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Functional magnetic resonance imaging of awake monkeys: some approaches for improving imaging quality

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Abstract

Functional magnetic resonance imaging (fMRI), at high magnetic field strength can suffer from serious degradation of image quality because of motion and physiological noise, as well as spatial distortions and signal losses due to susceptibility effects. Overcoming such limitations is essential for sensitive detection and reliable interpretation of fMRI data. These issues are particularly problematic in studies of awake animals. As part of our initial efforts to study functional brain activations in awake, behaving monkeys using fMRI at 4.7T, we have developed acquisition and analysis procedures to improve image quality with encouraging results.

We evaluated the influence of two main variables on image quality. First, we show how important the level of behavioral training is for obtaining good data stability and high temporal signal-to-noise ratios. In initial sessions, our typical scan session lasted 1.5 hours, partitioned into short (<10 minutes) runs. During reward periods and breaks between runs, the monkey exhibited movements resulting in considerable image misregistrations. After a few months of extensive behavioral training, we were able to increase the length of individual runs and the total length of each session. The monkey learned to wait until the end of a block for fluid reward, resulting in longer periods of continuous acquisition. Each additional 60 training sessions extended the duration of each session by 60 minutes, culminating, after about 140 training sessions, in sessions that last about four hours. As a result, the average translational movement decreased from over 500 μm to less than 80 μm , a displacement close to that observed in anesthetized monkeys scanned in a 7 T horizontal scanner.

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Another major source of distortion at high fields arises from susceptibility variations. To reduce such artifacts, we used segmented gradient-echo echo-planar imaging (EPI) sequences. Increasing the number of segments significantly decreased susceptibility artifacts and image distortion. Comparisons of images from functional runs using four segments with those using a single-shot EPI sequence revealed a roughly two-fold improvement in functional signal-to-noise-ratio and 50% decrease in distortion. These methods enabled reliable detection of neural activation and permitted blood-oxygenation-level-dependent (BOLD) based mapping of early visual areas in monkeys using a volume coil.

In summary, both extensive behavioral training of monkeys and application of segmented gradient-echo EPI sequence improved signal-to-noise and image quality. Understanding the effects these factors have is important for the application of high field imaging methods to the detection of sub-millimeter functional structures in the awake monkey brain.

Keywords

Functional MRI; Non-human primate; Macaque monkey; Awake; Visual cortex; Data stability

1. Introduction

Due to the many parallels in their brain organization and behavioral functions, non-human primates serve as a useful model for understanding functional brain organization in humans. Until recently, most studies have involved electrophysiological recordings or anatomical studies that required invasive techniques. However, the exciting development of functional magnetic resonance imaging (fMRI) in the last two decades has made it possible to non-invasively map the hemodynamic correlates of neural activity in the brain. This has led to an explosion of fMRI studies in humans as well as new studies in monkeys. The ability to conduct fMRI studies in monkeys promises to bridge the gap between human fMRI studies and the large body of electrophysiological and anatomical studies in monkeys. However, to properly link human fMRI data collected in the awake state to monkey studies, it is necessary to study brain function using fMRI methods in awake, behaving monkeys. In combination with invasive methods, such as electrical recording (1,2), electrical stimulation (3,4), local lesion (5,6), and reversible inactivation (7), these studies promise to greatly extend our understanding of the neural basis of perception and cognition.

Several challenges are encountered when adapting fMRI for use in awake, behaving monkeys. The first is that monkeys have relatively small brains and smaller functional structures compared to humans. The average area of the cerebral cortex in macaque monkeys is 100 cm² (8), compared to 2500 cm² in human. Functional domains in monkeys are also smaller. For example the width of ocular dominance columns in macaque monkeys (9) is half the size of those in humans (10,11). Thus, to observe the same functional structures in monkeys, images must be obtained with higher spatial resolution and higher signal specificity. Several groups have used standard clinical (12–15) or customized vertical (16–18) MR machines to conduct fMRI studies in awake monkeys. While many studies have been conducted at 1.5T and 3T machines, increasingly studies in awake monkeys are being conducted at higher field strengths (7,17,19–25).

