acquired in an anesthetized monkey viewing a flickering LED stimulus (Fig. 1B and C). All data reported here were acquired at 7 T using a vertical Bruker Biospec 70/60v scanner with 60 cm bore diameter, equipped with Siemens AC44 (40 mT/m) and more recently Bruker BGA38S gradients (75 mT/m). Custom designed and built radiofrequency (RF) coils were used. The scanner and experimental procedures are described in detail elsewhere [1,34]. The data in Fig. 1 were collected by using a single-shot gradient-echo echo planar imaging (GE-EPI) sequence with spatial resolution of $0.75 \times 0.75 \times 2\ mm^3$. The activation map is the average of four 8-min scans, although a single scan is already sufficient to obtain a similar map. In general, whether a BOLD signal can be detected depends on the SNR of the EPI images, the strength of the BOLD signal, the variability over the time series, and the number of images collected. The SNR depends on many factors, but arguably most important is spatial resolution, since the SNR decreases as the volume of the imaging voxel decreases. Other important factors include field strength, RF-coil properties, sequence parameters, etc. The image-to-image variability of awake monkey fMRI depends on physiological noise, animal motion and scanner stability. Although SNR and scanner stability are important factors determining whether a BOLD signal can be measured [35,36], ensuring that they are sufficient for fMRI is ideally addressed by using appropriate hardware and scanner optimization, leaving physiological variability and animal motion. Fig. 1D and E shows an example of the standard deviation (SD) of the image intensity over the time series in an anesthetized monkey. To generate the SD image, the SD over the entire time series is calculated for each voxel. Areas with a large SD denote areas where the variability of the time series is increased, and in such areas the BOLD signal may be obscured. In an anesthetized monkey the SD image is determined by the SNR of each image and the physiological variability. Hence, it shows a constant SD over the image except in vessels and ventricles, where the physiological variability is larger due to the heartbeat and flow/pulsation of the cerebrospinal fluid. Regions with very strong BOLD signal are also visible in the SD image. The amplitude of the BOLD signal in a voxel ranges from up to a few SDs above the noise level to below noise level. Due to the relatively low contrast-to-noise ratio (CNR) of the BOLD signal, averaging is needed, whether in the form of spatial averaging by filtering or ROI selection, or temporal averaging by acquiring a series of images.

The main advantages of fMRI in anesthetized preparations are: (1) the use of anesthetized monkeys allows longer scanning times, (2) the absence of animal motion allows the acquisition of images at higher spatial resolution, and (3) it can be more easily combined with long or complicated invasive procedures [24,37]. Disadvantages are the complicated anesthesia setup and management [1], the well-documented effects of general anesthetics on neural activity, which can vary depending on the type and depth of anesthesia [38], and the fact that processes involving attention or response are disrupted. All this can introduce difficulty interpreting the data. Despite this we did not observe a dramatic effect of anesthesia in primary visual cortex, or area V1: a comparable correlation between the BOLD and electrophysiological signals was found in awake and anesthetized monkeys [24,39]. Also, using our anesthesia protocols we did not observe marked differences in the extent of activated areas between awake animals that are passively viewing and anesthetized monkeys [1,39–43].

The awake monkey preparation has the obvious advantage that the added element of uncertainty arising from the depth of anesthesia and the physiological condition of the animal is absent and hence it allows a more straightforward comparison with human fMRI studies. Fig. 2 shows an example of functional activation in V1 in an awake monkey acquired at a spatial resolution of $0.75 \times 0.75\ mm^2$. The image was acquired in a single scanning session and shows that high-resolution maps can be obtained reliably in awake monkeys. Awake monkeys also have a higher BOLD signal than anesthetized monkeys, but drawbacks include problems due to animal movement and the time-consuming training necessary to ensure that the animal sits still and fixates for prolonged periods of time. In the following sections we discuss the procedures, common problems and solutions for awake monkey fMRI.

2. Methods and equipment

Different labs have successfully implemented fMRI in awake monkeys, and the different setups have been described in several reports [2,3,44–49]. A brief review of the methodology is provided below.

2.1. Scanner and field strength

Awake monkey fMRI has been performed at field strengths ranging from 1.5 T to 7 T and in horizontal and vertical scanners [1,5,7,41,47,50–52]. The most common approach is to use a human clinical scanner (1.5 T, 3 T or 7 T) while some labs, including ours, have dedicated vertical primate scanners (4.7 T or 7 T). In horizontal scanners the monkey sits in the so-called 'sphinx position' and can be trained to perform tasks, although the sphinx position is an unnatural position for them. The more natural upright position is more likely to encourage compliance and may be better for long scans or difficult tasks, but a direct comparison is difficult, since