The motivation for higher field imaging lies in the search for better temporal signal-to-noise ratios (*SNR*), functional contrast-to-noise ratio (*CNR*), and the associated potential for improved higher spatial resolution. Extending the work of Glover and colleagues (26,27), Triantafyllou et al. (28) demonstrated that in blood-oxygenation-level-dependent (BOLD) fMRI, the functional *CNR* is dominated by physiological noise for larger voxel volumes, and

remains roughly fixed as voxel sizes are reduced until thermal noise begins to dominate. These investigators further showed that the voxel volume at which this thermal noise threshold is reached decreases with increasing field strength. Thus the advantage of increasing field strength lies in the associated decrease in the voxel volume at which the maximum available temporal *SNR* and functional *CNR* can be maintained. In addition, at high magnetic fields, intravascular BOLD signals decrease, reducing the influence of blood volume changes in large veins, and in spin echo imaging the efforts of microvascular changes more closely associated with neural activity are enhanced. As a consequence, at high field fMRI can achieve sub-millimeter spatial resolution and a mapping signal that co-localizes well with the underlying changes in neural activity revealed by electrophysiological mapping and optical imaging (29).

For successful imaging of monkeys at high field, several factors contributing to image degradation must be overcome. First, artifacts which occur at interfaces between materials of different magnetic susceptibility must be addressed. Such artifacts are more difficult to remove by shimming at high magnetic fields, and may cause both geometric distortions and signal losses. Distortions are especially pronounced in single shot EPI acquisitions which are commonly used in human fMRI. Failure to correct for susceptibility artifacts can make it more difficult or even impossible to interpret functional images collected at high fields. The second problem is that of non-BOLD (e.g. motion) sources of “physiological noise”, i.e. dominant sources of variability in MR images that can have the same dependence on magnetic field strength as the BOLD mapping signal (26–28). Physiological effects such as respiration may alter the field uniformity in the brain (30,31) and usually limit the temporal *SNR* and BOLD *CNR*. Thus, increases in physiological noise can counteract gains in signal from increased magnetic field strength.

A second major challenge is to obtain good data stability during scans in behaving monkeys. In addition to movement, which can be reduced with a headpost to fix the head, there are additional noise sources in awake monkeys. As monkeys are rewarded with drops of juice to perform tasks during MR scans, each fluid reward may be accompanied by extra body and jaw movements (19). This results in added motion and physiological noise, leading to additional inhomogeneity in the local magnetic field. In addition, a scanning session is typically composed of several short runs. During the breaks between runs, monkeys may show considerable movements (32). These between-run movements may have different spatial distributions making correction and interpretation more difficult. To minimize these multiple sources of noise, a good behavioral paradigm is essential for data stability.

A third challenge derives from the head-fixation setup unique to the behaving animal preparation. Typically, monkeys will be implanted with a plastic headpost to eliminate head movements during experiments. However, MR compatible materials, such as ceramic and plastic, still have different magnetic susceptibility from bone and tissue (5), leading to magnetic field inhomogeneities at interfaces between headpost material and tissue. These, as well as other local air/tissue and bone/tissue boundaries (e.g. ear canals) and the associated local *B0* inhomogeneity can create variations in signal intensity and geometric distortions in images. These artifacts are greater at high magnetic fields and specific pulse sequences. In plane distortion artifacts are especially apparent with rapid imaging methods such as single shot echo-planar imaging (EPI) that have low pixel bandwidths in the phase encode direction, while the intra-voxel dephasing associated with through plane *B0* inhomogeneities can significantly compromise BOLD fMRI sensitivity. Carefully optimized parameters and pulse sequences must be chosen to minimize such susceptibility artifacts.

In this paper, we report our experiences in reducing noise and dealing with artifacts in fMRI data collected from awake, behaving monkeys imaged in a vertical bore 4.7T scanner. These

include adaptations to our monkey behavioral training paradigm, and adaptations in pulse sequences. By increasing the data stability, spatial resolution, anatomical *SNR*, functional *SNR* and minimizing spatial distortion, we have obtained much improved functional MRI data from awake, behaving monkeys at 4.7T.

2. Material and methods

2.1. Animal preparation and surgical procedures

Two Macaque monkeys (*Macaca mulatta*) were imaged over 15 sessions. All surgical and experimental procedures conformed to the guidelines of the National Institute of Health and were approved by the Institutional Animal Care and Use Committee of Vanderbilt University. Under general anesthesia (1~2% isoflurane), each animal was implanted with a custom-made plastic headpost secured by ceramic screws (Thomas Recording GmbH, Giessen, Germany) and dental cement. After a recovery period of six weeks or longer, monkeys were trained to sit in MR-compatible primate chairs with their heads fixed and were acclimated to the scanner testing environment.

2.2. The head-fixation design for awake monkeys

A good head-fixation design is one of the most important aspects that help to maximize data acquisition stability in awake, behaving monkeys (5,13,17,19,32). The design must be rigid so that no major motion noise will come from animal related motion and also be flexible enough that accessories like goggles and coils can be installed easily. We have established head-fixation setups for optical imaging in well-trained monkeys (33–35) in which the in-plane movements of the brain were less than 50 μm (36). We have adapted this design by making headposts from MR-compatible plastics and incorporating it into the MR-compatible monkey chair (Fig. 1). The monkey's chair is constructed from high density polycarbonate. The plastic head-post is secured to the chair by a head-post mounted onto a secure frame. Frame, head-post and head-post mount were made by Ultem (GE Polymershapes, Huntsville, AL). The position of the frame is adjustable to accommodate different animals and different types of coils used in the scanner. A nylon mount attaches a pair of plastic, fiber-optic binocular glasses to the frame. The position of each eye piece can be independently adjusted for each monkey. A plastic mouth piece, used to deliver fluid reward, was rigidly attached to the head piece to minimize reward related motion artifacts. A volume coil, which almost fully enclosed the monkey's head, both transmitted and received MR signals. This modified head-post mount and the eyepiece-mount were designed to be installed from the opening at the top of the chair without interfering with the volume coil.

2.3. Stimulus presentation and training paradigm

Visual stimuli were presented binocularly via a pair of plastic, fiber-optic glasses (SV-7021 Silent Vision system; Avotec, Stuart, Florida). To achieve binocular fusion, each eyepiece attached to a goggle mount was independently adjustable. The field of view of the system was 24.5° horizontal by 17° vertical visual angle with resolution of 1024 × 768 and 60 Hz refresh rate. Simultaneous binocular eye tracking was realized by the Real Eye RE-4601 eye monitor system (Avotec, Stuart, FL) and iView eye tracking system (SensoMotoric Instruments GmbH, Teltow, Germany). Visual stimuli consisted of monochromatic counter-phasing checkerboard patterns (6 Hz visual modulation at 90% contrast) alternating with periods of gray background of equal mean luminance (50 cd/m^2). Stimulation periods had duration of 20 s. For visual topographic mapping, we used annulus-shaped checkerboard patterns (3 annuli at 1°, 3°, and 5°, each with a width of 1°) to map isoeccentricities, and wedge-shaped checkerboard patterns to map the horizontal and vertical meridians (centered over 0°/180° and 90°/270° polarities, 30° wedge angle, extending from 0.26° to 8° eccentricity).

Before scanning in the vertical MR scanner, we first trained monkeys in a simulated MR environment. Animals were chaired and head-fixed in a chair with a modified head-fixation design. To mimic the actual MR environment, we darkened the room and played sounds recorded from real MR scans. Visual stimuli were presented on a monitor or through a pair of glasses placed in front of the eyes. Left and right eye positions were tracked by a CCD based eye tracking system. Monkeys were trained to do a fixation task and to minimize body movements. A second CCD camera and a motion sensor were used to monitor animal body movements. Fluid rewards were withheld if any large body movements were detected. With this pre-training paradigm, we found that monkeys can acclimate to the real scanner environment in as short as one week.

Monkeys were trained to maintain fixation within 1.5° radius of a centrally presented fixation spot ($0.2^\circ \times 0.2^\circ$) through the scan. They received a fluid reward for performing the fixation task. Their performance was estimated by the percentage of time their eye position remained within the fixation window. We included only scans in which monkey's performance were better than 90% for data analysis.

2.4. MRI acquisition and data analysis

Awake, behaving monkeys were scanned on a Varian 4.7T vertical MR scanner (Varian Medical Systems, Palo Alto, CA) using a 23-cm birdcage volume transmit-receive coil (Varian Medical Systems, Palo Alto, CA). The MR machine has a Magnex 60-cm bore magnet and high performance actively shielded gradient (7 G/cm, 175 μ s rise times to full gradient amplitude).

Single-shot or segmented T_2^* -weighted gradient-echo EPI sequences were used with an echo time between 10 and 20 ms to assess functional cortical activation. The repetition time for single-shot, two-shot, and four-shot EPI were 3,000 ms, 1,500 ms, and 750 ms, respectively. This resulted in an acquisition time of 3000 ms per acquired volume for different number of segments used in EPI. Up to 10 coronal slices were collected with an in-plane resolution of $1.5 \times 1.5 \text{ mm}^2$ or $1 \times 0.75 \text{ mm}^2$. The thickness of slices was 2 mm. T_2^* -weighted gradient-echo structural images were acquired from the same slices (repetition time = 600 ms, echo time = 10 ms, in-plane resolution = $0.75 \times 0.75 \text{ mm}^2$ or $0.5 \times 0.375 \text{ mm}^2$). Each scanning session consisted of 3–10 functional runs. Each run lasted from 450 to 2,400 s. In separate scan sessions, high resolution (0.625 mm isotropic voxels), T_1 -weighted anatomical images of the whole brain were collected from anesthetized animals using a Philips Achieva 7T whole-body MR scanner (Philips Healthcare, Cleveland, OH) with a 16-channel head coil (Nova Medical, Wilmington, MA). In the anesthetized monkey, an inversion prepared T_1 -weighted 3D turbo field echo (T1W 3D TFE) sequence was used.

Data were analyzed using SPM8 software (www.fil.ion.ucl.ac.uk/spm/) and custom code written in Matlab (Mathworks, Natick, MA). Functional data were not smoothed in the space domain, but low-frequency temporal fluctuations were removed by temporal filtering each series of images. The initial ten volumes of each run were discarded from data analysis so that only steady state signals were included. The correlation of each functional time course to a reference boxcar waveform was calculated, and functional maps were generated at a threshold of $p < 0.01$ (uncorrected for multiple comparisons). Only significant activation in clusters greater than five voxels were counted (29). The functional SNR was defined as the time-averaged value of signal divided by the temporal standard deviation of the signal. The CNR was calculated as the difference in T_2^* -weighted MRI signals between two states of the brain responding to two experimental conditions (with and without visual stimulation) divided by the standard variance of the MRI signal when no stimuli were presented.

Even well-trained monkeys can have large body or reward-related jaw movements during the scan even when their heads are fixed (37). These motion-related sources of noise can influence the data stability and may have disastrous influence on image quality (20). Data stability was estimated by a 3D rigid body model with six degrees of freedom (three translational movements $Tran_x$, $Tran_y$, and $Tran_z$ and three on rotational movements Rot_{Pitch} , Rot_{Yaw} , and Rot_{Roll}). All images within a run were aligned to the first image of each run. Two indices of motion *Displacement* and *Rotation* were defined as follows:

$$Displacement = \sqrt{Tran_x^2 + Tran_y^2 + Tran_z^2}$$

$$Rotation = \sqrt{Rot_{Pitch}^2 + Rot_{Yaw}^2 + Rot_{Roll}^2}$$

To estimate the extent of geometric distortion, functional images were non-rigidly co-registered with the anatomical image using an adaptive bases registration method (38). The deformation field generated from this procedure was used to calculate the distortion distance of every voxel in functional images.

Meridian mapping (39) aided by macaque atlases (40) was used to define areal borders in early visual areas.

3. Results

3.1. Strategies for improving data stability

Motion can contaminate the quality of fMRI images especially when attempting to increase the spatial resolution of the image. Post-processing methods, such as rigid body motion correction, can correct some small shifts but cannot fully correct for body movement (37).

The time course of data stability in one run is shown in Fig. 2A. Smaller displacements indicate greater stability. The translational and rotational motions are plotted as green and blue lines, respectively (see scale bar on right side of graph). For visual clarity, green and blue lines have been shifted vertically by an arbitrary amount. We found that with each fluid reward (pink triangle), there was a large corresponding motion artifact. This was observed in both the translational and rotational motion components. The extent of the body movement during reward can be ten to twenty times larger than the size of motion at time points between rewards.

Furthermore, we noticed that the data stability was directly related to the monkey's behavior. Behavioral performance was measured as the percentage of the time monkeys maintained good fixation (marked by the gray area in Fig. 2A). If monkeys broke fixation, they were not rewarded. In the run shown in Fig. 2, the monkey failed to maintain fixation five times (red arrows). Each break lasted from 20 to 50 seconds. Motion artifacts (calculated from displacement in the image) during the broken fixation period were greater than reward-related ones. The reward periods were also accompanied with a decrease in performance as monkeys blinked more frequently when they were rewarded (see enlarged inset). Better data stability is expected when animals maintain fixation well. Both translational and rotational motion artifacts were found to be inversely correlated with the percentage of the time animals maintained fixation (both $p < 10^{-10}$). Thus, in our subsequent image analysis, we only include runs collected during good behavioral performance (> 90% time of fixation).

We found that our imaging sessions fell into three stages. In the first stage, monkeys were trained to associate maintaining fixation with fluid reward. Animals were rewarded frequently, with an interval between rewards of less than ten seconds. The imaging sessions in this stage of training lasted around one and a half hours. The length of runs was short, mostly less than 10 minutes and the data stability at this stage was not good. The translational motion in one run at this stage (shown in Fig. 2B, red line) averaged $526 \pm 24 \mu\text{m}$ and reached a maximum displacement of a few millimeters. We also noticed that, within a run, the magnitude of motion changed over time. Typically, in the first and last two minutes of the run, the changes of displacement were larger than the remaining periods of the run (in this run, they were more than $500 \mu\text{m}$). Within a run, the extent of reward-related motions tended to increase over time.

In the second stage of training, we trained monkeys to adapt to sessions with longer runs with less frequent rewards. With training, the length of runs and the length of sessions improved over time. As a result, the functional images collected were more stable than those obtained in the first stage. In one run collected in this stage (shown in Fig. 2B, green line), the average displacement had fallen to $117 \pm 7 \mu\text{m}$. The motion artifacts in the first and last two minutes were still detectable but were smaller by more than half. Except for several large reward-related artifacts in the last two minutes, the remaining displacements were relatively small. As the length of runs were much longer in this stage, the influence from the initial and final periods within a run only accounted for a small number of volumes collected.

In the final stage, the interval between rewards was further lengthened such that monkeys received reward only at the end of each block. As shown in Fig. 2B (blue line), the length of a run increased to forty minutes. At this stage, the functional volumes collected in the first and last two minutes were as stable as volumes located in the middle of the run. The mean displacement of this run was $79 \pm 3 \mu\text{m}$. For comparison, a functional run collected from the same monkey in an anesthetized state in a 7T horizontal scanner had a mean displacement of $87 \pm 48 \mu\text{m}$, a magnitude which is not significantly different (data not shown). Thus a well trained monkey in the 4.7T machine can achieve as little displacement as an anesthetized preparation. This residual motion may in fact be an artifact resulting from quasiperiodic modulation of B0 associated with respiration.

For best results, monkeys need to be continuously trained for three to four months to achieve the third stage. As shown in Fig. 2C – 2E, repeated scans from one monkey revealed data stability from the first (red), the second (green), and the third (blue). The length of sessions (C), interval between rewards (D), and the length of runs (E) are plotted against the training session number. We found roughly each additional 30 training sessions improved the duration of a session by half an hour, the length of runs by about ten minutes, and the interval between rewards by five seconds.

3.2. Removal of susceptibility artifacts in high magnetic field artifacts by segmented EPI and sequence optimization

Our aim in using a vertical 4.7T scanner was to improve the temporal *SNR*, functional *CNR* and signal specificity of functional images. However, B0 inhomogeneities caused by susceptibility differences in high magnetic fields are difficult to shim to perfect homogeneity, even with the full second and third order shims provided with the MR machine. A T_2^* -weighted structural image collected with a multi-slice gradient-recalled echo sequence (GRE) sequence is presented in Fig. 3A. The influence of magnetic field inhomogeneity on the image distortion was negligible due to the high bandwidth readout window and relatively short TE (although some signal drop-out in areas of high B0 inhomogeneity is apparent). For functional mapping we used a T_2^* -weighted functional

gradient-echo echo-planar imaging (EPI). In single shot EPI, the whole of the required k -space is sampled following a single excitation pulse. Due to the long readout window, and the consequently very low pixel bandwidth in the phase encode direction, there are noticeable geometric distortions along the phase encode direction in the functional image (compare Fig. 3B with structural image in Fig. 3A).

We reduced the influence of local magnetic field inhomogeneity in two ways: replacing single-shot EPI with segmented EPI and optimizing echo time used in the EPI sequence. Rather than traversing the entire k -space, in 2-shot and 4-shot segmented EPI sequences, a half and a quarter of k -space, respectively, is traversed after a single excitation pulse. This significantly shortens the duration of the readout window. As a consequence, the degradation effect from inhomogeneities is reduced. A functional image collected with a two-shot EPI sequence with the same echo time used in the single-shot EPI sequence time (20 ms) is shown in Fig. 3C. The shape of the brain is closer to the structural image (seen in A) and has less geometric distortion.

We expected the SNR of two-shot EPI to be better than that of one-shot EPI. Indeed, compared the single-shot EPI image (Fig. 3E), the temporal SNR of some voxels in two-shot EPI was higher (Fig. 3F). However, we found, on average, the temporal SNR within cortical areas from four hemispheres we tested were not significantly different (20.2 ± 3.6 for single-shot and 23.5 ± 5.6 for two-shot EPI, $p = 0.35$). These results were due to serious signal losses at the edge of the brain (Fig. 4F). Such signal losses can be minimized by shortening the echo time, the time interval between an excitation pulse and the collection of data from the center of k -space (41). Figure 3D shows the image collected with a four-shot EPI sequence with echo time of 10 ms. The extent of distortion was significantly reduced ($p < 0.001$), falling from 2.9 ± 0.7 voxels with single-shot EPI to 1.5 ± 0.1 voxels with a four-shot EPI sequence. At the same time, the signal losses at the edges of the brain were largely recovered. As a result, the temporal SNR of voxels in the cortex increased more than 50% to 31.5 ± 1.7 ($p < 0.01$). Furthermore, the percentage of voxels with temporal SNR greater than 30 increased almost three times from $14.1 \pm 10.8\%$ to $55.8 \pm 7.0\%$ (Fig. 3G). These results indicate that our ability to discriminate signal from noise improved greatly by using segmented EPI and optimized parameters.

3.3. Contrast-to-noise ratio and spatial specificity of functional images

In addition to the temporal signal-to-noise ratio (SNR), the functional contrast-to-noise ratio (CNR) is a useful indicator of the imaging quality. Here, we used the BOLD signal change associated with activation from visual cortex in awake, behaving monkeys to estimate the functional CNR of our setup. Monkeys were presented binocularly with a large checkerboard pattern on a gray background (stimulus periods indicated by blue shaded stripes in Fig. 4A) with interleaved “blank” intervals with no stimulation (white stripes). MR signals from seven coronal slices over visual cortex were collected. Each of these slices revealed detectable visual stimulation induced activation (Fig. 4A). The time courses of the averaged BOLD signals from all significantly activated voxels in each slice are plotted as red lines. Every stimulation block caused a change in BOLD signal.

In Fig. 4B, the BOLD signals from the first slice are plotted relative to the stimulus presentation period (blue line). Individual trial responses are plotted as gray lines and the mean response of 25 repeats are shown by the black line (error bars indicate standard deviation). The time course of the activation from this slice has a typical shape of the hemodynamic response. Upon presentation of the visual stimulation, the activation increased sharply, reaching a plateau after about 6 seconds. The mean visual activation of all repeats was 3.2% with a range from 1.9% to 4.8%. The functional CNR in units of standard deviation was 4.0 for the first slice. The amplitudes of responses were different from slice to

slice. The averaged activations were $2.1 \pm 0.6\%$ with a range from 1.5% to 3.2% over seven slices we collected. In contrast, the functional *CNR* was relatively constant among slices at 4.0 ± 0.2 with a range of 3.7 to 4.2. The behavior of the monkey was very good in this 20 minute run and proceeded without any breaks in fixation, marked only by very brief blinks or saccades.

To further assess the spatial specificity of our setup, we increased the in-plane spatial resolution to $1000 \times 750 \mu\text{m}$. Compared to the voxel size of that in Fig. 4 ($1500 \times 1500 \mu\text{m}$), this decreases the volume of each voxel by two thirds. As shown in Fig. 5B, we can detect visual related activation fairly well with these small voxels. Previously, other studies have mapped the border between primary visual cortex (V1) and the second visual cortex (V2) in awake, behaving monkeys using surface coils (21,42) or contrast agents (39,43). We tested whether the visual field can be reasonably mapped using this smaller voxel size in a volume coil (which has one-fifth or less signal-to-noise ratio compared with surface coils, Logothetis et al 2002) and without contrast agents. Our stimuli consisted of checkerboard patterns overlying the vertical meridian (B1), the horizontal (B2) meridian, and annuli at three different eccentricities (B3: 1° , B4: 3° , B5: 5°). As shown in Fig. 5B, these stimuli produced activations consistent with previous studies of visual field maps in V1 and V2 of the macaque monkey (44). Activation in B1 is consistent with the location of the vertical meridian which is known to lie at the V1/V2 border, dorsally, close to and parallel to the lunate sulcus. The horizontal meridian wraps around from the caudal pole to the lateral (and foveal) extent of the operculum. B2 reveals a slice with activation biased towards the lateral pole, as expected at this antero-posterior location. As the visual stimulus location shifts from near foveal to more eccentric locations, the activation shifts from more lateral to more medial (B3 to B4 to B5). The overall activation map is illustrated in Fig. 5C–D and the boundaries between V1 and V2 (transitions from red to orange) in Fig. 5E (consistent with atlas, see Methods). These activated areas are consistent with previously published fMRI results (43) and atlas maps (40).

4. Discussion

4.1. Summary

We report on our methods for improving signal-to-noise ratio and contrast-to-noise ratio in fMRI images collected from awake, behaving monkeys in a 4.7T vertical bore scanner. The primary challenges of high field imaging in alert monkeys relate to field inhomogeneity due to susceptibility artifacts, increased variability due to motion and physiological noise, and image acquisition stability related to monkey behavior. In this study, we quantify behavioral performance improvement over multiple imaging sessions and its accompanying effect on motion-related noise reduction. Consistent training over a period of a few months reduced displacement to an average of less than $100 \mu\text{m}$. We also found that multi-shot EPI sequences coupled with short echo times significantly reduced susceptibility artifacts. This resulted in larger signal-to-noise ratios and reduced edge-related signal dropout. These methods achieved contrast ratios of about 4.0 (in units of standard deviation). Importantly, such measurable improvements led to higher spatial resolution mapping of visual topography. Neural activation with in-plane resolution of $1000 \times 750 \mu\text{m}$ could be reliably detected using a volume coil. This is significant as higher spatial resolution will lead to improved ability to map functional structures in the macaque monkey that are submillimeter in size.

4.2. Behavioral Training

Most fMRI studies in awake, behaving monkeys use data collection runs of short length. Monkeys are trained to maintain continuous fixation for less than ten minutes. During that

period, animals are given frequent fluid reward, typically one reward every two seconds at the beginning of a run to twice per second towards the end of runs. The direct influence on MR signal is hard to detect and to correct. This is especially true if the sampling rate of functional volumes is lower than the reward frequency, as the motion influence inherent to all volumes (5). By increasing the interval between rewards to twenty seconds, we are able to estimate the data stability of volumes with reward and those without. By quantifying translational and rotational displacement, we found that fluid reward does have a great influence on data stability. Even in well trained monkeys, the reward related motion noise can be as large as 1 mm. Inclusion of such noise in all volumes will inevitably degrade the image quality and limit the achievable spatial resolution. Our solution was to increase the interval between two rewards and to take advantage of periods without reward related noise. We also synchronized visual stimulation with the reward so that animals receive fluid reward only at the end of each block. The other training paradigm is that monkeys will be rewarded at the end of each trial. Animals are free to move their body until they are ready for the next trial. Keliris and colleagues reported that large body motions, which happen more frequently during breaks, have a direct current influence that is hard to correct (19). The body movement could be small within each trial. But the body motion between trials could be much larger and be destructive to the image quality of following volumes. Therefore, a continuous fixation paradigm will be able to eliminate the excess body movement between trials. Furthermore, with continuous fixation with long runs monkeys will spend most of their scan time to perform task.

4.3. Acquisition With Segmented EPI

The method of segmented EPI, originally proposed by McKinnon (45), was designed to minimize the influence from magnetic field inhomogeneity by reducing the duration of the readout window, thereby increasing signal-noise-ratio (in both anatomical and functional imaging) and increasing the spatial resolution. Segmented EPI has been applied to fMRI data acquisition in anesthetized monkeys (16,46,47) and in awake monkeys (15,20,21,24,48). This study extends these previous reports by quantifying, for the first time, the image quality and the number of segments used in the GE-EPI sequence. As we have shown in this paper, with our training methods, monkeys can perform the task long enough to obtain a good activation map with segmented EPI. At the same time, the data stability is sufficient to prevent significant ghosting artifacts. We found that with 4-shot segmented EPI, both the functional SNR and the number of voxels with high SNR can increase significantly compared with single-shot EPI. With segmented EPI, the functional images, even collected with a volume coil, can have signal-to-noise ratio of 30 and contrast-to-noise ratio of 4, ratios which have been previously achieved with surface coils and/or contrast agents (49).

4.4. Volume vs. Surface Coils

In this study, we used a volume coil for both transmission and reception. As this coil is large enough to fit the whole monkey head in it, it is capable of generating homogeneous fields which encompass all cortical areas in the monkey brain. As a consequence, the noise from all these regions may contribute to the signal collected. In comparison, the sensitive volume of a surface coil is small, with the depth of penetration dependent on the size of the coil. Surface coils achieve good signal to noise ratio by excluding noise signal from outside the region of interest. To help resolve the dilemma between the coverage and sensitivity, some coil array designs use a volume coil for transmission and an array of surface coils to receive. However, such a combination is accompanied by the cost of additional special hardware and software. In this study, we used a volume coil for both transmitting and receiving. We demonstrate here that, even without independent transmission and receiving coils, a volume coil is capable of achieving fairly high SNR and high spatial resolution. Combinations of

volume coil and surface coils would achieve even better results. For example, parallel imaging with a coil array could subsample k -space from individual coils, which would lead to increases in the speed of image acquisition. Coil arrays have more localized noise origin than volume coils, so we expect that the functional SNR of a coil array may be high enough to detect sub-millimeter structures in the brain of awake, behaving monkeys. At the same time, the faster speed in image acquisition will be critical to detect rapidly changing functional signals such as the initial dip.

5. Conclusions

Using a well-designed training paradigm, improved head-fix setup, and optimized sequence, we have demonstrated significant improvement of 4.7T fMRI imaging quality in awake, behaving monkeys. Our results are the first to quantitatively analyze the image quality of different training stage and sequence parameters in awake, behaving monkeys. Segmented EPI greatly improves the quality, signal-to-noise ratio and the spatial resolution of collected images. Neural activation with in-plane resolution of $1000 \times 750 \mu\text{m}$ could be reliably detected using a volume coil. We expect that these improvements, in combination with more sensitive surface coil or coil array systems, will lead to mapping of sub-millimeter structures in the awake, behaving monkey brain.

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Figure 1.

Setup for functional MRI in awake behaving monkeys.

Visual stimulation was delivered by a pair of plastic, fiber-optic glasses (4). The position of each eyepiece could be adjusted independently by an adjustable mount (3). Animals were head fixed by a headpost holder (1) secured by a frame (2) to the monkey chair. A volume coil (5) was used for both excitation and reception.

Figure 2.

Effect of fluid reward and behavioral training on data stability.

(A) Data stability declined when monkeys received fluid reward or broke fixation. Data taken from one run. Performance measured the percentage of time within each trial that the animal maintained fixation (gray shaded areas). Measured translational (green) and rotational (blue) motion are plotted in synchrony with performance (scale bars at right of graph). Pink triangles mark when fluid reward was given. Red arrows indicate breaks in fixation. Both fluid reward and break in fixation events were accompanied with larger translational and rotational displacements. We trained monkeys to perform the fixation task for longer periods per trial and to adapt to less frequent rewards. These training periods fell into three stages. Data stability improved from the first to second to third stages. (B) The translational motion measured from one session in each of the three stages: the first stage (session 42, red), second stage (session 115, green), and third stage (session 139, blue). The length of sessions (C), interval between rewards (D), and length of runs (E) improved with time. All three parameters increased with the number of training sessions. Each star indicates a single session. Three sessions show in (B) are marked as circles.

Figure 3. Effect of two-shot and 4-shot EPI sequence on the signal loss and distortion in functional images. (A–D) images obtained in awake, fixating macaque in vertical 4.7T machine. (A) anatomical image, (B) single-shot EPI image, (C) two-shot EPI image, (D) four-shot EPI image. Both B and C exhibit regions of signal loss and distortion due to susceptibility artifacts. These artifacts were reduced by increasing the number of segment to four (D). Concurrent with reduction in susceptibility artifact were increases in averaged signal-to-noise ratios in functional images (E – G). Scale: 10 mm. Color scale: functional signal-to-noise ratio.

Figure 4.

Functional activation of the brain revealed by fMRI.

(A) Areas of statistically significant activation to a flashing full field checkerboard are indicated in color. Time courses of averaged response in each slice are plotted as red lines. Time courses were shifted 6 s to accommodate for hemodynamic delay. Scale in left-upper panel: 10 mm. (B) Time course of averaged responses of all activated voxels in the first slice. The stimulation duration is shown in light blue in (A) and (B).

Figure 5.

Functional maps of the brain produced by fMRI.

(A) Acquisition plane of coronal slice shown in B. (B) Areas of statistically significant activation in one coronal slice in response to five different checkerboard patterns. B1: vertical meridian, B2: horizontal meridian, B3: 1° annulus, B4: 3° annulus, B5: 5° annulus. (C) and (D) Summary maps of B1–B5 illustrating the activations produced by color-coded regions of the visual field (inset) in visual cortex. (E) Mapped boundaries between V1 and V2 derived from imaging data and published functional atlases.